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 (OS), and mutational status of the Janus kinase 2 (*JAK2*), calreticulin (*CALR*), and myeloproliferative leukemia virus oncogene (*MPL*) genes. We compared hematologic and clinical features of $JAK2^{V617F}$-ET vs. CALR-mutated ET vs. $JAK2^{V617F}$-PV patients. $JAK2^{V617F}$-patients had higher values of erythrocytes, hemoglobin, and hematocrit compared to *CALR*-mutated patients ($p < 0.05$). The mutant allele burden in $JAK2^{V617F}$-PV and $JAK2^{V617F}$-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 $JAK2^{V617F}$-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 World Health Organization (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 molecular basis [10]. Around 5% of patients suffer from progression to more advanced disease, including transformation to acute myeloid leukemia (AML) [11].

The 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 the *JAK2* (Janus kinase 2), *CALR* (calreticulin), and *MPL* (myeloproliferative leukemia virus oncogene) genes [13,14]. A 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]. 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% *CALR*, and 7% *MPL*) [12,13]. $JAK2^{V617F}$ PV patients have significantly higher mutant allele burden (MAB) in comparison to $JAK2^{V617F}$ ET patients [19]. Mutually exclusive somatic

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mutations in the *JAK2*, *CALR*, and *MPL* genes gave a new insight into the pathogenesis and diagnostics of MPN.

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

MATERIALS AND METHODS

Study population

A cohort of 138 patients diagnosed with MPN and treated at the Department of Hematology, Clinical Center of the University of Sarajevo and the Department of Internal Medicine, Cantonal Hospital Zenica, in the period January 2000–June 2018 was 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 WHO 2008 or 2016 classification criteria for hematopoietic malignancies, according to the criteria at the time of diagnosis [4]. 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 the WHO classification were collected, except for erythropoietin (EPO) levels which had not been determined 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). Patient characteristics included complete blood count (CBC), bone marrow morphology, hepato/splenomegaly, and overall survival (OS).

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 patient peripheral blood using standard protocol (Qiagen QIAamp® DNA mini kit, USA). *JAK2* mutational analysis and MAB were performed by quantitative PCR (qPCR) using a 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 allele-specific oligonucleotide (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 GAA GTC TGA CCC TTT-3′ and R 5′-ACA GAG CGA ACC AAG AAT GCC TGT-3′ (Invitrogen, Thermo Fisher Scientific, USA).

Statistical analysis

Numerical variables were presented by their range and median, and categorical variables by count and relative frequency (%) of each category (Tables S1 and 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 ρ (ρ) coefficient.

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 and compared using the log-rank test. Data were analyzed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). Statistical significance was determined at the level $p < 0.05$ (2-tailed).

RESULTS

Clinical characteristics

We included 138 MPN patients in this study, diagnosed with PV (n = 41), ET (n = 56), PMF (n = 10), and MPN-U (n = 31). Patient characteristics are shown in Table S1, 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 (Table S1); 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 shows clinical parameters of each MPN subtype at diagnosis according to the presence of *JAK2*^{V617F}, *CALR*, and *MPL* mutations.

