A dynamic prognostic model to predict survival in post-polycythemia vera myelofibrosis

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Introduction

Post-polycythemia vera myelofibrosis (post-PV MF) is a late evolution of PV. In 647 patients with PV, we found that leukocytosis leukocyte count > (15 × 10⁹/L) at diagnosis is a risk factor for the evolution of post-PV MF. In a series of 68 patients who developed post-PV MF, median survival was 5.7 years. Hemoglobin level less than 100 g/L (10 g/dL) at diagnosis of post-PV MF was an independent risk factor for survival. The course of post-PV MF, however, is a dynamic process that implies a progressive worsening of clinical parameters. Using a multivariate Cox proportional hazard regression with time-dependent covariates, we found that a dynamic score based on hemoglobin level less than 100 g/L (10 g/dL), platelet count less than 100 × 10⁹/L, and leukocyte count more than 30 × 10⁹/L is useful to predict survival at any time from diagnosis of post-PV MF. The resulting hazard ratio of the score was 4.2 (95% CI: 2.4-7.7; P < .001), meaning a 4.2-fold worsening of survival for each risk factor acquired during follow up. In conclusion, leukocytosis at diagnosis of PV is a risk factor for evolution in post-PV MF. A dynamic score based on hemoglobin level, and platelet and leukocyte count predicts survival at any time from diagnosis of post-PV MF. (Blood. 2008;111:3383-3387)

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Methods

Patients

In 76 consecutive patients previously diagnosed as post-PV MF according to the criteria of the International Working Group on Myelofibrosis Research and Treatment (IWG-MRT),1 the aim of this study is to define survival of patients with post-PV MF and to identify prognostic factors for survival. We developed a dynamic prognostic model useful to predict survival at any time from diagnosis.
**JAK2 (V617F) mutational analysis**

Granulocytes were obtained from the neutrophil fraction by osmotic lysis of red cells. Genomic DNA was obtained using the Puregene Blood DNA isolation kit (Genta Systems, Minneapolis, MN). A quantitative real-time polymerase chain reaction–based allelic discrimination assay was used to detect the V617F mutation of JAK2 gene.17

**Flow cytometric analysis of circulating CD34⁺ cells**

Circulating CD34⁺ cells were enumerated by flow cytometry using a single-platform assay as previously described,18 following the cell-gating guidelines recommended by the International Society for Hematothepmy and Graft Engineering (ISHAGE)19 and the subsequent modifications of the European Working Group of Clinical Cell Analysis (EWGCCA).20 Daily instrument quality control, including fluorescence standardization, linearity assessment, and spectral compensation, was performed to ensure identical operation from day to day.

**Statistical analysis**

The cumulative probability of survival was estimated using the Kaplan-Meier method. Comparison between survival curves was performed using the Gehan-Wilcoxon test. Survival analysis was performed using Cox models with time-dependent covariates to assess the effect of the variables of interest on overall survival (OS). Cox regression models were also applied to carry out multivariate survival analyses. Standardized mortality ratios (SMRs) were calculated to compare the patients’ mortality with the mortality of the general population in Italy. The Italian mortality rates by age, sex, and calendar year were provided by the Istituto Nazionale di Statistica (ISTAT, Rome, Italy). Statistical analyses were performed using Microsoft Excel 2000 (Redmond, WA), Statistica 7.1 (Stat-Soft, Tulsa, OK), and Stata 9.2 (StataCorp, College Station, TX).

**Results**

**Disease information prior to post-PV MF**

A total of 647 patients with PV were evaluated at the 2 institutions between 1970 and 2007. The median interval between the diagnosis of PV and that of post-PV MF was 13 years (range, 2.4–29.6 years). We found that the longer the follow-up of patients with PV, the higher the risk of developing post-PV MF (P < .001). During PV, myelosuppressive agents were given to 65 (96%) of 68 patients who developed post-PV MF and to 501 (86.4%) of 579 patients who did not, while the remaining patients received phlebotomy alone. The rate of patients receiving myelosuppression was significantly higher among those who developed post-PV MF (P = .01). On the other hand, patients receiving myelosuppression had a significantly longer follow-up than those treated with phlebotomy alone (7.1 years and 2.9 years, respectively; P < .001).

