Drivers and high-molecular-risk mutations in Argentine patients with primary myelofibrosis

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Abstract

Primary myelofibrosis (PMF), a BCR-ABL negative myeloproliferative neoplasm, is a heterogeneous clinical and genetic disorder with a poor prognosis. We aimed to study the mutational profile of driver genes (JAK2, CALR, and MPL) and high-molecular-risk (HMR) genes (ASXL1, EZH2, IDH1/2, and SRSF2) and their prognostic impact in 65 Argentine patients with PMF. Mutually exclusive driver mutations were identified in 88% of the cases and HMR mutations were detected in 37% of patients, including 37.5% of triple negative cases. ASXL1 and SRSF2 were the most frequently mutated HMR genes, and a significant association between these mutations was observed (P = 0.04). Additionally, age >65 years, lower hemoglobin level, constitutional symptoms, male sex, and bone marrow (BM) fibrosis grade ≥ 2 were correlated with ASXL1 mutations, and circulating blasts with SRSF2 mutations. HMR mutations were significantly associated with inferior overall survival (P < 0.001). These mutations were more frequent with increasing risk of DIPSS score and allowed us to identify patients with poor survival in intermediate DIPSS groups. The application of the MIPSS70 score stratified our patients into three risk groups, with significant differences in overall survival (P < 0.001). Interestingly, 85% of the patients who were upgraded to a higher risk category compared to the DIPSS score showed a HMR profile. In conclusion, our study contributes to the understanding of the mutational landscape in MFP, supports the integration of molecular data to improve prognostic models and reinforces the adverse prognostic impact of HMR mutations, particularly in ASXL1 and SRSF2 genes.

Keywords: Mutations; Myeloproliferative neoplasm; Primary myelofibrosis; Prognostic impact

1. Introduction

Primary myelofibrosis (PMF), a BCR-ABL negative myeloproliferative neoplasm (MPN) characterized by clonal proliferation of hematopoietic stem cells, progressive bone marrow fibrosis, extramedullary hematopoesis, anemia, splenomegaly, constitutional symptoms, leukemic progression, and shortened survival. Although JAK1/2 inhibitors are available for patients with PMF, the only treatment with curative potential is allogeneic stem cell transplant [1].

Driver mutations occurring in JAK2, CALR, or MPL genes lead to constitutive activation of the JAK-STAT pathway, a key pathogenetic event in all MPNs [2]. These mutations are often mutually exclusive and their frequencies in PMF are 50–60% for JAK2 V617F, 20–25% for CALR (approximately 75% type 1/1-like and 15% type 2/2-like), and 6–7% for MPL (predominantly MPL W515L/K). Approximately 10% to 15% of patients lack any of the 3 driver mutations and are referred to as being triple-negative (TN) [3, 4]. Distinctive clinical features and outcomes have been observed among
driver mutations. In fact, CALR mutations in PMF are associated with younger age, higher platelet count, and lower frequencies of anemia, leukocytosis, and spliceosome mutations than JAK2 V617F mutation. In addition, type 1/1-like CALR mutations have been correlated with superior survival [5, 6].

Other somatic non-driver mutations have been increasingly detected in PMF with the use of high-throughput sequencing. These additional mutations predominantly affect genes involved in epigenetic regulation and RNA splicing machinery, are commonly found in all types of myeloid neoplasms, and often contribute to the clinical phenotype and overall outcome [7]. Screening for non-driver mutations may help to determine the clonal nature of the disease in TN cases and has been included in the World Health Organization (WHO) major diagnostic criteria for PMF [8]. ASXL1, EZH2, SRSF2, and IDH1/2 mutations have been associated with inferior survival and an enhanced risk of leukemic transformation, and are referred to as high molecular risk (HMR) mutations [9]. The presence of HMR mutations, the occurrence of two or more HMR mutations and the absence of type 1/1-like CALR mutations have been incorporated as unfavorable risk factors into new prognostic models in PMF [10–12]. MIPSS70 (Mutation-enhanced international prognostic scoring system) for transplant-age patients is based on clinical and mutational risk factors, and is the only useful score in the absence of cytogenetic information, which is only available for a fraction of patients with this disease.

Herein, we examined the mutational profile of driver genes (JAK2, CALR, and MPL) and HMR genes (ASXL1, EZH2, IDH1/2, and SRSF2), and investigated their prognostic impact in 65 Argentine patients with PMF.