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

Characteristics (Normal range)	PV		ET					PMF			
	JAK2+ (n=25)	NA (n=9)	*JAK2*+ (n=31)	*CALR*+ (n=8)	*MPL*+ (n=2)	Triple negative (n=4)	NA (n=11)	*JAK2*+ (n=6)	*CALR*+ (n=2)	*MPL*+ (n=1)	Triple negative (n=1)
Male/Female (% Male)	9/16 (36%)	8/1 (89%)	9/22 (29%)	5/3 (63%)	1/1 (50%)	0/4 (0%)	4/7 (36%)	4/2 (67%)	1/1 (50%)	0/1 (100%)	1/0 (100%)
Age, years	70 (29–84)	69 (61–81)	66 (40–82)	68 (37–84)	62 (56–68)	67.5 (28–87)	69 (39–78)	63.5 (25–78)	56 (47–65)	56	32
RBC (4.34–5.72×10^{12}/L)	6.67 (2.87–8.32)	5.82 (4.60–7.77)	5.01 (3.22–7.29)	4.85 (3.35–5.03)	4.5 (4.29–4.71)	4.51 (4.41–5.14)	4.73 (2.68–7.94)	4.88 (3.57–5.63)	4	4.46	1.89
Hemoglobin (138–175 g/L)	169 (118–217)	173 (155–207)	146 (78–169)	143.5 (118–156)	130.5 (129–132)	144.5 (134–151)	147 (118–194)	138 (89–149)	121.5 (118–125)	115	35
Hematocrit (0.415–0.530)	0.55 (0.35–0.66)	0.53 (0.47–0.63)	0.44 (0.25–0.55)	0.43 (0.36–0.45)	0.38	0.43 (0.39–0.45)	0.44 (0.33–0.59)	0.41 (0.31–0.47)	0.34	0.29	/
MCV (83–97.2 fL)	82 (56.8–124)	87 (80.6–101)	87.5 (73.4–102)	89.5 (85–95.6)	89	92.05 (88.2–95.2)	88 (74–114)	80.8 (78–103)	86	77	/
WBC (3.4–9.7×10^{9}/L)	10 (5.2–16.9)	7.10 (5.13–13.00)	11.15 (6.06–15.97)	9.98 (6.5–18.01)	11.09 (10.98–11.2)	9.63 (5.1–12.5)	9.35 (3.3–19.4)	18.55 (1.63–40.8)	8.16	9.85	5.5
Neutrophils (44–72%)	68.5 (41.7–82)	59.11 (42–80.2)	68.50 (43.2–75.6)	60.8 (46–85.6)	65.00	66.68 (61.45–71.9)	71.93 (53.9–90.9)	75.9 (62.6–84)	/	69.64	/
Eosinophils (0–7%)	2 (0.3–6.15)	1.85 (1–2.93)	1.60 (0.129–5.3)	1.49 (1–1.98)	1.80	2.25	2.38 (0.161–4.68)	2.63 (2–3.43)	2.14	1.42	/
Basophils (0–0.43%)	0.8 (0.1–3.01)	1 (0.83–1)	1.03 (0.263–3.57)	0.34 (0.04–0.64)	0.91	1.49 (1.06–1.92)	0.68 (0.102–1.25)	1.8 (1.18–3)	/	1.3	/
Plt (158–424×10^{9}/L)	471 (97.3–1650)	266 (151–532)	834 (367–1438)	1155.5 (848–1250)	977 (922–1032)	648 (448–1051)	927 (530–1643)	342 (158–2250)	284.5 (157–412)	1479	140
Splenomegaly	5/14 (36%)	3/3 (100%)	3/14 (21%)	1/6 (17%)	1/2 (50%)	1/3 (30%)	1/9 (11%)	4/4 (100%)	1/1 (100%)	1/1 (100%)	/
Hepatomegaly	1/10 (10%)	0/4 (0%)	2/12 (17%)	0/5 (0%)	1/2 (50%)	0/2 (0%)	1/8 (13%)	2/3 (67%)	/	1/1 (100%)	1/1 (100%)
LDH (125–241 U/L)	381.5 (217–1876)	321 (159–721)	282 (167–521)	345 (183–553)	261.5 (175–348)	387 (161–613)	337 (235–427)	609 (254–1697)	1166.5 (1102–1231)	410	/
Bilirubin (3–20 µmol/L)	12.7 (7.6–19.5)	33.1	10	/	23.1 (7.4–38.8)	/	7.2	31.95 (22–41.9)	/	/	/
Therapy	Hydroxyurea, litalir, allopurinol, controloc, aspirin protect	Hydroxyurea, litalir, allopurinol, controloc, aspirin protect	Hydroxyurea, allopurinol, controloc, aspirin protect	Hydroxyurea, allopurinol, controloc, aspirin protect	Hydroxyurea, allopurinol, controloc, aspirin protect	Hydroxyurea, allopurinol, controloc, aspirin protect	Hydroxyurea, allopurinol, controloc, aspirin protect	Surea, folacin aspirin protect	Surea, folacin aspirin protect	Surea, folacin aspirin protect	Surea, folacin aspirin protect
BM cellularity	3/4 (75%)	0/1 (0%)	11/16 (69%)	2/2 (100%)	1/2 (50%)	0/1 (0%)	4/5 (80%)	4/5 (80%)	/	1/1 (100%)	1/1 (100%)
Follow-up months	24 (2–218)	113 (11–164)	19 (4–131)	69 (17–159)	40.5 (4–77)	43 (19–102)	90 (68–170)	40.5 (1–100)	109	69	1
Deceased	2/25 (8%)	3/9 (33%)	2/31 (6%)	/	/	/	1/11 (9%)	0/6 (0%)	0/2 (0%)	0/1 (0%)	0/1 (0%)

