Zakar Mnjoyan, Jun Li, Vahid Afshar-Kharghan
Letters to the Editor

express PRV-1 mRNA? Is there a difference in the expression of PRV-1 on hematopoietic progenitor cells between normal subjects and patients with myeloproliferative diseases? Is there a correlation between the level of mRNA and surface expression of PRV-1? There are controversial data about the answer to this last question.57 Besides being a possible diagnostic marker for PV and ET, PRV-1 overexpression may alter cellular function. We found that expression of PRV-1 on CHO cells decreased the dependency of these cells on serum for survival and proliferation. The role of PRV-1 in proliferation of myeloid cells and in the pathogenesis of PV and ET should be studied further in other in vitro systems and in animal models.

The current study of 153 subjects with both classic and atypical myeloproliferative disorders suggests that neutrophil polycythemia rubra vera-1 (PRV-1) over-expression is a non-specific feature of clonal myeloproliferation that displays significant correlation with leukocyte alkaline phosphatase score. These observations undermine the utility of the PRV-1 assay as a diagnostic test of additional value.

Zakar Mnjoyan, Jun Li, Vahid Afshar-Kharghan
Thrombosis Research Section, Baylor College of Medicine, Houston, TX, USA

Key words: myeloproliferative disorders, PRV-1, cell proliferation, growth factors.

Correspondence: Vahid Afshar-Kharghan, M.D., Thrombosis Research Section, Department of Medicine, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.
Phone: international +1.713.798.3395. Fax: international +1.713.798.3345. E-mail: vahid @bcm.tmc.edu

References


Chronic Myeloproliferative Disorders

Neutrophil polycythemia rubra vera-1 expression in classic and atypical myeloproliferative disorders and laboratory correlates

Previous studies have found a strong association between neutrophil PRV-1 over-expression and polycythemia vera (PV) which is neither invariable (test sensitivity ranging from 69-100%) nor exclusive (a substantial minority of patients with either essential throm-
bocythemia (ET) or myelofibrosis with myeloid metaplasia (MMM) also display the specific abnormality). Nevertheless, neutrophil PRV-1 over-expression in myeloproliferative diseases (MPD) other than PV has been interpreted by some to represent a biological link with PV suggesting either an inevitable progression to PV, in the case of ET, or a marker of antecedent PV, in the case of MMM. In order to address the issue further, we prospectively studied neutrophil PRV-1 expression patterns in a large cohort of patients with typical MPD (PV, ET, MMM) as well as in a smaller cohort with atypical MPD including hypereosinophilic syndrome (HES) and systemic mastocytosis (SM). In addition, we explored for laboratory correlates of neutrophil PRV-1 expression in both PV and ET.

A total of 153 subjects were accrued to the study between April 2003 and July 2004. The diagnoses of PV, ET, MMM, HES, SM, and chronic myelomonocytic leukemia (CMML) were made according to the World Health Organization (WHO) diagnostic criteria. SP represented both secondary polycythemia (a co-morbidity known to be associated with secondary polycythemia was identified) and apparent polycythemia (the diagnosis of either PV or secondary polycythemia could not be made and the stability of hematocrit values was documented by serial measurements). Neutrophil PRV-1 transcript level was quantitatively measured by reverse transcriptase polymerase chain reaction (RT-PCR) and interpreted according to previously published methods.

Among the 153 study patients, 90 had classic MPD including 49 with PV, 23 with ET, and 18 with MMM (Table 1). It should be emphasized that cases of PV included both newly diagnosed cases and patients with a previously established diagnosis. The 18 MMM patients included 11 with agnogenic myeloid metaplasia (AMM), 4 with post polycythemic myeloid metaplasia (PPMM), and 3 with post-thrombocythemic myeloid metaplasia (PTMM). Twelve patients had atypical MPD (Table 2). Forty patients had either secondary or apparent polycythemia (SP) and 11 were healthy volunteers (Table 1).

### Table 1. Neutrophil PRV-1 transcript levels in typical myeloproliferative disorders, secondary or apparent polycythemia, and controls.