2. Material and methods

This study, approved by the bioethics committee at Faculty of Biochemical and Pharmaceutical Sciences, National University of Rosario, included 65 patients with PMF diagnosed according to the 2016 WHO criteria. A total of 51 (78%) cases were studied within 1 year of diagnosis, while other 14 (22%) cases were studied more than one year after diagnosis (mean 57.2 months, median 50.2, range 12.2-150 months).

DNA was extracted from peripheral blood samples using the phenol-chloroform method. JAK2 V617F and MPL W515L/K mutations were assessed as previously described [13]. CALR exon 9 and other MPL exon 10 mutations were evaluated using high-resolution melt assays and Sanger sequencing [14, 15]. ASXL1 was analyzed using a Sanger sequencing approach, by splitting exon 13 into 2 overlapping fragments. Mutational analysis of the entire coding regions of EZH2 and previously reported mutational hotspots for IDH1 (exon 4), IDH2 (exon 4), and SRSF2 (exon 1) was performed by high-resolution melting analysis, as described previously, with modifications [16–19]. Products showing abnormal melt patterns were directly sequenced. Variants were cross-referenced with COSMIC [20], ClinVar [21], and dbSNP [22] databases to identify clinically relevant mutations. In addition, a systematic literature review was performed to identify any possible novel variants.

All statistical analyses included clinical and laboratory parameters obtained at the time of referral for the molecular study. Differences in the distribution of continuous variables between categories were analyzed by either the Mann-Whitney or the Kruskal-Wallis test. Patient groups with nominal variables were compared using the Chi-square test or Fisher’s exact test, when appropriate. Overall survival (OS) was calculated from the date of referral to the date of the last follow-up or death. Survival curves were generated using the Kaplan-Meier method and compared by the log-rank test. In addition, a Cox proportional hazards regression model was used for univariate and multivariate analyses. P values less than 0.05 were considered significant. All analyses were conducted using GraphPad Prism 9.4.1 software.

3. Results

3.1. Clinical characteristics and mutation spectrum

Clinical and laboratory characteristics of the 65 study patients are outlined in Table 1. The median follow-up was 69.8 months (range: 3.4 - 163 months). The number of documented deaths was 37 (57%) and the median survival was 60 months.
Table 1 Main clinical and laboratory characteristic of 65 patients with PMF

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years; median (range)</td>
<td>64 (40-89)</td>
</tr>
<tr>
<td>Male sex; n (%)</td>
<td>36 (55)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL; median (range)</td>
<td>10.3 (5.4-15.2)</td>
</tr>
<tr>
<td>Leukocytes x 10⁹/L; median (range)</td>
<td>12.9 (2.1-85.2)</td>
</tr>
<tr>
<td>Platelets x 10⁹/L; median (range)</td>
<td>257 (19-1350)</td>
</tr>
<tr>
<td>DIPSS; n (%)</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>11 (19)</td>
</tr>
<tr>
<td>Intermediate-1</td>
<td>20 (34)</td>
</tr>
<tr>
<td>Intermediate-2</td>
<td>18 (31)</td>
</tr>
<tr>
<td>High</td>
<td>9 (16)</td>
</tr>
</tbody>
</table>

DIPSS, dynamic international prognostic scoring system.; clinical data was available for 58 patients.

Only 8% (n=5) of patients did not present any mutation in the studied genes (Figure 1). Driver mutation distribution was 57% (n=37) for JAK2 V617F, 22% (n=14) for CALR, 9% (n=6) for MPL, and 12% (n=8) for triple negative (TN). All these mutations were found to be mutually exclusive. A total of 31 HMR mutations categorized as pathogenic or likely pathogenic were identified in 37% (n=24) of the patients studied; 17 (26%) subjects harbored one and 7 (11%) two. Among driver mutational categories, these mutations were present in 38% of JAK2, 21% of CALR, 67% of MPL, and 38% of TN cases. The frequency of HMR variants was 26% (n=17) for ASXL1, 15% (n=10) for SRSF2, 5% (n=3) for EZH2 and 2% (n=1) for IDH2. No mutations were detected in IDH1.

Figure 1 Mutational spectrum in PMF.