PV: Polycythemia vera; ET: Essential thrombocythemia; PMF: Primary myelofibrosis; JAK2: Janus kinase 2; CALR: Calreticulin; MPL: Myeloproliferative leukemia virus oncogene; RBC: Red blood cell; MCV: Mean corpuscular volume; WBC: White blood cell; Plt: Platelets; LDH: Lactate dehydrogenase; Hb: Hemoglobin; BM: Bone marrow; NA: Not available (JAK2 status unknown)

Mutational status vs. CBC in PV patients

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

Mutational status vs. CBC in ET patients

Hematologic parameters in ET patients compared to mutational status (*JAK2*^{V617F}, *JAK2*^{WT}, *CALR*-mutated, *CALR*^{WT}, *MPL*-mutated, and *MPL*^{WT}) are presented in Figure 2. RBC levels were higher in *JAK2*^{V617F} compared to *JAK2*^{WT} ET patients. Between *JAK2*^{V617F} vs. *CALR*-mutated ET patients, *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 *JAK2*^{V617F} mutation, we compared the clinical parameters among *JAK2*^{V617F} ET vs. *JAK2*^{V617F} PV patients; we found that RBC, Hb, and Hct values were higher in PV patients, but platelet count values were lower; however, the frequency of splenomegaly was higher ($p < 0.001$). Interestingly, we found the same results when we compared *JAK2*^{V617F} PV patients and *CALR*-mutated ET patients ($p < 0.05$).

Mutational status vs. CBC in PMF patients

Hematologic parameters in PMF patients compared to mutational status are shown in Figure S1. The presence of either *JAK2*^{V617F} or *CALR* mutation did not have an impact on the 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. A comparison between *CALR*-mutated PMF vs. *CALR*-mutated ET patients showed that *CALR*-mutated PMF patients had lower Hb ($p < 0.05$) and platelet (Plt) values ($p < 0.001$).

