Evaluation of bone marrow morphology is essential for assessing disease status in recombinant interferon α-treated polycythemia vera patients

Polycythemia vera (PV) is a clonal myeloproliferative neoplasm (MPN) almost always associated with mutations in *JAK2*, most commonly the gain-of-function missense variant V617F. Results of recombinant interferon α-2b (rIFNα-2b) or pegylated rIFNα-2α (peg-rIFNα-2a) therapy in patients with PV have shown the efficacy of this treatment in inducing hematologic and molecular responses.[1-4] *JAK2*^{V617F} allelic burden (%V617F), determined by quantitative RT-PCR, has been used increasingly as a surrogate marker of disease response to treatment.[5-7] Reduction in allele burden has also been used as a unique criterion for discontinuation of therapy.[8,9] However, it has been demonstrated that hematologic response does not always correlate with a significant decrease in %V617F after rIFNα-2b therapy.[10] To date, no studies have evaluated the relationship of change in %V617F with other indicators of disease such as bone marrow cellularity and/or degree of fibrosis.

At our institution, we encourage annual marrow examinations of our PV patients to assess cellularity and degree of fibrosis as one of the parameters we use for maintaining or changing the dose of interferon therapy. This is because we have observed persistent marrow hypercellularity and/or progressive fibrosis in PV patients receiving rIFNα despite developing a significantly reduced %V617F in peripheral blood (PB). This has therapeutic importance and relevance since increasing the dose of rIFNα may retard the progression of disease irrespective of marrow fibrosis.[5,11]

To gain a better understanding of the disease, we examined the correlation of %V617F with marrow cellularity and degree of fibrosis in patients treated with rIFNα. We also assessed immunohistochemical expression of pSTAT5 as a marker of JAK2 activation. Constitutive activation of JAK2 in patients harboring the V617F mutation results in phosphorylation of several downstream factors, including STAT5.[12] A previous study found significantly higher nuclear pSTAT5 expression in marrow from JAK2 mutation positive patients compared with those who were JAK2 negative; however, the correlation between staining and allele burden is unclear.[13]

We identified 15 patients with PV based on our criteria, which includes an increased Cr^{51} red cell mass and bone marrow abnormalities, who had at least 2 marrow examinations and quantitative *JAK2* levels measured at a median of 1.2 months from the bone marrow (BM) biopsy date.[14] Marrow abnormalities included hypercellularity and morphological changes in megakaryocytes consistent with PV. Clinical, hematologic and molecular responses were graded according to European LeukemiaNet criteria: complete molecular response (CMR) as a decrease of %V617F to less than 10%; partial molecular response (PMR) as a decrease of %V617F by more than 50% if the baseline %V617F was less than 50% or by more than 25% when the baseline %V617F was more than 50%.[7]

Bone marrow biopsies were fixed in Bouin's solution and evaluated for cellularity and degree of marrow fibrosis, as assessed by reticulin and trichrome stains. Marrow cellularity was assessed by 2 pathologists (AO and EM) and a hematomorphologist (RTS), by subtracting the estimated percentage of fatty marrow from 100.

Marrow fibrosis was scored according to the WHO 3-tiered semi-quantitative grading system (MF-1,2,3).[15] Immunohistochemical staining for phospho-STAT5 was performed on clot sections using previously established protocols for optimal detection of nuclear pSTAT5.[16] Nuclear expression of pSTAT5 was assessed in megakaryocyte nuclei.

Serial quantified measurements of the *JAK2*^{V617F} allele burden (%V617F) were performed from DNA purified from total white blood cells collected from PB at the time of the BM biopsy. %V617F was determined by pyrosequencing, a method for quantifying %V617F when the mutant allele is over 5%.

For continuous variables, a paired *t*-test was used; $P<0.05$ was considered statistically significant. For correlation of categorical variables, a Cohen kappa statistic was calculated. Analysis was performed in Microsoft Excel (Redmond, Washington, USA). This study received approval from the Institutional Review Board.

Out of 106 PV patients treated with rIFN, we identified 15 patients who had at least 2 BM examinations with paired *JAK2* V617F allele burden testing: 10 men and 5 women, median ages 62.1 (48-66.5) and 47.5 (34.4-72.7) years, respectively. The median allele burden at the time of the initial biopsy was 62.2% (18.2%-100%), which decreased to a median of 33% (0-93.7%) at the time of the subsequent biopsy (P=0.02).

Patients were initially treated with peg-rIFNα-2a (n=13) or rIFNα-2b (n=2). The initial dose of peg-rIFNα-2a was 45 mcg weekly and for rIFNα-2b, 1 MU ($1x10^6$ units) thrice weekly, both increased as necessary, and titrated for efficacy and toxicity as previously elaborated.[1,3] The median maintenance doses of peg-rIFNα2a and rIFNα-2b were 90 mcg and 3 MU weekly, respectively. The median durations of treatment between marrows were 3.4 (0.6-8.4) years for patients treated with rIFNα-2b and 4.8 (0.8-6.6) years for patients treated with peg-rIFNα–2a. The overall median duration of treatment between marrows was 4.2 (0.6-8.4) years and the total median duration of rIFN treatment was 5.5 years. One patient discontinued therapy due to adverse reactions after 3.3 years of rIFNα–2a treatment.

We first assessed the relationship between clinical and molecular response. All patients achieved either a complete (CHR) (n=8) or a partial (PHR) (n=7) hematologic response. Of these 15 patients, 3 achieved CMR, 4 PMR, and 8 patients no molecular response (NMR) (Table 1). We observed no correlation between clinical and molecular response since, of the 8 patients who achieved CHR, 2 achieved a CMR and 6 did not achieve any molecular response. There was no correlation between clinical response and fibrosis or cellularity, since of 8 patients

Table 1. Comparison of clinical response with molecular and morphological findings.