To investigate potential risk factors of transformation in post-PV MF present at diagnosis of PV, we evaluated the clinical features at diagnosis in the whole cohort of patients (n = 647). Parameters taken into account were age, hemoglobin level, platelet count, white blood cell count, spleen size (all considered as continuous numeric variables), leukocytosis (white blood cell count > 15 × 10⁹/L), calendar year at diagnosis, and institutional location. Univariate survival analysis showed that white blood cell count as numeric variable (P < .001) and white blood cell count more than 15 × 10⁹/L (P = .002) were significant risk factors for transformation in post-PV MF.

**Clinical features at diagnosis of post-PV MF**

Table 1 summarizes clinical and hematologic data at diagnosis of 68 patients with post-PV MF. IWG-MRT criteria and patients’ distribution per single criterion are outlined in Table 2. Regarding spleen, one patient underwent splenectomy before diagnosis of post-PV MF. Another patient with no spleen enlargement at diagnosis of post-PV MF showed anemia and leukoerythroblastic peripheral picture in addition to required criteria. Among 47 patients studied for the JAK2 (V617F) mutation at different intervals from diagnosis, all carried the mutation. In 27 patients evaluated at diagnosis, 21 (78%) had more than 50% mutant alleles. In all patients, the number of circulating CD34⁺ cells and serum lactate dehydrogenase (LDH) level exceeded the upper reference value (10 cells/μL for CD34⁺ cells and 450 U/L for LDH).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
<th>68</th>
<th>Age at diagnosis, y† (range)</th>
<th>65 (44-81)</th>
<th>Male/female</th>
<th>45/23</th>
<th>WBC count, × 10⁹/L,† (range)</th>
<th>12.2 (2.3-98)</th>
<th>Hemoglobin level, g/L,† (range)</th>
<th>123 (78-148)</th>
<th>PLT count, × 10⁹/L,† (range)</th>
<th>369 (50-1827)</th>
<th>Spleen size, cm below left costal margin</th>
<th>7 (0-25)</th>
<th>Bone marrow findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marrow cellularity, †% (range)</td>
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<td></td>
<td></td>
<td></td>
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<td>Marrow myeloblast, †% (range)</td>
<td>(0-5)</td>
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<tr>
<td>Reticulin fibrosis, grade 2:grade 3</td>
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<td></td>
<td>Lactate dehydrogenase, U/L,† (range), n = 41</td>
<td>837 (460-3151)</td>
<td>Circulating CD34⁺ cells/μL, †% (range), n = 39</td>
</tr>
<tr>
<td>Favorable</td>
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<td>Normal range of circulating CD34⁺ cells: less than 10/μL; normal range of LDH: less than 450 mU/mL.</td>
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<tr>
<td>Unfavorable</td>
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<td>*Favorable indicates normal, 20q; 13q–; unfavorable, other than favorable.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>IWG-MRT required criteria</th>
<th>No. of patients</th>
<th>68 (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Previous diagnosis of polycythemia vera (WHO criteria)</td>
<td></td>
<td>68 (100)</td>
</tr>
<tr>
<td>2. Bone marrow fibrosis grade 2-3 (on 0-3 scale)</td>
<td></td>
<td>68 (100)</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>IWG-MRT additional criteria (2 are required)</th>
<th>No. of patients</th>
<th>68 (100)</th>
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</thead>
<tbody>
<tr>
<td>1. Anemia* or</td>
<td></td>
<td>43 (63)</td>
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<tr>
<td>Sustained loss of requirement of phlebotomy or</td>
<td></td>
<td>25 (37)</td>
</tr>
<tr>
<td>cytoreduction</td>
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<td></td>
</tr>
<tr>
<td>2. Leukoerythroblastic peripheral blood picture</td>
<td></td>
<td>66 (87)</td>
</tr>
<tr>
<td>3. Increasing splenomegaly</td>
<td></td>
<td>54 (86)</td>
</tr>
<tr>
<td>Palpable spleen more than 5 cm from left costal margin</td>
<td></td>
<td>12 (18)</td>
</tr>
<tr>
<td>Appearance of a newly palpable splenomegaly</td>
<td></td>
<td></td>
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<tr>
<td>4. Development of more than 1 of the constitutional symptoms†</td>
<td></td>
<td>26 (38)</td>
</tr>
</tbody>
</table>

