Correspondence

To the editor:

Endogenous erythroid and megakaryocytic circulating progenitors, HUMARA clonality assay, and PRV-1 expression are useful tools for diagnosis of polycythemia vera and essential thrombocytopenia

Recently, Liu et al. and Kralovics et al. reported in this journal the possible diagnostic value of some biologic markers in chronic myeloproliferative disorders such as polycythemia vera (PV) and essential thrombocytopenia (ET). Briefly, they described a strong correlation between endogenous erythroid colony growth in the absence of added erythropoietin (Epo) and polycythemia rubra vera (PRV-1) expression in PV patients.

Our experience demonstrates very similar results using comparable methodologies. We studied the correlations among endogenous growth of circulating erythroid burst-forming unit (eBFU-E) and megakaryocytic colony-forming unit (eCFU-MK) progenitors, clonality by human androgen receptor–polymerase chain reaction (HUMARA-PCR) assay, and expression of PRV-1 in females with PV (n = 11) and ET (n = 17). All patients fulfilled the diagnostic criteria of the Polycythemia Vera Study Group (PVSG). At the time HUMARA and PRV-1 assays were performed, 5 of 17 ET and 2 of 11 PV patients were receiving myelosuppressive/platelet-lowering agents. BFU-E and CFU-MK were determined at diagnosis, except for 2 ET patients who were receiving angagelide.

In vitro cultures were performed as previously reported. HUMARA assay was analyzed on granulocytes and CD34+ lymphocytes. PRV-1 expression was quantified by real-time reverse-transcriptase (RT)–PCR in RNA from granulocytes.

In PV patients, 11 (100%) of 11 showed eBFU-E and 4 (36%) of 11, eCFU-MK. Clonality was demonstrated in 5 (50%) of 10 patients, and 1 patient was homozygous. PRV-1 overexpression was detected in 10 (91%) of 11 patients. The correlation among the 3 assays gave a concurrent positive result in 5 patients, but considering the 3 assays separately, we found a positive result in all PV patients (Table 1).

In ET patients, 5 (29%) of 17 had eBFU-E, 7 (41%) of 17 had eCFU-MK, and 9 (53%) of 17 had eBFU-E and/or eCFU-MK. Clonality was demonstrated in 6 (40%) of 15 patients, and the other 2 were homozygous. PRV-1 overexpression was observed in 10 (59%) of 17 patients (Table 1). Correlation between eBFU-E and/or eCFU-MK and overexpression of PRV-1 were observed in 7 (41%) of 17 patients. When we compared the 3 assays, a coinciding positive result was observed in 2 patients, but if they were considered separately, a positive result was found in 15 (88%) of 17 ET patients.

In our preliminary results, the best and most reliable correlation was observed between eBFU-E and PRV-1 expression in PV (91%) of patients. In ET, a lower correlation (41%) between the 2 assays was found; but if we consider all assays separately, patients with a positive result increase to 88%. Cultures and PRV-1 results are in agreement with Liu et al. and Kralovics et al. except for eCFU-MK, which was not studied. Clonality by HUMARA gene is lower than that obtained by Liu et al. using G6PD, IDS, MPP1, BTK, and FHL1 genes.

We conclude that endogenous BFU-E remains the single most useful technique for diagnosis of PV and, together with PRV-1 expression, the most reliable biologic markers for PV. In ET, eBFU-E and eCFU-MK with PRV-1 expression are useful diagnostic tests in more than 70% of ET patients. We suggest, in line with others, that the concomitant implementation of in vitro cultures of BFU-E and/or CFU-MK and PRV-1 expression should be applied in PV and ET for their high diagnostic yield and that, whenever possible, HUMARA assay should be added.