Each column represents one patient. Variants are depicted by representative colored bars. JAK2 V617F (dark red), CALR type 1 (blue), CALR type 2 (dark blue), CALR indeterminate (turquoise), MPL W515L/K (green), p.L513_R514insTSWGLLLL (light green), ASXL1 frameshift and nonsense mutations (yellow), the three patients in light yellow presented unpublished ASXL1 mutations. SRSF2 P95 (pink), IDH2 p.R140Q (violet), EZH2 (red). Bar graphs on the right of the plot show the frequencies of mutations in each gene.

The only significantly associated mutations were those of ASXL1 and SRSF2 (P = 0.04). Additionally, ASXL1 mutations were correlated with age >65 years (76% vs 40%; P = 0.01), male sex (70% vs 48%; P = 0.05), lower hemoglobin level (9.2 vs 10.8 g/dL; P = 0.002), constitutional symptoms (63% vs 31%; P = 0.04), and bone marrow fibrosis grade ≥2 (76% vs 52%; P = 0.04). In turn, SRSF2 mutations showed a significant association with the presence of circulating blasts (70% vs 30%; P = 0.03) and were not found in CALR-mutated patients.

3.2. Mutations as prognostic factors for OS

Overall survival (OS) was not affected by status of driver mutations (P = 0.5), although the median survival was higher for CALR-mutated patients than for those with JAK2 V617F mutation (99 vs 44.7 months, respectively; P = 0.09).
Patients who displayed at least one HMR mutation were significantly associated with inferior OS than those without high-risk mutations (median, 92 vs 29 months; \( P < 0.001 \); Figure 2); however, patients with 1 and 2 HMR mutations showed similar survival (\( P = 0.9 \)). When ASXL1 and SRSF2 mutations were considered individually, both were of poor prognosis in the univariate analysis (\( P < 0.001 \) and \( P = 0.002 \), respectively), and their independent prognostic value was confirmed in the multivariate analysis that included both mutations as covariates (HR: 2.9, 95% CI: 1.3-6.4, \( P = 0.007 \) for ASXL1 and HR: 2.6, 95% CI: 1.0-6.5, \( P = 0.04 \) for SRSF2). The limited number of patients with EZH2 (n=3) and IDH1/2 (n=1) mutations prevented us from studying the clinical impact of these mutations individually.

\[ \text{Figure 2:} \text{Overall survival curves in 65 patients with PMF, stratified by the presence or absence of high molecular risk mutations.} \]

Median survival was assessed using the Kaplan-Meier method and compared using log-rank method. HR was estimated by Cox regression analysis.

The DIPSS model was predictive of OS (\( P < 0.001 \); Figure 3); however, no significant difference was observed between intermediate 2 and high-risk categories (\( P = 0.56 \)). Only one patient in the low-risk group presented a mutation in HMR genes, whereas these genes were more frequently mutated in the higher DIPSS groups: 35% of intermediate-1, 50% of intermediate-2, and 56% of high-risk cases. Patients in intermediate-1 and intermediate-2 groups with HMR mutations showed poorer OS than those from the same group without these mutations (median OS of 30 vs 99 months, \( P = 0.003 \) for intermediate-1 and median OS of 19 vs 55 months, \( P < 0.05 \) for intermediate-2; Figure 3).

\[ \text{Figure 3:} \text{Overall survival (OS) curves in 58 patients with PMF stratified by DIPSS score (A) OS curves in intermediate-1 (B) and intermediate-2 patients (C) stratified by the presence of high molecular risk (HMR) mutations.} \]

Median survival was assessed using the Kaplan-Meier method and compared using log-rank method.

The application of the MIPSS70 model to patients \( \leq 70 \) years (n=43) resulted in low, intermediate, and high-risk categories, composed of 11 (26%), 19 (44%), and 13 (30%) patients, respectively. The 5-year OS was 100% (median 163 months), 68% (median 77 months) and 31% (median 21 months) for low, intermediate, and high-risk patients,
respectively (\(P < 0.001\), Figure 4A). The model remained effective even after the inclusion of older patients in the analysis (\(P < 0.001\), Figure 4B). Figure 5 shows that one patient (with a HMR mutation) initially classified as low risk according to DIPSS was upgraded to the intermediate MIPSS category, and 12 patients (10 with a HMR profile) in intermediate DIPSS risk were reclassified into the high-risk MIPSS category. The median OS of the latter patients was 19.4 months, indicating that they were appropriately identified by MIPSS70 as being at high risk. Therefore, a HMR profile was responsible for the change in risk category in 19% (\(n=11\)) of the evaluated patients. Conversely, 5 patients without HMR mutations, 4 in the intermediate and 1 in the high DIPSS groups, were downgraded to the lowest and intermediate MIPSS categories, respectively.