*Defined as hemoglobin value less than 120 g/L (12 g/dL) for female and less than 135 g/L (13.5 g/dL) for male. |
†Defined as 10% or more weight loss in 6 months, night sweats, unexplained fever (>37.5°C). |
‡One patient underwent splenectomy before diagnosis, so the calculation was provided on 67 patients.
Disease complications and outcome

Patients with post-PV MF were observed for 181 person-years of follow up after diagnosis and received palliative treatments. During follow-up, the incidence of thrombosis was 42 × 1000 person-years (95% CI: 19.9-35.5): 3 patients had deep venous thrombosis, 2 had stroke, and 1 had myocardial infarction. Two patients had splenic infarction. The incidence of leukemia was 50 × 1000 person-years (95% CI: 26.115), and the 3-year leukemia-free survival was 82%. Univariate analysis performed on clinical parameters at diagnosis of post-PV MF identified as significant risk factors for leukemia the low platelet count (P = .041) and the high circulating CD34+ cell count (P = .016). In a multivariate Cox proportional hazard regression, only circulating CD34+ cell count retained a significant impact on leukemia-free survival (P = .036).

The median survival of patients with post-PV MF was 5.7 years. The standardized mortality ratio (SMR) was 6.5 (95% CI: 4.2-10.1), indicating a significantly higher mortality for patients with post-PV MF in comparison with the general Italian population matched for age, sex, and calendar year (P < .001). We compared the survival of patients with post-PV MF (mortality: 11.1 per 100 person-years) with the survival of 291 patients with PMF (mortality: 10.1 per 100 person-years). Gehan-Wilcoxon test showed that survival of patients with post-PV MF was not significantly different from that of patients with PMF (P = .32). In addition, after adjustment for white blood cell count, hemoglobin level, platelet count, spleen size, and age in a multivariate Cox proportional hazard regression model, there was no difference in survival between the 2 conditions.

Finally, to evaluate whether transformation to myelofibrosis affects the overall survival of patients with PV, a Cox proportional hazard regression model with transformation to myelofibrosis as time-dependent covariate was applied to the whole series of PV patients. We found that survival of patients with PV was significantly worsened after progression to post-PV MF (HR = 2.17; 95% CI: 1.27-3.72; P = .005). This finding retained statistical significance also after adjustment for age, white blood cell count, hemoglobin level, platelet count, and spleen size in a multivariate Cox proportional hazard regression model.

Prognostic factors at diagnosis of post-PV MF

The parameters we evaluated at diagnosis of post-PV MF to investigate potential predictors of survival were age, hemoglobin level, platelet count, white blood cell count, spleen size, year duration of PV, serum lactate dehydrogenase level, granulocyte JAK2-V617F mutation burden, circulating CD34+ cells (all considered as continuous numeric variables), hemoglobin value less than 100 g/L (10 g/dL),21 white blood cell count less than 4 × 10^9/L,21 white blood cell count more than 30 × 10^9/L,21 platelet count less than 100 × 10^9/L,22 and karyotype23 (according to the categorization in use for PMF). Univariate survival analysis showed that hemoglobin value less than 100 g/L (10 g/dL; P < .001) and circulating CD34+ cell count (P = .009) were significant risk factors for survival. Multivariate Cox regression model including the parameters available in all patients at diagnosis of post-PV MF (hemoglobin value, white blood cell count, platelet count, spleen size, age) indicated that only hemoglobin level less than 100 g/L (10 g/dL) was an independent risk factor for survival (P < .001). Using this hemoglobin level as cutoff, patients could be stratified into 2 risk categories with significantly different survival: 6.6 years for those with hemoglobin value 100 g/L (10 g/dL) or higher and 1.9 years for those with hemoglobin value less than 100 g/L (10 g/dL; P = .0001).