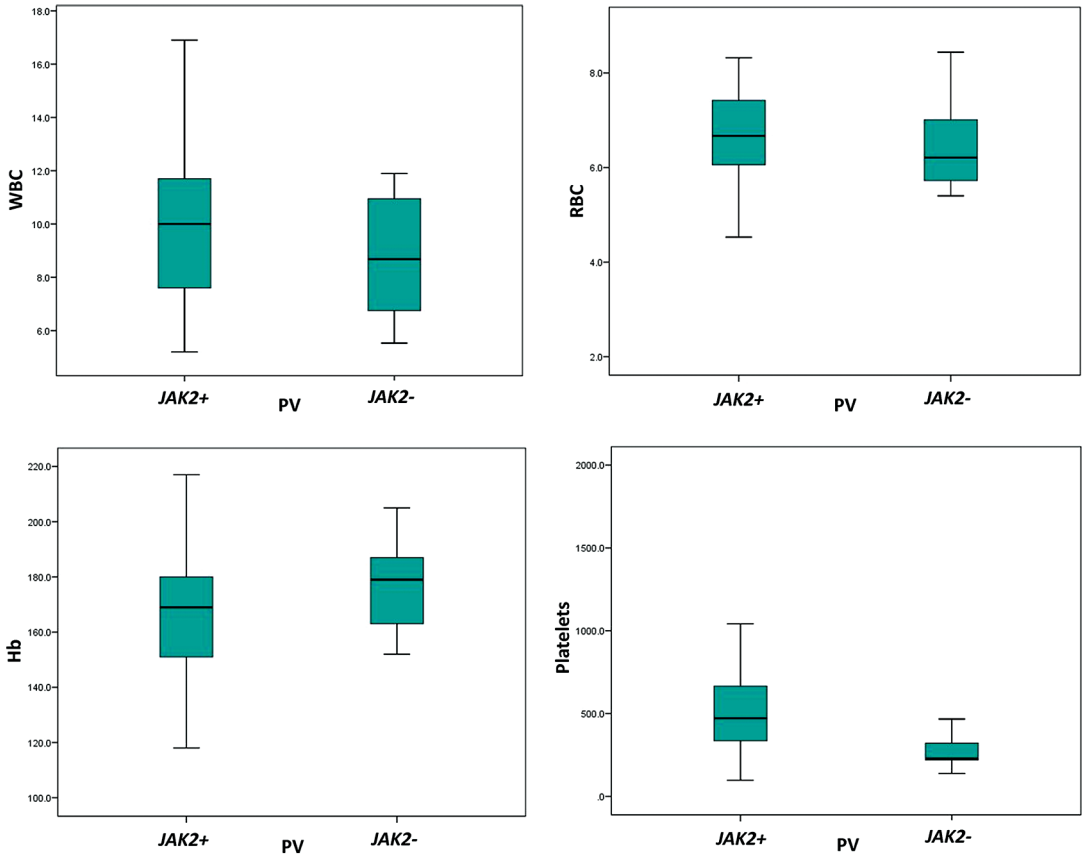

FIGURE 1. Hematologic parameters in JAK2^{V617F} and JAK2^{WT} PV patients. Data are presented in a box and whisker plot showing the upper and lower values (highest and lowest horizontal line, respectively) and upper and lower quartile (box) with median value. Patients with JAK2^{V617F} PV had markedly higher platelet count values ($p < 0.05$) than patients without JAK2^{V617F} mutation. WBC and RBC levels were higher in JAK2^{V617F} PV. *JAK2*: Janus kinase 2; PV: Polycythemia vera; WBC: White blood cell; RBC: Red blood cell.

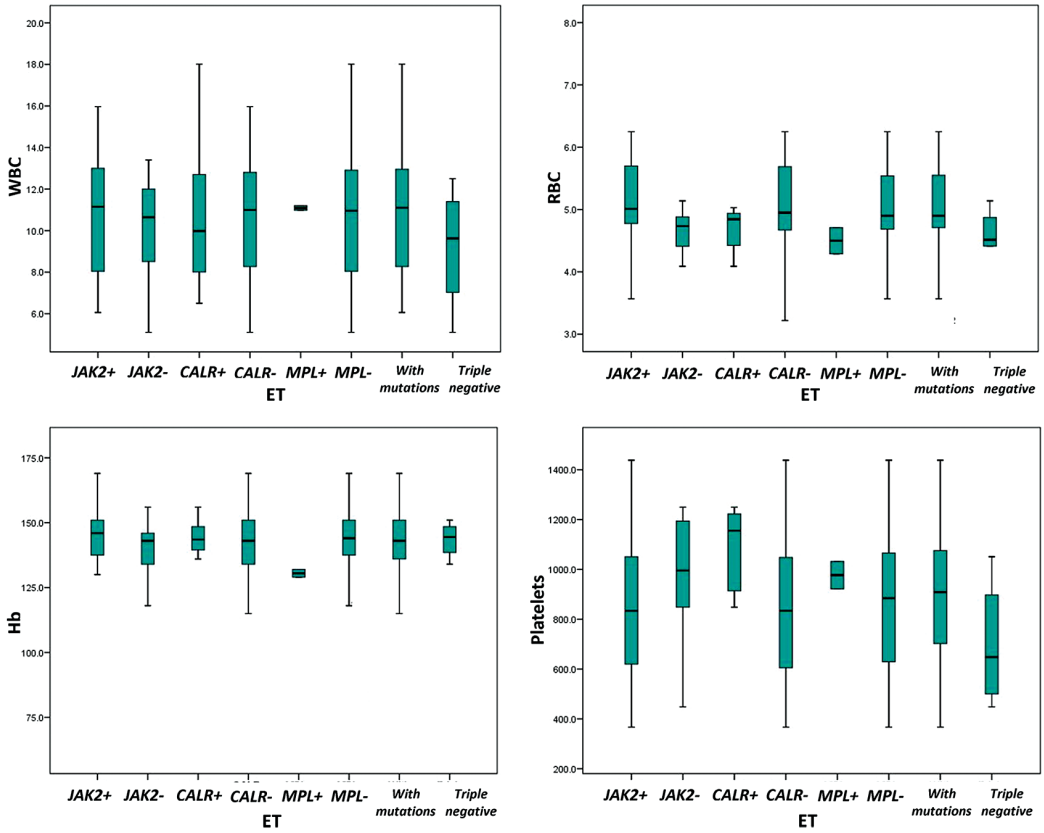

FIGURE 2. Hematologic parameters in *JAK2*^{V617F}, *JAK2*^{WT}, *CALR*-mutated, *CALR*^{WT}, *MPL*-mutated, *MPL*^{WT} triple-negative ET patients, and ET patients with driver mutation. Data are presented in a box and whisker plot showing the upper and lower values (highest and lowest horizontal line, respectively) and upper and lower quartile (box) with median value. *JAK2*^{V617F} ET patients had markedly higher RBC values ($p < 0.05$) than *JAK2*^{WT} patients and *CALR*-mutated patients had significantly higher platelet count compared to *JAK2*^{V617F} ET patients. *JAK2:* Janus kinase 2; *CALR*: Calreticulin; *MPL:* Myeloproliferative leukemia virus oncogene; ET: Essential thrombocythemia; RBC: Red blood cell.

