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Amina Kurtovic-Kozaric, et al.: Clinical outcomes in MPN mutational profiles

RESEARCH ARTICLE

The effects of mutational profiles on phenotypic presentation of myeloproliferative neoplasm subtypes in Bosnia: 18 year follow-up

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ABSTRACT

The identification of mutually exclusive somatic mutations shared among myeloproliferative neoplasm (MPN) subtypes has provided a powerful tool for studying disease evolution. Clinical features, gene mutations, and survival over 18 years were analyzed in MPN patients. One hundred thirty-eight MPN patients were subcategorized according to MPN subtypes: essential thrombocythemia (ET, n = 41), polycythemia vera (PV, n = 56), primary myelofibrosis (PMF, n = 10), and MPN unclassified (MPN-U, n = 31). Patient characteristics included clinical parameters, overall survival, and mutational status of the JAK2, CALR, and MPL genes. We compared hematologic and clinical features of JAK2\textsuperscript{V617F}-ET vs. CALR-mutated ET vs. JAK2\textsuperscript{V617F}-PV patients. JAK2\textsuperscript{V617F}-patients had higher values of erythrocytes, hemoglobin, and hematocrit compared to CALR-mutated patients (p < 0.05). The mutant allele burden in JAK2\textsuperscript{V617F}-PV and JAK2\textsuperscript{V617F}-ET patients directly correlated with erythrocyte, hemoglobin, and hematocrit values, but it inversely correlated with platelet count. Thus, mutant allele burden was an indicator of the clinical phenotype in JAK2\textsuperscript{V617F}-MPN patients. OS was not affected by the mutational status. In general, mutated JAK2, CALR, and MPL genes left specific hematological signatures.

KEYWORDS: MPN; myeloproliferative neoplasm; JAK2; janus kinase 2; CALR; calreticulin; MPL; myeloproliferative leukemia virus; mutant allele burden
INTRODUCTION

Myeloproliferative neoplasms (MPNs) are a group of clonal myeloid disorders that affect normal blood cell production in the bone marrow [1-3]. According to the 2016 revision of the WHO classification of tumors of hematopoietic and lymphoid tissues, MPNs are categorized into chronic myeloid leukemia (*BCR-ABL1* positive), polycythemia vera (PV), primary myelofibrosis (PMF, prefibrotic/early and overt fibrotic stage), essential thrombocythemia (ET), chronic eosinophilic leukemia (CEL), chronic neutrophilic leukemia (CNL), mastocytosis, and unclassifiable MPNs (MPN-U) [4-9]. The most common *BCR-ABL1* negative MPNs include PV, ET, and PMF, while the other subtypes are rare [4]. These diseases have shared clinical features and a molecular basis [10]. Around 5% of patients suffer from progression to more advanced disease, including transformation to acute myeloid leukemia (AML) [11].

WHO 2016 revision lists the presence of driver mutations as one of several major criteria in the diagnosis of these diseases [12]. *BCR-ABL1* negative MPN patients carry driver mutations in *JAK2* (Janus kinase), *CALR* (calreticulin) and *MPL* (myeloproliferative leukemia virus oncogene) genes [13,14]. High proportion of MPN patients (75%) carries the unique *JAK2*V617F mutation in exon 14; subsequently exon 12 mutations were found in 5% of patients with PV [15]. Somatic *MPL* exon 10 mutations include W515L and W515K, and were first described in 2006 [16]. Recently in 2013, *CALR* mutations were found in nonmutated *JAK2* and *MPL* ET and PMF patients [17]. In fact, about 80% of *CALR*-mutated patients harbor one of two mutually exclusive mutation variants: type 1 (52-bp deletion) or type 2 (5-bp TTGTC insertion) [18].

Overall, the incidence of the mutations within individual subtypes of MPN is: PV (98% of patients with *JAK2* mutations), ET (60% *JAK2*, 22% *CALR* and 3% *MPL*) and PMF (58% *JAK2*,
25% \textit{CALR} and 7\% \textit{MPL}) [12,13]. \textit{JAK2}^{V617F} PV patients have significantly higher mutant allele burden (MAB) in comparison to \textit{JAK2}^{V617F} ET patients [19].

Mutually exclusive somatic mutations in \textit{JAK2}, \textit{CALR} and \textit{MPL} genes gave new insight into pathogenesis and diagnostics of MPN. In this study, we compared hematologic and clinical characteristics of \textit{JAK2}^{V617F} vs \textit{CALR}-mutated ET patients and correlated those findings with \textit{JAK2}^{V617F} PV patients.

Even though the disease presentation might be different, recent work has shown that \textit{JAK2}^{V617F} ET and PV are diseases that evolve from a single neoplasm [10]. On the other hand, ET with \textit{CALR} mutations is a separate disease category, both clinically and molecularly, from \textit{JAK2}^{V617F} ET. Thus, \textit{JAK2} and \textit{CALR} mutations determine the evolution of the MPN subtype. Therefore, the aim of this study was to evaluate disease phenotypes and evolution in regards to the detected \textit{JAK2}, \textit{CALR} and \textit{MPL} mutations in the population of MPN patients in Bosnia and Herzegovina.

