Rapid decline of *JAK2*V617F levels during hydroxyurea treatment in patients with polycythemia vera and essential thrombocythemia

In polycythemia vera (PV) and essential thrombocythemia (ET) the discovery of the presence of *JAK2*V617F mutations indicates that tyrosine kinase activation is a common pathogenetic mechanism in Ph- MPD.[1] The main therapeutic goal is to diminish the risk of clinical complications without increasing the risk of the development of myelofibrosis or acute leukemia. Hydroxyurea (HU) is widely used as a first line myelosuppressive therapy for patients with PV and ET.[2,3] The control of the thrombocytosis is usually rapid and effective with initial treatment with HU,[2] but the effect upon the *JAK2*V617F allele burden has not been studied. The *JAK2*V617F expression has been shown to decrease with IFN therapy and in some patients the mutated clone even seems to be eradicated.[4] The aim of this study was to investigate the *JAK2*V617F response of HU treatment in newly diagnosed patients with PV or ET. Nine patients with PV and 9 with ET positive for *JAK2*V617F with high risk MPD3 and need for myelosuppressive treatment were included. The PV patients were treated with phlebotomy prior to myelosuppressive treatment and all patients were on aspirin prophylaxis. Clinical characteristics at inclusion are presented in Table 1. The dose of HU was adjusted to achieve the stipulated platelet goal, platelets (PLT) ≥400×10^9/L, without neutropenia or anemia. The *JAK2*V617F level, measured as percentage mutated alleles, was determined before treatment and subsequently analyzed during treatment. The study was approved by the local ethics committee at the University of Gothenburg. Ten ml of EDTA anticoagulated peripheral blood was collected from 18 PV and ET patients at diagnosis and during HU treatment. Blood samples from 50 healthy controls were also collected. After isolation of total leukocytes, DNA was extracted by the BioRobot M48 workstation (QIAGEN, Solna, Sweden).

The percentage of *JAK2*V617F alleles was measured by real-time PCR under standard conditions on the ABI 7500HT system (Applied Biosystems, Stockholm, Sweden) and amplified by allelic-specific PCR and detected with Power SYBR Green PCR Master Mix (Applied Stockholm, Sweden). The JAK2V617F allelic specific primers used were, forward; 5'-GCA TTT GGT TTT AAA TTA TGG AGT ATa TT-3' with an introduced mismatch at the 3´-minus 2-position, and reverse; 5'-ACC TAG CTG TGA TCC TGA AAC TGA AT-3'. A standard curve, (InVivoScribe Technologies) containing DNA from a *JAK2*V617F clonal cell line, was used in serial dilutions in normal control DNA. The calculated mean day-to-day coefficient of variation was 9%. The Wilcoxon signed non-parametric ranks test and the Mann-Whitney U test were used for statistical analysis. The sensitivity of the quantitative *JAK2*V617F real-time PCR assay was estimated to be 0.25% by dilution of *JAK2*V617F clonal control DNA in normal DNA and an assay cut-off limit of 1% was set. All the tested 50 healthy controls had less than 0.25% *JAK2*V617F alleles (range 0.01-0.2%).

The PV patients had significantly higher *JAK2*V617F levels at diagnosis than ET patients (median 29.5% and 10.6% respectively, p=0.003).

After initiation of HU therapy the percentage of *JAK2*V617F in peripheral blood was subsequently tested after approximately 1, 4 and 12 months. The response to HU treatment was also followed with measurement of the hemoglobin (Hb) concentration and the PLT and white blood cell (WBC) counts (Table 1). All but 4 patients achieved the therapeutic goal within four months. A major reduction of the *JAK2*V617F levels was seen after four months of HU treatment (Figure 1). In both PV and ET patients significantly lower median *JAK2*V617F levels were detected (19.0%, p=0.0020 and 4.3%, p=0.012, respectively) compared to the levels at diagnosis. There were still significantly lower *JAK2*V617F levels after 12 months (17.8%, p=0.047 and 4.9%, p=0.039 respectively), the inter-individual changes were less than 20% in all evaluable patients but 3 who had a renewed increase of *JAK2*V617F. There was no significant difference in degree of reduction between PV and ET patients either after four or 12 months. In 3 PV and 2 ET patients the levels of *JAK2*V617F decreased less than 20% after four and/or 12 months of HU treatment, compared to typically more than a 40% reduction in the other cases. There was no significant difference in *JAK2*V617F, Hb, PLT or WBC levels at diagnosis between responders and non-responders. However, 2 of the patients (one with ET and one with PV) with low or no *JAK2*V617F reduction had an insufficient hematologic response.

Since the discovery of the *JAK2*V617F mutations in MPD in 2005[1] specific JAK2 inhibitors have been rapidly developed and some are already in clinical trials.[5] The effects of interferon therapy on *JAK2*V617F levels have recently been published.[4] In 24 out of 27 PV patients, with a median follow-up of 11 months, a 49% decrease in *JAK2*V617F levels was seen on treatment with pegylated IFN (Pegasys).[4] In contrast, the Swedish MPD study group

Table 1. Clinical characteristics of 18 patients with essential thrombocythemia or polycythemia vera treated with hydroxyurea.