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Table 1. In vitro growth of eBFU-E and eCFU-MK, clonality analysis and PRV-1 expression in patients with polycythemia vera and essential thrombocytopenia

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*eBFU-E indicates endogenous erythroid colony growth in the absence of added Epo; eCFU-MK, endogenous megakaryocytic colony growth in the absence of added LCM; and ND, not done. Clonality was calculated after correcting granulocytes value for the degree of lyonization of CD34+ cells. Clonality was considered when the corrected allele ratio was less than 0.30, which corresponds to more than 70% expression of one allele.

*Obtained using G6PD, IDS, MPP1, BTK and FHL1 genes.

†Obtained using HUMARA gene.
To the editor:

Usefulness of the quantitative assessment of PRV-1 gene expression for the diagnosis of polycythemia vera and essential thrombocythemia patients

In a recent article in this journal, Klippel et al.1 reported that overexpression of the polycythemia rubra vera 1 gene (PRV-1) in purified granulocytes can distinguish patients with polycythemia vera (PV) from those with secondary erythrocytosis (SE) and from healthy subjects. However, as Klippel et al. pointed out, the frequency of PRV-1 overexpression in PV patients still remains to be precisely established; whereas they observed PRV-1 overexpression in all the patients who met the World Health Organization (WHO) criteria, other groups2-4 reported PRV-1 overexpression in only a percentage of PV patients, ranging from 69% to 91%. These discrepancies may be due to the small number of cases studied, to the different procedures used, and to the fact that the PRV-1 expression has been evaluated in some cases on sorted granulocytes while in others on unfractioned peripheral blood (PB). Finally, the question concerning the PRV-1 expression in essential thrombocythemia (ET) and secondary thrombocytosis (ST) still remains to be answered.

To establish the significance of the PRV-1 expression as molecular marker of PV and ET, we carried out the quantitative assessment of the PRV-1 transcript in 119 unfractioned PB samples collected from 34 PV patients; 12 secondary erythrocytosis (SE) cases, represented by 10 patients with lung and heart diseases and 2 cases with familial erythrocytosis; 32 ET patients; 16 cases of secondary thrombocytosis (ST); and 25 healthy volunteers. PV and ET diagnosis was established according to the WHO criteria.

To evaluate the PRV-1 transcript amount, we used a real-time quantitative polymerase chain reaction (PCR) assay based on a specific set of primers and probe (Assays-on-Demand, Gene Expression Products) supplied by Applied Biosystems (Foster City, CA). The values obtained were normalized using Abelson (ABL) as control gene,5 and the results were expressed using the ΔΔCt method as the efficiencies of both PCR reactions were determined and found to be equal. Normal samples expressed almost constant PRV-1 transcript amount (mean value of 2ΔΔCt, 5.3; range, 1-14), and PRV-1 transcript levels in PB from SE, including the 2 cases of familiar erythrocytosis, and ST patients were not significantly different from those detected in healthy subjects: the mean value of 2ΔΔCt was 4.8 (range, 1-12) in SE and 4.9 (range, 1-12) in ST (P = .4 and P = .45, respectively, by t test; Figure 1). By contrast, in all PV patients we detected high levels of PRV-1 transcript (mean value of 2ΔΔCt, 7517; range, 46-40 342). The difference is highly significant with respect to normal PB samples (P = .005) and to SE (P = .006).

Similar results were obtained by analyzing the PB samples from ET patients: the mean value of 2ΔΔCt was 3949 (range, 29-38 967; Figure 1). Also, for ET patients the difference of expression is highly significant with respect to healthy subjects (P < .001) and to ST patients (P < .001). Our data, in accordance with those reported by Klippel et al.,6 clearly confirm that PRV-1 is a sensitive marker for diagnosis of PV and, in addition, demonstrate that this marker may be useful also for diagnosis of ET. Therefore, PRV-1 represents a sort of universal molecular marker useful in distinguishing between myeloproliferative disorders and secondary polyclonal disorders characterized by erythrocytosis and/or thrombocytosis. Finally, our data show that the quantitative assessment of PRV-1 can also be performed using unfractioned PB samples, making the procedure easier and faster.

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References