We compared hematologic and clinical phenotypes of ET patients carrying either \textit{JAK2} or \textit{CALR} mutations with \textit{JAK2}^{V617F} PV patients in order to determine the correlation of clinical features and disease evolution.

\textbf{MATERIALS AND METHODS}

\textbf{Study population}

A cohort of 138 patients diagnosed with MPN and treated at Department of Hematology, Clinical Center of the University of Sarajevo, and Department of Internal Medicine, Cantonal Hospital Zenica, in the period January 2000-June 2018 were included in this study. Procedures performed in the study were in accordance with the 1964 Helsinki Declaration. Diagnoses of PV, ET, PMF, and MPN-U were made according to the World Health Organization 2008 or 2016 classification criteria for hematopoietic malignancies, according to the criteria at the time of
Briefly, 24 patients were diagnosed before 2008, 57 patients were diagnosed between 2008 and 2016, and 56 patients were diagnosed after 2016. All major and minor criteria for MPN diagnosis according to WHO classification were collected, except for EPO levels which had not been performed routinely for all patients. Analyzed patients were subcategorized according to $BCR-ABL1$ negative MPN subtypes: ET (n=56), PV (n=41), PMF (n=10), and MPN-U (n=31). Patients' characteristics included full blood count, bone marrow morphology, hepato/splenomegaly, and overall survival.

**JAK2, MPL, and CALR mutation analysis**

All molecular analyses were performed at the Clinical Center of the University of Sarajevo, Department of Clinical Pathology, Cytology and Human Genetics, Laboratory for Human Genetics. Genomic DNA was extracted from patients’ peripheral blood using standard protocol (Qiagen QIAamp® DNA mini kit, USA). JAK2 mutational analysis and MAB were performed by qPCR using quantitative allelic discrimination assay (Ipsogen MutaQuant and MutaScreen kit, Qiagen, USA). $JAK2^{V617F}$ positive patients were not tested for $CALR$ and $MPL$ mutation. $JAK2^{WT}$ patients were tested for $CALR$ mutations in exon 9. Primers for ASO-PCR were designed to detect mutations type 1 and 2 in one reaction, as previously described [20]:

```
F1 5'-GCA GCA GAG AAA CAA ATG AAG G-3',
F2 5'-GCA GAG GAC AAT TGT CGG A-3' and
R 5'-AGA GTG GAG GAG GGG AAC AA-3' (Invitrogen, Thermo Fisher Scientific, USA).
```

Double negative patients ($JAK2$ and $CALR$) were sequenced to detect mutations, type 1 (W515L,1544G>T) or type 2 (W515K, 1543_1544TG>AA), in $MPL$ gene (exon 10). We used primers for the amplification of 212 bp region;

```
F 5'-TGG GCC AAG GTC TGA TGT CGG A-3',
and
R 5'-ACA GAG CGA ACC AAG AAT GCC TGT-3' (Invitrogen, Thermo Fisher Scientific, USA).
```
**Statistical analysis**

Numerical variables have been presented by their range and median, and categorical variables by count and relative frequency (%) of each category, as presented in Supplemental Table 1 and Table 1. Comparisons of quantitative variables between groups of patients were carried out by the nonparametric Wilcoxon rank-sum test. Correlation between numerical variables was tested by the nonparametric Spearman’s $\rho$ ($\rho$) coefficient.

Overall survival (OS) was estimated using the Kaplan-Meier method, and survival curves were compared by the log-rank test. Survival probabilities were estimated with the Kaplan-Meier method using and compared using the log-rank test. Data were analyzed using IBM SPSS v.21 (IBM Corp., Armonk, NY, USA). Statistical significance was determined at the level $p<.05$ (2-tailed).

**RESULTS**

**Clinical characteristics**

We included 138 MPN patients, diagnosed with PV (n=41), ET (n=56), PMF (n=10) and MPN-U (n=31) in this study. Patients characteristics are shown in Supplemental Table 1, which reports clinical parameters and mutational status of each MPN subtype at diagnosis. Median follow-up was 33 months (60 45.5, 69 and 20 months for PV, ET, PMF and MPN-U subtypes, respectively). Age at diagnosis among different MPN subtypes did not show significant differences ($p>0.05$). Regarding mutational analysis, three genes were analyzed: $JAK2$, $CALR$, and $MPL$. $JAK2^{V617F}$ mutation was found in 71% of all MPN patients (Supplemental Table 1); $CALR$ mutations, type 1 and 2, were detected in 13% of all MPN patients; $MPL$ mutations, type 1 (W515L) and type 2 (W515K), were found in 4% of all MPN patients. Table 1 categorized
clinical parameters of each MPN subtype at diagnosis according to the presence of \textit{JAK2}^{V617F}, \textit{CALR}, and \textit{MPL} mutations.