Diagnosis	*Essential thrombocythemia*	*Polycythemia vera*
Number of patients	9	9
Female/Male	7/2	6/3
Age, years mean (range)	70 (52-81)	74 (66-83)
Hemoglobin conc. (g/L)		
At start of HU treatment	131 (105-150)	147 (126-178)
After 4 months of HU	133 (113-159)	131 (118-148)
After 12 months of HU	129 (110-147)	139 (131-151)
WBC count (×10^9/L)		
At start of HU treatment	9.8 (6.2-14.8)	20.8 (10.4-55)
After 4 months of HU	6.4 (4.0-9.4)	8.8 (4.5-26.4)
After 12 months of HU	6.9 (4.0-9.2)	9.5 (6.1-18.3)
PLT count (×10^9/L)		
At start of HU treatment	934 (412-1170)	720 (207-910)
After 4 months of HU	404 (277-742)	290 (91-563)
After 12 months of HU	364 (268-444)	325 (246-406)
JAK2V617F allele burden %		
At start of HU treatment	12 (1.4-20)	25 (16-62)
After 1 month of HU	10 (1.4-20)	26 (11-57)
After 4 months of HU	1.9 (0.8-16)	14 (3.5-57)
After 12 months of HU	1.9 (0.4-16)	10 (3.8-46)

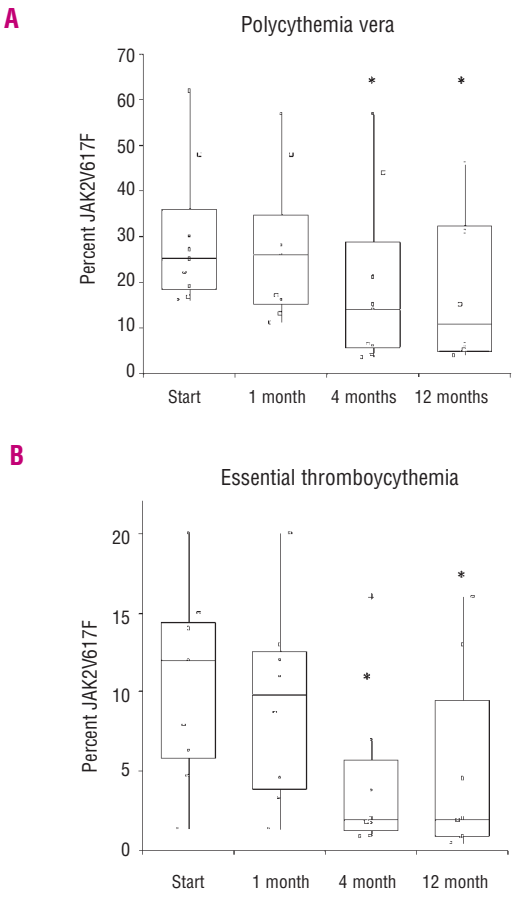

Figure 1. ***JAK2*V617F allele burden in nine patients with PV and nine patients with ET. The *JAK2*V617F was measured at start with hydroxyurea therapy, after one, four and twelve months, respectively. *indicate significant reduction of *JAK2*V617F allele burden compared with start, $p<0.05$.**

could only report a limited effect on the *JAK2*V617F levels during two years of pegylated IFN (PegIntron) therapy.[6] In the present study a rapid decrease in the *JAK2*V617F levels was seen in 72% of the patients and this effect was statistically significant after four months of HU therapy in both groups of patients. Only one of the patients had a higher level of *JAK2*V617F at four months compared with the level at start of HU therapy. The mean *JAK2*V617F levels in the patients decreased with 55% during the first four months. Among the patients who responded to treatment (i.e. *JAK2*V617F allele burden reduction more than 20%) the mean level of *JAK2*V617F was reduced with 71% (range 43-87%) in the present study. There was no significant change of the mean level of *JAK2*V617F between four and 12 months of therapy. Campbell *et al.* have reported a better hematologic response in ET patients with *JAK2*V617F mutation compared to patients with wild type JAK2 with HU treatment.[7] This finding may be due to the rapid decrease in *JAK2*V617F allele burden shown in this study. The influence of JAKV617F allele burden in myeloproliferative disorders and the risk of thrombosis is an issue of debate.[8-10] Antoniolio *et al.*[10] demonstrated a higher frequency of arterial thrombosis in patients with ET and high *JAK2*V617F allele burden at diagnosis. Pemmaraju *et al.*[8] could, however, not find any correlation between *JAK2*V617F allele burden and the risk of thrombosis in ET patients. However, the clinical relevance of the reduction of *JAK2*V617F levels has yet to be studied, but it could be hypothesized that a lower *JAK2*V617F allele burden has effect on the long-term clinical outcome.

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