<table>
<thead>
<tr>
<th></th>
<th>PV (n=49)</th>
<th>ET (n=23)</th>
<th>AMM (n=11)</th>
<th>PPMM (n=4)</th>
<th>PTMM (n=3)</th>
<th>SP (n=40)</th>
<th>Controls (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRV-1 /GAPDH (median)</td>
<td>1.01</td>
<td>1.24</td>
<td>1.26</td>
<td>1.04</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>PRV-1 /GAPDH (range)</td>
<td>0.83-1.28</td>
<td>0.9-1.44</td>
<td>1.08-1.43</td>
<td>0.93-1.11</td>
<td>1.23-1.27</td>
<td>0.99-1.41</td>
<td>1.14-1.43</td>
</tr>
<tr>
<td>% with increased PRV-1 expression</td>
<td>76%</td>
<td>18%</td>
<td>17%</td>
<td>100%</td>
<td>0%</td>
<td>15%</td>
<td>9%</td>
</tr>
</tbody>
</table>

PV: polycythemia vera; ET: essential thrombocythemia; AMM: agnogenic myeloid metaplasia; PPMM, post-polycythemic myeloid metaplasia; PTMM, post-thrombocythemic myeloid metaplasia; SP, secondary or apparent polycythemia; NA, not applicable. Neutrophil PRV-1 expression was considered increased when the neutrophil PRV-1/GAPDH ratio was below 1.17.

### Table 2. Neutrophil PRV-1 transcript levels in 12 patients with atypical myeloproliferative disorders.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>Neutrophil PRV-1 /GAPDH ratio</th>
<th>Neutrophil PRV-1 expression*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CMML associated with myelofibrosis</td>
<td>63</td>
<td>F</td>
<td>1.05</td>
<td>Increased</td>
</tr>
<tr>
<td>2</td>
<td>FIP1L1-PDGFRA+ eosinophilic disorder</td>
<td>34</td>
<td>M</td>
<td>1.39</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>FIP1L1-PDGFRA+ eosinophilic disorder</td>
<td>52</td>
<td>M</td>
<td>1.19</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>FIP1L1-PDGFRA+ eosinophilic disorder</td>
<td>49</td>
<td>M</td>
<td>1.10</td>
<td>Increased</td>
</tr>
<tr>
<td>5</td>
<td>HES with cardiac involvement</td>
<td>68</td>
<td>F</td>
<td>1.15</td>
<td>Increased</td>
</tr>
<tr>
<td>6</td>
<td>HES with cardiac involvement</td>
<td>57</td>
<td>M</td>
<td>1.13</td>
<td>Increased</td>
</tr>
<tr>
<td>7</td>
<td>HES with sinus involvement</td>
<td>53</td>
<td>F</td>
<td>1.29</td>
<td>Normal</td>
</tr>
<tr>
<td>8</td>
<td>Aggressive SM associated with CMML</td>
<td>71</td>
<td>M</td>
<td>1.08</td>
<td>Increased</td>
</tr>
<tr>
<td>9</td>
<td>Aggressive SM with myelofibrosis</td>
<td>63</td>
<td>M</td>
<td>1.09</td>
<td>Increased</td>
</tr>
<tr>
<td>10</td>
<td>Aggressive SM with circulating mast cells</td>
<td>74</td>
<td>M</td>
<td>1.29</td>
<td>Normal</td>
</tr>
<tr>
<td>11</td>
<td>Indolent SM</td>
<td>39</td>
<td>M</td>
<td>1.20</td>
<td>Normal</td>
</tr>
<tr>
<td>12</td>
<td>Indolent SM</td>
<td>34</td>
<td>F</td>
<td>1.18</td>
<td>Normal</td>
</tr>
</tbody>
</table>

PRV-1: polycythemia rubra vera-1; CMML: chronic myelomonocytic leukemia; HES: hypereosinophilic syndrome; SM: systemic mastocytosis; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. *Neutrophil PRV-1 expression was considered increased when the neutrophil PRV-1/GAPDH ratio was below 1.17. **