**Mutational status vs CBC in PV patients**

Hematologic parameters in PV patients compared to mutational status (\textit{JAK2}^{V617F} and \textit{JAK2}^{WT}) were summarized in Figure 1. Among PV patients, the presence of \textit{JAK2}^{V617F} mutation was associated with higher platelet count (p<0.05). However, no effect was found on clinical parameters such as WBC, RBC, Hb, Hct. WBC and RBC values were higher in \textit{JAK2}^{V617F} PV patients, although difference was not significant.

**Mutational status vs CBC in ET patients**

Hematologic parameters in ET patients compared to mutational status (\textit{JAK2}^{V617F}, \textit{JAK2}^{WT}, \textit{CALR}-mutated, \textit{CALR}^{WT}, \textit{MPL}-mutated, \textit{MPL}^{WT}) were presented in Figure 2. RBC levels were higher in \textit{JAK2}^{V617F} compared to \textit{JAK2}^{WT} ET patients. Between \textit{JAK2}^{V617F} vs \textit{CALR}-mutated ET patients, \textit{JAK2}^{V617F} mutation was present in patients with lower platelet count (p<0.05). We also categorized ET patients based on the presence or absence of driver mutations (triple negative vs patients with driver mutations) and found no significant differences in hematologic parameters.

Since PV and ET patients share \textit{JAK2}^{V617F} mutation, we compared the clinical parameters among \textit{JAK2}^{V617F} ET vs \textit{JAK2}^{V617F} PV patients; we found that RBC, Hb, and Hct values were higher in PV patients t, but platelet count values were lower; however, the frequency of splenomegaly was higher (p<0.001). Interestingly, we found the same results if we compared \textit{JAK2}^{V617F} PV patients and \textit{CALR}-mutated ET patients (p<0.05).

**Mutational status vs CBC in PMF patients**

Hematologic parameters in PMF patients compared to mutational status were summarized in Supplemental Figure 1. The presence of either \textit{JAK2}^{V617F} or \textit{CALR} mutation did not have an
impact on analyzed clinical parameters (p>0.05). When we compared $JAK2^{V617F}$ PMF, ET, and PV patients, we found that $JAK2^{V617F}$ PV patients had higher RBC (p<0.001), Hb, and Hct (p<0.05) values; $JAK2^{V617F}$ PMF patients had higher WBC values compared to $JAK2^{V617F}$ PV and ET patients. Comparison between CALR-mutated PMF vs CALR-mutated ET patients showed that CALR-mutated PMF patients had lower Hb (p<0.05) and Plt values (p<0.001).

Mutational status vs CBC in MPN-U patients

Values of hematologic parameters in MPN-U patients compared to mutational status were presented in Supplemental Figure 2. Platelet count was statistically different between $JAK2^{V617F}$ and $JAK2^{WT}$ MPN-U patients. Comparison among $JAK2^{V617F}$ MPN-U, PV, ET, PMF patients showed that $JAK2^{V617F}$ MPN-U patients had higher RBC and Hb values (p<0.05) and lower WBC values (p<0.05) than $JAK2^{V617F}$ PMF patients, and lower Hb values than $JAK2^{V617F}$ PV patients (p<0.05). In $JAK2^{V617F}$ MPN-U patients platelet count was lower than in $JAK2^{V617F}$ ET patients (p<0.05).

Driver gene MPN patients

We hypothesized that the presence of a specific mutation in $JAK2$, $CALR$, or $MPL$ gene is the driver of MPN pathogenesis. Thus, we combined data for all PV/ET/PMF/MPN-U patients that carried $JAK2^{V617F}$ mutation. Accordingly, we organized data for $CALR$ and $MPL$ positive MPN patients (Figure 3). We found that $JAK2^{V617F}$ patients had higher values for RBC, Hb, and Hct compared to $CALR$-mutated patients (p<0.05). $MPL$-mutated patients had higher values for Plt compared to $JAK2^{V617F}$ patients (p<0.05).

Allele burden

We assessed the MAB in 45 $JAK2^{V617F}$ MPN patients. The median $JAK2^{V617F}$ MAB at diagnosis was significantly lower in $JAK2^{V617F}$ ET than in PV patients (21% vs 40%, p<0.05). In other
words, in 12% of $JAK2^{V617F}$ ET patients, a MAB was higher than 50%, compared to 47% of PV patients.

Correlation between hematologic parameters (RBC, Hb, Hct, Plt) and MAB was calculated for $JAK2^{V617F}$ PV vs $JAK2^{V617F}$ ET patients, as illustrated in Figure 4. Direct correlation was found between the MAB and RBC; however, Hb and Hct were directly correlated and Plt count was inversely correlated with MAB but without statistical significance. These findings present that the MAB is an indicator of the phenotypic presentation of $JAK2^{V617F}$ MPN.