	CHR (n=8)	PHR (n=7)
CMR	2	1
PMR	0	4
NMR	6	2
Increase in cellularity	1	3
No change in cellularity	4	0
Decrease in cellularity	3	4
Increase in fibrosis	2	4
No change in fibrosis	4	2
Decrease in fibrosis	2	1

CHR: complete hematologic response; PHR: partial hematologic response; CMR: complete molecular response; PMR: partial molecular response; NMR: no molecular response.

Table 2. Relationship of morphological changes and molecular responses.

	CMR (n=3)	PMR (n=4)	NMR (n=8)
Cellularity			
Increase	0	2	2
No change	2	0	2
Decrease	1	2	4
Fibrosis			
Increase	2	2	2
No change	1	2	3
Decrease	0	0	3

Table 3. Comparison of change in JAK2 allele burden with change in pSTAT5 expression in megakaryocyte nuclei, as assessed by immunohistochemical staining.

	JAK2 VAF (%)		pSTAT5 staining	
Case ID	Initial	End	Initial	End
1	100	44	6/26	0/15
2	36	2	4/23	5/18
3	44	11	0/16	0/6
4	55	66	0/14	0/33

who achieved CHR, 5 showed an increased or unchanged cellularity and 6 had increased or unchanged fibrosis. Of the 3 patients with CMR, all had persistent hypercellularity (range: 60%-90%). Marrow fibrosis increased in 2 patients from MF-1 to MF-2 and MF-2 to MF-3, respectively, and remained unchanged in the third patient (MF-1). Among the 4 patients who achieved a PMR, 2 had an increase in cellularity and 2 had a significant decrease in cellularity (mean: -42.5%). However, fibrosis progressed in 2 of these patients and remained the same in the other 2.

We then assessed the relationship between morphological changes on BM biopsy and molecular response (Table 2). Of the 3 patients with CMR, 2 had persistent hypercellular marrows and one normocellular (range: 30%-100%). Marrow fibrosis increased in 2 of these patients from MF-1 to MF-2 and MF-2 to MF-3, respectively, and remained unchanged in the third (MF-1). Among the 4 patients who achieved a PMR, 2 had an increase in cellularity and 2 had a significant decrease in cellularity. However, fibrosis progressed in 2 of these patients and remained the same in the other 2. Among the 8 patients with no molecular response, 2 had an increase in marrow cellularity, 2 no change, and 4 a decrease. Fibrosis increased in 2 of the NMR patients, was unchanged in 3, and decreased in 3. Using Cohen's kappa statistic, no significant correlations were observed between change in %V617F and cellularity (κ=0.02) and fibrosis (κ=0.04) (*data not shown*).

To assess whether the persistent hypercellularity in rIFNa treated marrows was due to expansion of *JAK2* mutated cells, we assessed nuclear expression of pSTAT5 by immunohistochemistry in 4 patients with paired clot sections. Two patients showed no expression of pSTAT5. One patient who achieved CMR had a slight increase in pSTAT5 expression from 18% to 28% of megakaryocyte nuclei. One individual in PMR showed a marked reduction of pSTAT5 expression from 23% to 0%. Overall, we found no correlation between %V617F and the presence of p-STAT5 staining (Table 3).

Monitoring %V617F by quantitative RT-PCR has been proposed as a surrogate marker of disease status or 'tumor burden' in patients with PV, analogous to the use of *BCR-ABL1* transcript levels used to monitor patients with chronic myeloid leukemia. High %V617F has been associated with a poorer prognosis.[5,6] Thus, it is not unreasonable to assume that %V617F may be a non-invasive marker of disease burden in PV patients, and that by maximally reducing the biomarker, disease regression may occur. Recent reports have suggested that patients with PV with a sustained molecular response to interferon treatment may be candidates for discontinuation of therapy.[3,8,9] Similarly, the use of qualitative PCR (qPCR) to monitor disease and as a basis for rIFNα discontinuation has been proposed for CALR-mutated essential thrombocythemia.[17] Based on our findings, it may be premature to recommend PCR-based monitoring of these patients.

Based on our findings, it may be premature to recommend PCR-based monitoring of these patients as the sole criteria for treatment. In all patients with CMR, we observed progressive or persistent hypercellularity or fibrosis by marrow examination, suggesting to us a need for continued therapy in these patients. Furthermore, in patients who achieved CHR and/or PHR with decrease in %V617F, we observed persistent erythroid and megakaryocytic hypercellularity and no change or progressive fibrosis.

Our observations have unique implications for therapy. Our results question the use of *JAK2*^{V617F} allele burden as the sole criterion for discontinuation of therapy. Inappropriate discontinuation of rIFNα therapy may prematurely abrogate the value of its treatment. For the moment, the prognostic significance of failing to attain a morphological response in patients who have achieved a hematologic and/or molecular response remains unresolved. It is important to resolve these issues with longer follow up in a prospective trial, since premature discontinuation of rIFNα may allow the pathogenic mechanisms of the disease to progress unfettered.

Our findings add credence to recent World Health Organization criteria stressing the importance of the marrow biopsy at diagnosis, specifically recommended first by the Polycythemia Vera Study Group (PVSG).[14] Whereas it may be argued that a baseline BM biopsy is not a necessary diagnostic procedure for patients with a significantly increased red cell count and a demonstrable *JAK2*^{V617F} allele burden, a biopsy establishes initial degree of cellularity and fibrosis which should be used in clinical decision making during the course of the illness.

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