**Figure 4** Overall survival (OS) curves according to MIPSS70 score (A) in patients \(\leq\) 70 years and (B) in all evaluable patients.

Median survival was assessed using the Kaplan-Meier method and compared using the log-rank method.

**Figure 5** Categorization of all evaluable patients (\(n=58\)) according to DIPSS versus MIPSS70 score.

Green, yellow, and red bars represent low, intermediate, and high-risk MIPSS70 groups, respectively, in the context of the DIPSS stratification (represented by the rows).

4. **Discussion**

In the present study, driver and HMR mutations were analyzed in 65 Argentine patients by traditional methods, which included ARMS and ASO PCR, high resolution melting and Sanger sequencing. The detection of the studied mutations allowed genetic confirmation of clonal proliferation in 92% of the patients: 55% displayed only a driver mutation, 32% showed co-occurrence of driver and HMR mutations, and 5% (37.5% of TN cases) had only HMR mutations.
Notably, a novel driver mutation was identified in MPL exon 10, p.L513_R514insTSWGLLLL (c.1540_1541ins[1518_1524;1524_1540]). This in-frame insertion affects the RWQFP amphipathic domain of MPL, which has been demonstrated to be important in maintaining the receptor inactive in the absence of thrombopoietin [23] In addition, 3 previously undescribed frameshift mutations were found in ASXL1 gene: p.E676Tfs*41 (c.2025_2028delinsCA), p.V771Nfs*3 (c.2310_2311delinsCAA), and p.I901Tfs*7 (c.2702del).

Although type 1/1-like CALR mutations are described to be predominant in PMF [5, 24, 25], in this study, consistently with our previous report [13], their frequency was lower than that of type 2/2-like mutations (36% vs 57%, respectively). Moreover, no association between type 1/1-like mutations and a favorable impact on overall survival was observed. These discrepancies may be due to the small sample size used in this work.

The most common mutated HMR gene was ASXL1 (26%); it was also the second most frequent mutated gene after JAK2. SRSF2 was mutated in 15% of cases, whereas EZH2 and IDH1/2 mutations were present in only 5% and 2% of patients, respectively. These findings are consistent with next-generation sequencing (NGS) reports [26–30]. The presence of ASXL1 mutations was associated with phenotypic characteristics representative of high-risk disease (age >65 years, lower hemoglobin level, constitutional symptoms, male sex, and BM fibrosis grade ≥ 2) and with inferior survival. SRSF2 variants were associated with ≥1% circulating blasts and with inferior survival. Additionally, as previously reported [9, 30], ASXL1 and SRSF2 mutations were found to cluster together.

HMR profile, found in 37% of the patients, influenced OS negatively; however, no differences in survival were observed between patients with 1 and 2 HMR mutations. The combination of HMR profile and DIPSS model allowed us to demonstrate the prognostic importance of HMR mutations in intermediate-1 and 2 risk groups. The limited number of cases prevented us from drawing any conclusions for the other groups. The three-tiered MIPSS70 model was able to stratify our cohort of patients ≤70 years old in three categories with significant differences in OS. Moreover, the model proved to be applicable to all patients, irrespective of age, suggesting its potential to be used for any patient with PMF. A total of 13 (22%) patients were upgraded in category compared to the DIPSS score; 85% of those patients presented a HMR profile.

5. Conclusion

Our prospective single-center study presents the mutational landscape of a cohort of 65 Argentine patients with PMF. Driver and HMR mutations were identified using traditional methods at frequencies comparable to those reported by NGS studies. Mutually exclusive driver mutations were found in 88% of the cases and HMR mutations were detected in 37% of patients, including 37.5% of TN cases. Furthermore, our findings reinforce the adverse prognostic role of HMR mutations, particularly in ASXL1 and SRSF2 genes, and support the concept that the integration of molecular data enhances the effectiveness of conventional prognostic models based on clinical and cytogenetic variables.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No author has a conflict or duality of interests to report.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References