**Overall survival**

Kaplan–Meier estimates were performed to generate and analyze survival-time data. We compared survival rates among different MPN subtypes (PV, ET, PMF, MPN-U). At 120 months, overall survival for PV, ET, PMF and MPN-U patients was 80%, 90%, 100%, and 77%, respectively. Also, all MPN patients were categorized according to presence of driver mutation ($JAK2^{V617F}$, $CALR$-mutated and $MPL$-mutated). Interestingly, $JAK2^{V617F}$ patients had worse survival compared to $CALR$-mutated and $MPL$-mutated patients, even though significant differences were not found (p>0.05). The presence of $JAK2^{V617F}$ mutation in PV patients did not confer better survival. No significant differences in OS were found among ET patients who were triple negative or carried a driver mutation (Supplemental Figure 3).

**DISCUSSION**

MPN driver mutations in Janus kinase 2 ($JAK2$), calreticulin ($CALR$), and myeloproliferative leukemia virus oncogene ($MPL$) genes, upregulate JAK-STAT signaling pathway [21]. Ectopic expression of $JAK2^{V617F}$ stimulates proliferation of erythroid progenitor in Epo-dependent manner, delays final erythropoiesis due to low expression level of erythroid-related genes, and activates abnormal STAT signaling through activation of Stat1 protein [22]. $JAK2$, $MPL$, and
CALR mutations in BCR-ABL1 negative MPN patients are usually mutually exclusive [23]. The discovery of these genes in MPN pathogenesis has led to the introduction of targeted therapy [23]. Therefore, it has become crucial to test all BCR-ABL1–negative MPN patients for these mutations for management and prognosis of the disease [24-27].

When we analyzed blood parameters such as RBC, WBC, Hb, Hct and Plt of all MPN patients regardless of the disease subtype, we found the following: RBC count was higher in JAK2V617F PV patients vs CALR-mutated ET patients, suggesting that the presence of JAK2V617F helps RBC production. Furthermore, when we compared JAK2V617F PV vs JAK2V617F ET vs JAK2V617F PMF patients, we found that the highest RBC values were in JAK2V617F PV patients. Similarly, higher JAK2V617F expression (allele burden) was associated with increased RBC values in JAK2V617F PV vs JAK2V617F ET patients, supporting the hypothesis that higher JAK2V617F expression leads to increased RBC values.

Higher platelet counts were associated with the presence of CALR mutation, similar to other published studies [23,28,29]. When we compared CALR-mutated ET patients vs JAK2V617F ET patients, we found that CALR mutation is associated with increased platelet counts. Similarly, our results showed that CALR-mutated ET patients had higher platelet counts than CALR-mutated PMF patients, suggesting that there may be an additional factor that increases platelet count in ET patients.

Previous studies have hypothesized that JAK2V617F, CALR and MPL are the drivers of MPN pathogenesis [10,30-33]. Since JAK2V617F is present in both PV and ET patients, we compared their blood parameters. Regarding platelet count, JAK2V617F ET patients vs JAK2V617F PV patients had higher platelet values, which suggests that even though there is no CALR mutation, there might be an additional factor that increases platelet values in ET patients, differentiating
them from PV patients [34]. Similar to our results, Rumi and colleagues [10] showed that $JAK2^{V617F}$ MPN patients had lower platelet counts than $CALR$-mutated patients. Also, the same study found that hematologic parameters of $JAK2^{V617F}$ ET and $JAK2^{V617F}$ PV patients were associated with the MAB, leading them to conclude that $JAK2^{V617F}$ PV and ET present distinct phenotypes of a single MPN ($JAK2^{V617F}$ MPN), whereas $CALR$-mutated ET is another disease category.

We found that within $JAK2^{V617F}$ disease, WBC was higher in PMF than PV patients, which could imply that there is additional factor in $JAK2^{V617F}$ PMF patients that increases WBC count. Regarding overall survival, we found that $CALR$-mutated patients had higher survival rates compared to $JAK2^{V617F}$ patients, which is similar to results published by Tefferi et al., and Kourie et al. [23,35]; however, statistical significance was not reached. ET patients had better survival than PV patients and MPN-U patients ($p<0.05$). The presence of $JAK2^{V617F}$ in PV patients did not confer better survival compared to $JAK2^{WT}$ PV patients. Similarly, triple negative ET patients did not have worse survival compared to mutation-positive ET patients ($p>0.05$). In a study performed by Tefferi et al., triple negative status in PMF patients did not show additional prognostic information for overall survival [36].