Time-dependent analysis of prognostic factors

Sixty-four patients with post-PV MF had longitudinal blood cell count measurements at regular intervals from diagnosis. We studied this cohort of patients to assess whether variation of hematologic parameters during follow-up may further help in predicting survival at any time from diagnosis. The acquisition of the following parameters was studied: hemoglobin level less than 100 g/L (10 g/dL),212 platelet count less than 100 × 10^9/L,22 and white blood cell count less than 4 × 10^9/L or more than 30 × 10^9/L.21 Modification of therapy was not involved in the acquisition of risk factors. During follow-up of post-PV MF, hemoglobin level dropped lower than 100 g/L (10 g/dL) in 17 (26%) patients, platelet count lower than 100 × 10^9/L in 23 (36%), and white blood cell count lower than 4 × 10^9/L in 7 (11%) and higher than 30 × 10^9/L in 14 (22%).

As a first step, we evaluated univariate survival analysis with Cox regression models using hemoglobin value less than 100 g/L (10 g/dL), platelet count less than 100 × 10^9/L, white blood cell count less than 4 × 10^9/L, and white blood cell count more than 30 × 10^9/L as time-dependent covariates. The HRs were 5.8 (95% CI: 2.2-15.2; P < .001) for hemoglobin, 4.5 (95% CI: 1.67-12, P = .003) for platelets, and 8.2 (95% CI: 3.2-22; P < .001) for white blood cell count more than 30 × 10^9/L, while white blood cell count less than 4 × 10^9/L did not significantly affect survival (P = .115). After adjustment for age in a multivariate Cox proportional hazard regression with time-dependent covariates, hemoglobin value less than 100 g/L (10 g/dL), platelet count less than 100 × 10^9/L, and white blood cell count more than 30 × 10^9/L retained statistical significance on survival.

So, we defined a dynamic scoring system based on these 3 independent risk factors. As the 95% CIs of the 3 HRs did not differ, we assigned the same weight (presence = 1; absence = 0) to the 3 factors. As a consequence, the resulting score can be easily calculated by simply counting the number of risk factors acquired at any time during follow-up. The lower risk group includes patients who never acquire risk factors during follow-up (ie, hemoglobin level ≥ 100 g/L [10 g/dL], platelets ≥ 100 × 10^9/L, and white blood cells < 30 × 10^9/L). Conversely, higher risk categories include patients with 1, 2, or 3 risk factors, respectively. To assess the impact on survival of this dynamic scoring system, we analyzed the score as a continuous time-dependent covariate in a Cox survival regression model, obtaining an HR of 4.2 (95% CI: 2.4-7.7; P < .001). This implies a 4.2-fold increase of risk when the patient acquires one risk factor at any time from the diagnosis of post-PV MF. The time-dependent prognostic model retained statistical significance after adjustment for age (HR: 6.7, 95% CI: 3.14-7.7; P < .001). Figure 1 exemplifies the impact of this dynamic prognostic model on survival, showing the estimated survival curves for the resulting 4 risk groups according to the Cox time-dependent model.

Discussion

In this study, we evaluated 68 patients who developed post-PV MF in a cohort of 647 patients with PV. Diagnosis of post-PV MF is based on distinctive criteria, recently proposed by the IWG-MRT.3 These criteria combine histopathologic (bone marrow fibrosis),
alleles, as previously reported. In PMF, the rate of homozygosity patients at diagnosis of post-PV MF had more than 50% mutant carried the mutation with a high mutational burden. In fact, 78% of patients with PV after fibrotic transformation.

In conclusion, this study demonstrates that patients with PV showing a white blood cell count more than 15 $\times$ 10$^9$/L at diagnosis have higher risk of developing post-PV MF. When patients with PV develop post-PV MF, a dynamic prognostic model based on hemoglobin level, platelet count, and white blood cell count may predict survival at any time after diagnosis.

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**Authorship**

Contribution: F.P. and M.L. conceived the study, collected, analyzed, and interpreted data, and wrote the paper; E.M. and M. Cazzola analyzed and interpreted data; E.R. collected and analyzed data; M. Caramella, C.E., L.A., and C.D. collected clinical data; C.E. performed bone marrow evaluation; D.P. performed JAK2 mutation analysis; L.V. performed CD34+ cell count; P.B. performed cytogenetic analysis; C.P. did statistical analyses.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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**References**


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