PV (76%) and PPMM (100%), which was significantly different from the levels seen in both the other classic MPD and SP (Table 1; p < 0.0001). On the other hand, the incidence of PRV-1 over-expression was similar among SP (15%), ET (18%), AMM (17%), PTMM (0%), and con-

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Our experience is further supported by recent evidence from another large prospective study involving 99 patients with PV that reported similar sensitivity (68%) and specificity (60%) values. Although the small sample size does not allow definitive conclusions, the current study also confirms previous observations regarding the high prevalence of the specific abnormality in PFFM as opposed to in other subtypes of MMM. One of the original observations from the current study is the demonstration of neutrophil PRV-1 over-expression in a substantial proportion (50%) of patients with atypical MPD. This information strongly suggests that altered neutrophil PRV-1 expression is a non-specific marker of clonal myeloproliferation. Furthermore, in the current study, we were able to demonstrate that a PRV-1/GAPDH ratio of < 1.17 (i.e. increased PRV-1 expression) was almost always associated with an increased LAP score (>100) in both PV and ET. Obviously, this raises the issue of added value regarding consideration of the particular assay as a new diagnostic test and warrants careful evaluation in a larger study.

Shireen Sirhan, Terra L. Lasho, Michelle A. Elliott, Ayalew Tefferi
From the Division of Hematology, Mayo Clinic, Rochester, MN, USA

Key words: PRV-1, PRV-1 assay, diagnostic value.

Correspondence: Dr. Ayalew Tefferi, Division of Hematology and Internal Medicine, Mayo Clinic, 200 First St SW, Rochester, MN 55905, USA. Phone: international +1.507.2843159. Fax: international +1.507.2664972. E-mail: tefferi.ayalew@mayo.edu

References


**Chronic Myeloproliferative Disorders**

**Mutation of the prothrombin gene and thrombotic events in patients with polycythemia vera or essential thrombocythemia: a cohort study**

The association between a prothrombin mutation and the risk of thrombosis was analyzed in 214 patients with polycythemia vera or essential thrombocythemia. The rate for venous thrombotic events was 14.7/100 patient-years in patients with the prothrombin mutation compared to 0.8 in patients without the mutation (rate ratio 17.5).

Thromboembolism remains the major cause of morbidity and mortality in polycythemia vera (PV) and essential thrombocythemia (ET). It has been shown that the diagnosis of PV is frequently preceded by thromboembolic events with an increasing incidence during the last 7 years before diagnosis. Once the diagnosis has been established, the incidence of thrombosis has been estimated to range from 4 to 11 events per 100 patient-years. A similar frequency of thromboembolic complications was reported for ET.

The G20210A mutation in the prothrombin gene is associated with elevated levels of this zymogene and was identified as a congenital risk factor for deep venous thrombosis. Heterozygous carriers of the mutation have a 2- to 9-fold higher risk of deep venous thrombosis than do individuals with a normal genotype.

We performed a retrospective cohort study to assess the association between this prothrombin mutation and the risk of thrombotic events in PV or ET. The primary end-points were the occurrence of a venous thromboembolic event (i) in the seven years preceding the diagnosis of PV or ET and (ii) after diagnosis.

We determined the G20210A mutation of the prothrombin gene, factor V Leiden mutation, total homocysteine levels, plasma folate, vitamin B12 levels, protein C activity, free protein S antigen and antithrombin activity as previously described. Poisson regression was used to model the association between the prothrombin mutation and the incidence of venous thrombosis/embolism.

We assessed 214 patients with a chronic myeloproliferative disorder. The clinical characteristics of patients without and with the prothrombin mutation appeared to be comparable (Table 1).

Prothrombin mutation and venous thrombosis seven years before the diagnosis of PV or ET. Within 1,509 total observation years at risk, the incidence rate for a venous thrombotic event was 6.2 per 100 patient-years in patients with the