**ACKNOWLEDGMENTS**

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**DECLARATION OF INTERESTS**

The authors declare no conflict of interests.
REFERENCES


TABLE 1. Demographic, hematologic and clinical features at diagnosis of patients with PV, ET, PMF, subdivided according to JAK2, CALR or MPL mutational status

<table>
<thead>
<tr>
<th>CHARACTERISTICS (normal range)</th>
<th>PV JAK2+ (N=25)</th>
<th>PV NA (N=9)</th>
<th>ET JAK2+ (N=31)</th>
<th>ET CALR+ (N=8)</th>
<th>ET MPL+ (N=2)</th>
<th>PMF TRIPLE NEGATIVE (N=4)</th>
<th>PMF NA (N=11)</th>
<th>PMF JAK2+ (N=6)</th>
<th>PMF CALR+ (N=2)</th>
<th>PMF MPL+ (N=1)</th>
<th>PMF TRIPLE NEGATIVE (N=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALE/ FEMALE (% MALE)</td>
<td>9/16 (36%)</td>
<td>8/1 (89%)</td>
<td>9/22 (29%)</td>
<td>5/3 (63%)</td>
<td>1/1 (50%)</td>
<td>0/1 (0%)</td>
<td>4/7 (36%)</td>
<td>4/2 (67%)</td>
<td>1/1 (50%)</td>
<td>0/1 (100%)</td>
<td>1/0 (100%)</td>
</tr>
<tr>
<td>AGE, Y</td>
<td>70 (29-84)</td>
<td>69 (61-81)</td>
<td>66 (40-82)</td>
<td>68 (37-84)</td>
<td>62 (56-68)</td>
<td>67.5 (28-87)</td>
<td>69 (39-78)</td>
<td>63.5 (25-78)</td>
<td>56 (47-65)</td>
<td>56 (32)</td>
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<tr>
<td>RBC (4.34-5.72 x 10^{12}/L)</td>
<td>6.67 (2.87-8.32)</td>
<td>5.82 (4.60-7.77)</td>
<td>5.01 (3.22-7.29)</td>
<td>4.85 (3.35-5.03)</td>
<td>4.5 (4.29-4.71)</td>
<td>4.51 (4.41-5.14)</td>
<td>4.73 (2.68-7.94)</td>
<td>4.88 (3.57-5.63)</td>
<td>4 (3.57-5.63)</td>
<td>4.46 (1.89)</td>
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<tr>
<td>HEMOGLOBIN (138-175 g/L)</td>
<td>169 (118-217)</td>
<td>173 (155-207)</td>
<td>146 (78-169)</td>
<td>143.5 (118-156)</td>
<td>130.5 (129-132)</td>
<td>144.5 (134-151)</td>
<td>147 (118-194)</td>
<td>138 (89-149)</td>
<td>121.5 (118-125)</td>
<td>115 (35)</td>
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<tr>
<td>HEMATOCRIT (0.415-0.530)</td>
<td>0.55 (0.35-0.66)</td>
<td>0.53 (0.47-0.63)</td>
<td>0.44 (0.25-0.55)</td>
<td>0.43 (0.36-0.45)</td>
<td>0.38 (0.39-0.45)</td>
<td>0.43 (0.39-0.45)</td>
<td>0.44 (0.33-0.59)</td>
<td>0.41 (0.31-0.47)</td>
<td>0.34 (0.29)</td>
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<td>MCV (83-97.2 fL)</td>
<td>82 (56.8-124)</td>
<td>87 (80.6-101)</td>
<td>87.5 (73.4-102)</td>
<td>89.5 (85-95.6)</td>
<td>89 (88.2-95.2)</td>
<td>88 (74-114)</td>
<td>80.8 (78-103)</td>
<td>86 (77)</td>
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<tr>
<td>WBC (3.4-9.7 x 10^{9}/L)</td>
<td>10 (5.2-16.9)</td>
<td>7.10 (5.13-13.00)</td>
<td>11.15 (6.06-15.97)</td>
<td>9.98 (6.5-18.01)</td>
<td>11.09 (10.98-11.2)</td>
<td>9.63 (5.1-12.5)</td>
<td>9.35 (3.3-19.4)</td>
<td>18.55 (1.63-40.8)</td>
<td>8.16 (9.85)</td>
<td>5.5</td>
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<tr>
<td>NEUTROPHILS (44-72%)</td>
<td>68.5 (41.7-82)</td>
<td>59.11 (42-80.2)</td>
<td>68.50 (43.2-75.6)</td>
<td>60.8 (46-85.6)</td>
<td>65.00 (61.45-71.9)</td>
<td>71.93 (53.9-90.9)</td>
<td>75.9 (62.6-84)</td>
<td>/ (69.64)</td>
<td>/</td>
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<tr>
<td>EOSINOPHILS (0-7%)</td>
<td>2 (0.3-6.15)</td>
<td>1.85 (1-2.93)</td>
<td>1.60 (1.029-5.3)</td>
<td>1.49 (1-1.98)</td>
<td>1.80 (2.016-4.68)</td>
<td>2.38 (2-3.43)</td>
<td>2.63 (2-3.43)</td>
<td>2.14 (1.42)</td>
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<td>BASOPHILS (0-0.43%)</td>
<td>0.8 (0.1-3.01)</td>
<td>1 (0.83-1)</td>
<td>1.03 (0.263-3.57)</td>
<td>0.34 (0.04-0.64)</td>
<td>0.91 (0.04-0.64)</td>
<td>1.49 (1.06-1.92)</td>
<td>0.68 (0.102-1.25)</td>
<td>1.8 (1.18-3)</td>
<td>/ (1.3)</td>
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<td></td>
<td>PLT (158-424 x 10^9/L)</td>
<td>471 (97.3-1650)</td>
<td>266 (151-532)</td>
<td>834 (367-1438)</td>
<td>1155.5 (848-1250)</td>
<td>977 (922-1032)</td>
<td>648 (448-1051)</td>
<td>927 (530-1643)</td>
<td>342 (158-2250)</td>
<td>284.5 (157-412)</td>
<td>1479</td>
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<td><strong>SPLENOMEGALY</strong></td>
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<td></td>
<td>5/14 (36%)</td>
<td>3/3 (100%)</td>
<td>3/14 (21%)</td>
<td>1/6 (17%)</td>
<td>1/2 (50%)</td>
<td>1/3 (30%)</td>
<td>1/9 (11%)</td>
<td>4/4 (100%)</td>
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</tr>
<tr>
<td><strong>HEPATOMEGALY</strong></td>
<td></td>
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<td></td>
<td>1/10 (10%)</td>
<td>0/4 (0%)</td>
<td>2/12 (17%)</td>
<td>0/5 (0%)</td>
<td>1/2 (50%)</td>
<td>0/2 (0%)</td>
<td>1/8 (13%)</td>
<td>2/3 (67%)</td>
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<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td><strong>LDH (125-241 U/L)</strong></td>
<td></td>
<td>381.5 (217-1876)</td>
<td>321 (159-721)</td>
<td>282 (167-521)</td>
<td>345 (183-553)</td>
<td>261.5 (175-348)</td>
<td>387 (161-613)</td>
<td>337 (235-427)</td>
<td>609 (254-1697)</td>
<td>1166.5 (1102-1231)</td>
<td>410</td>
</tr>
<tr>
<td><strong>BILIRUBIN</strong></td>
<td></td>
<td>12.7 (7.6-19.5)</td>
<td>33.1</td>
<td>10</td>
<td>/</td>
<td>23.1 (7.4-38.8)</td>
<td>/</td>
<td>7.2</td>
<td>31.95 (22-41.9)</td>
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<td>/</td>
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<tr>
<td><strong>THERAPY</strong></td>
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<td>Hydroxiurea, Litalir, Allopurinol, Controloc, Aspirin Protect</td>
<td>Hydroxiurea, Allopurinol, Controloc, Aspirin Protect</td>
<td>Hydroxiurea, Allopurinol, Controloc, Aspirin Protect</td>
<td>Hydroxiurea, Allopurinol, Controloc, Aspirin Protect</td>
<td>Hydroxiurea, Allopurinol, Controloc, Aspirin Protect</td>
<td>Hydroxiurea, Allopurinol, Controloc, Aspirin Protect</td>
<td>Sura, Folicin Aspirin Protect</td>
<td>Sura, Folicin Aspirin Protect</td>
<td>Sura, Folicin Aspirin Protect</td>
<td>Sura, Folicin Aspirin Protect</td>
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<td><strong>BM CELLULARITY</strong></td>
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<tr>
<td></td>
<td>3/4 (75%)</td>
<td>0/1 (0%)</td>
<td>11/16 (69%)</td>
<td>2/2 (100%)</td>
<td>1/2 (50%)</td>
<td>0/1 (0%)</td>
<td>4/5 (80%)</td>
<td>4/5 (80%)</td>
<td>/</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td><strong>FOLLOW UP MONTHS</strong></td>
<td></td>
<td>24 (2-218)</td>
<td>113 (11-164)</td>
<td>19 (4-131)</td>
<td>69 (17-159)</td>
<td>40.5 (4-77)</td>
<td>43 (19-102)</td>
<td>90 (68-170)</td>
<td>40.5 (1-100)</td>
<td>109</td>
<td>69</td>
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<tr>
<td><strong>DECEASED</strong></td>
<td></td>
<td>2/25 (8%)</td>
<td>3/9 (33%)</td>
<td>2/31 (6%)</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>1/11 (9%)</td>
<td>0/6 (0%)</td>
<td>0/2 (0%)</td>
<td>0/1 (0%)</td>
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<td></td>
<td>0/1 (0%)</td>
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</tbody>
</table>
FIGURE 1. Hematologic parameters in JAK2\textsuperscript{V617F}, and JAK2\textsuperscript{WT} PV patients. Data are presented in a box and whisker plot showing the upper and lower values (highest and lowest horizontal line, respectively) and upper and lower quartile (box) with median value. Patients with JAK2\textsuperscript{V617F} PV had markedly higher platelet count values (p<0.05) than patients without JAK2\textsuperscript{V617F} mutation. WBC and RBC levels were higher in JAK2\textsuperscript{V617F} PV.
FIGURE 2. Hematologic parameters in JAK2<sup>V617F</sup>, JAK2<sup>WT</sup>, CALR-mutated, CALR<sup>WT</sup>, MPL-mutated, MPL<sup>WT</sup>, triple negative ET patients and ET patients with driver mutation. Data are presented in a box and whisker plot showing the upper and lower values (highest and lowest horizontal line, respectively) and upper and lower quartile (box) with median value. JAK2<sup>V617F</sup> ET patients had markedly higher RBC values (p<0.05) than JAK2<sup>WT</sup> patients, and CALR-mutated patients had significantly higher platelet count compared to JAK2<sup>V617F</sup> ET patients.
FIGURE 3. PV/ET/PMF/MPN-U patients are categorized according to detected driver gene mutation ($JAK2$, $CALR$ and $MPL$). Presence of driver mutation in $JAK2$ gene had impact on higher RBC and Hb values (*) compared to $CALR$-mutated patients, and $MPL$ mutation had impact on higher Plt values (**) compared to $JAK2$-mutated patients.
FIGURE 4. Relationship between $JAK2^{V617F}$ allele burden and hematologic parameters in ET and PV patients. The mutant allele burden was directly correlated with RBC values ($\rho = 0.362$, $p<0.05$), Hb level ($\rho = 0.140$, $p>0.05$) and hematocrit ($\rho = 0.206$, $p>0.05$), and inversely correlated with Plt count ($\rho = -0.12$, $p>0.05$).
### TABLE S1. Clinical characteristics and mutational status of 138 MPN patients at diagnosis in Bosnia and Herzegovina

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>MPN (n=138)</th>
<th>PV (n=41)</th>
<th>ET (n=56)</th>
<th>PMF (n=10)</th>
<th>MPN-U (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALE/FEMALE (% MALE)</td>
<td>67/71 (49%)</td>
<td>22/19 (54%)</td>
<td>19/37 (34%)</td>
<td>6/4 (60%)</td>
<td>20/11 (65%)</td>
</tr>
<tr>
<td>AGE, Y</td>
<td>66 (22-89)</td>
<td>69 (29-84)</td>
<td>66 (28-87)</td>
<td>59.5 (25-78)</td>
<td>61.50 (22-89)</td>
</tr>
<tr>
<td>RBC *</td>
<td>5.40 (1.89-8.44)</td>
<td>6.34 (2.87-8.44)</td>
<td>4.88 (2.68-7.94)</td>
<td>4.46 (1.89-5.63)</td>
<td>5.69 (2.12-8.32)</td>
</tr>
<tr>
<td>HEMOGLOBIN *</td>
<td>150 (35-217)</td>
<td>170 (118-217)</td>
<td>145 (78-194)</td>
<td>121.5 (35-149)</td>
<td>150 (76-184)</td>
</tr>
<tr>
<td>HEMATOCRIT *</td>
<td>0.45 (0.25-0.66)</td>
<td>0.54 (0.36-0.66)</td>
<td>0.43 (0.25-0.59)</td>
<td>0.37 (0.29-0.47)</td>
<td>0.47 (0.31-0.62)</td>
</tr>
<tr>
<td>MCV *</td>
<td>86.5 (56.8-124)</td>
<td>83 (56.8-124)</td>
<td>88.9 (73.4-114)</td>
<td>80.8 (77-103)</td>
<td>85.5 (59-106)</td>
</tr>
<tr>
<td>WBC *</td>
<td>10.00 (1.63-40.8)</td>
<td>8.96 (5.13-16.9)</td>
<td>10.95 (3.3-19.4)</td>
<td>9.85 (1.63-40.8)</td>
<td>9.99 (2.8-26.6)</td>
</tr>
<tr>
<td>NEUTROPHILS *</td>
<td>66.00 (42-90.9)</td>
<td>65.5 (42-82)</td>
<td>67 (43.2-90.9)</td>
<td>75.9 (62.6-84)</td>
<td>64.57 (47.7-82.2)</td>
</tr>
<tr>
<td>EOSINOPHILS *</td>
<td>1.7 (0.129-6.15)</td>
<td>1.85 (0.3-6.15)</td>
<td>1.8 (0.129-5.3)</td>
<td>1.71 (1.42-3.43)</td>
<td>1.50 (1.3-2.3)</td>
</tr>
<tr>
<td>BASOPHILS *</td>
<td>0.87 (0.04-3.57)</td>
<td>0.91 (0.1-3.01)</td>
<td>0.90 (0.04-3.57)</td>
<td>1.8 (1.18-3.0)</td>
<td>0.60 (0.37-1.2)</td>
</tr>
<tr>
<td>PLT *</td>
<td>563.5 (76-2250)</td>
<td>371 (97-1650)</td>
<td>918 (367-1643)</td>
<td>342 (140-2250)</td>
<td>407.5 (76-887)</td>
</tr>
<tr>
<td>SPLENOMEGALY</td>
<td>26/78 (33%)</td>
<td>9/24 (38%)</td>
<td>7/34 (21%)</td>
<td>6/6 (100%)</td>
<td>4/14 (29%)</td>
</tr>
<tr>
<td>HEPATOMEGALY</td>
<td>11/66 (17%)</td>
<td>2/21 (10%)</td>
<td>4/29 (14%)</td>
<td>4/5 (80%)</td>
<td>1/11 (9%)</td>
</tr>
<tr>
<td>LDH *</td>
<td>341 (155-1876)</td>
<td>356 (155-1876)</td>
<td>317 (161-613)</td>
<td>855.5 (254-1697)</td>
<td>312.5 (171-815)</td>
</tr>
<tr>
<td>BILIRUBIN *</td>
<td>16.3 (7.6-33.1)</td>
<td>16.3 (7.6-33.1)</td>
<td>8.7 (7.2-38.8)</td>
<td>31.95 (22-41.9)</td>
<td>16.4 (10.2-28.5)</td>
</tr>
<tr>
<td>THERAPY</td>
<td>Hydroxiurea, Allopurinol, Controloc, Aspirin Protect</td>
<td>Hydroxiurea, Litalir, Allopurinol, Controloc, Aspirin Protect</td>
<td>Hydroxiurea, Allopurinol, Controloc, Aspirin Protect</td>
<td>Surea, Folacin Aspirin Protect</td>
<td>Hydroxiurea, Allopurinol, Controloc, Aspirin Protect</td>
</tr>
<tr>
<td>JAK2V617F</td>
<td>82/117 (71%)</td>
<td>25/32 (78%)</td>
<td>31/45 (69%)</td>
<td>6/10 (60%)</td>
<td>20/30 (67%)</td>
</tr>
<tr>
<td>CALR+</td>
<td>11/85 (13%)</td>
<td>/</td>
<td>8/45 (18%)</td>
<td>2/10 (20%)</td>
<td>1/30 (3%)</td>
</tr>
<tr>
<td>MPL+</td>
<td>3/85 (4%)</td>
<td>/</td>
<td>2/45 (4%)</td>
<td>1/10 (10%)</td>
<td>0/30 (0%)</td>
</tr>
<tr>
<td>Triple negative</td>
<td>7/85 (8%)</td>
<td>/</td>
<td>4/45 (9%)</td>
<td>1/10 (10%)</td>
<td>2/30 (7%)</td>
</tr>
<tr>
<td>NA</td>
<td>21/138 (15%)</td>
<td>9/41 (22%)</td>
<td>11/56 (20%)</td>
<td>0/10 (0%)</td>
<td>1/31 (3%)</td>
</tr>
<tr>
<td>HYPERCELLULAR BONE MARROW</td>
<td>32/46 (70%)</td>
<td>3/5 (60%)</td>
<td>19/26 (73%)</td>
<td>6/7 (86%)</td>
<td>4/8 (50%)</td>
</tr>
<tr>
<td>FOLLOW UP MONTHS</td>
<td>33 (1-218)</td>
<td>50 (2-218)</td>
<td>45.5 (4-170)</td>
<td>69 (1-109)</td>
<td>20 (3-145)</td>
</tr>
<tr>
<td>DECEASED</td>
<td>18/138 (13%)</td>
<td>8/41 (20%)</td>
<td>3/56 (5%)</td>
<td>0/10 (0%)</td>
<td>7/31 (23%)</td>
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<td>------------</td>
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</table>

* RBC is given in $10^{12}$/L; Hb in g/L; Hct in percentages; MCV in fL; WBC as $10^9$/L; NE, EO and BA are given in percentages; Plt in $10^9$/L; LDH in U/L; bilirubin in μmol/L.
**FIGURE S1.** Hematologic parameters in $JAK2^{V617F}$, $JAK2^{WT}$, $CALR$-mutated, $CALR^{WT}$, $MPL$-mutated, $MPL^{WT}$, triple negative PMF patients and PMF patients with driver mutation. Data are presented in a box and whisker plot showing the upper and lower values (highest and lowest horizontal line, respectively) and upper and lower quartile (box) with median value. $JAK2^{V617F}$ PMF patients had higher RBC, WBC, Hb values and platelet count than $JAK2^{WT}$ patients, and $CALR$-mutated PMF patients, eventhough difference was not statistically different (p>0.05).
FIGURE S2. Hematologic parameters in $JAK2^{V617F}$, $JAK2^{WT}$, $CALR$-mutated, $CALR^{WT}$, triple negative MPN-U patients and MPN-U patients with driver mutation. Data are presented in a box and whisker plot showing the upper and lower values (highest and lowest horizontal line, respectively) and upper and lower quartile (box) with median value. $JAK2^{V617F}$ MPN-U patients had higher RBC and platelet count than $JAK2^{WT}$ MPN-U patients, and $CALR$-mutated MPN-U patients, even though difference was not statistically different ($p>0.05$).
FIGURE S3. Kaplan–Meier estimates of overall survival rate in A) MPN subtypes B) PV, ET, PMF and MPN-U patients categorized according to presence of driver mutation (JAK2V617F, CALR-mutated and MPL-mutated) C) PV patients with and without JAK2 mutation D) Triple negative and ET patients with detected driver mutation (JAK2V617F, CALR-mutated and MPL-mutated). Statistically significant values regarding survival rate were found only for comparison among different MPN subtypes (p<0.05).