Mutational status vs. CBC in MPN-U patients

The values of hematologic parameters in MPN-U patients compared to mutational status are presented in Figure S2. Platelet count was statistically different between *JAK2*^{V617F} and *JAK2*^{WT} MPN-U patients. A comparison among *JAK2*^{V617F} MPN-U, PV, ET, and 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 specific mutations 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, the MAB was higher than 50%, compared to 47% of PV patients.

A correlation between hematologic parameters (RBC, Hb, Hct, and Plt) and MAB was calculated for *JAK2*^{V617F} PV vs. *JAK2*^{V617F} ET patients, as illustrated in Figure 4. A 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 suggest 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, and MPN-U). At 120 months, OS for PV, ET, PMF, and MPN-U patients was 80%, 90%, 100%, and 77%, respectively. Also, all MPN patients were categorized according to the presence of driver mutation

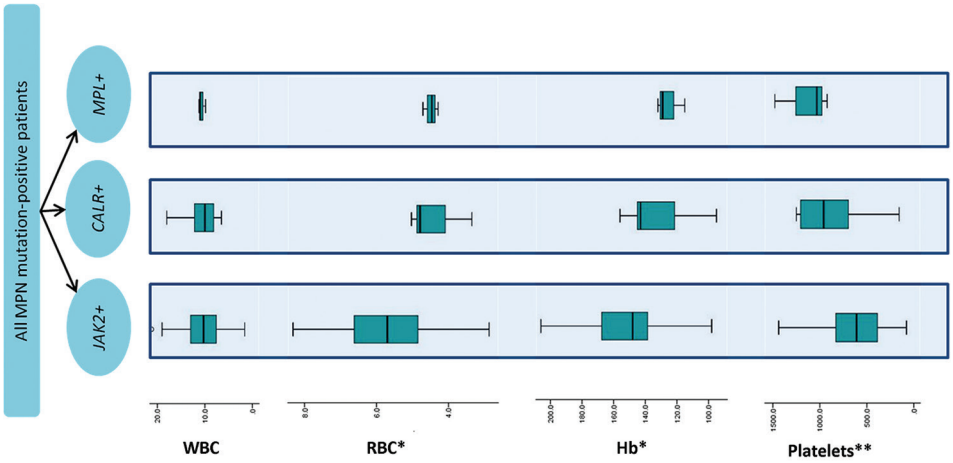

FIGURE 3. PV/ET/PMF/MPN-U patients are categorized according to detected driver gene mutation (*JAK2, CALR*, and *MPL*). Presence of driver mutation in *JAK2* gene had an impact on higher RBC and Hb values (*) compared to *CALR*-mutated patients and *MPL* mutation had an impact on higher Plt values (**) compared to *JAK2*-mutated patients. PV: Polycythemia vera; ET: Essential thrombocythemia; PMF: Primary myelofibrosis; MPN: Myeloproliferative neoplasm; MPN-U: Unclassifiable MPNs; *JAK2*: Janus kinase 2; *CALR*: Calreticulin; *MPL:* Myeloproliferative leukemia virus oncogene; RBC: Red blood cell; Hb: Hemoglobin; Plt: Platelet.

(*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 (Figure S3).

DISCUSSION

MPN driver mutations in the JAK2, CALR, and MPL genes upregulate the 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 of erythroid-related genes, and activates abnormal STAT signaling through the activation of signal transducer and activator of transcription 1 (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 *JAK2*^{V617F} PV patients vs. *CALR*-mutated ET patients, suggesting that the presence of *JAK2*^{V617F} helps RBC production. Furthermore, when we compared *JAK2*^{V617F} PV vs. *JAK2*^{V617F} ET vs. *JAK2*^{V617F} PMF patients, we found that the highest RBC values were in *JAK2*^{V617F} PV patients. Similarly, higher *JAK2*^{V617F} expression (allele burden) was associated with increased RBC values in *JAK2*^{V617F} PV vs. *JAK2*^{V617F} ET patients, supporting the hypothesis that higher *JAK2*^{V617F} 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. *JAK2*^{V617F} 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 *JAK2*^{V617F}, *CALR*, and *MPL* are the drivers of MPN pathogenesis [10,30-33]. Since *JAK2*^{V617F} is present in both PV and ET patients, we compared their blood parameters. Regarding platelet count, *JAK2*^{V617F} ET patients vs. *JAK2*^{V617F} 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 et al. [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 an additional factor in *JAK2*^{V617F} PMF patients that increases WBC count.

Regarding OS, we found that *CALR*-mutated patients had higher survival rates compared to *JAK2*^{V617F} patients, which is similar to the 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 ($p < 0.05$).

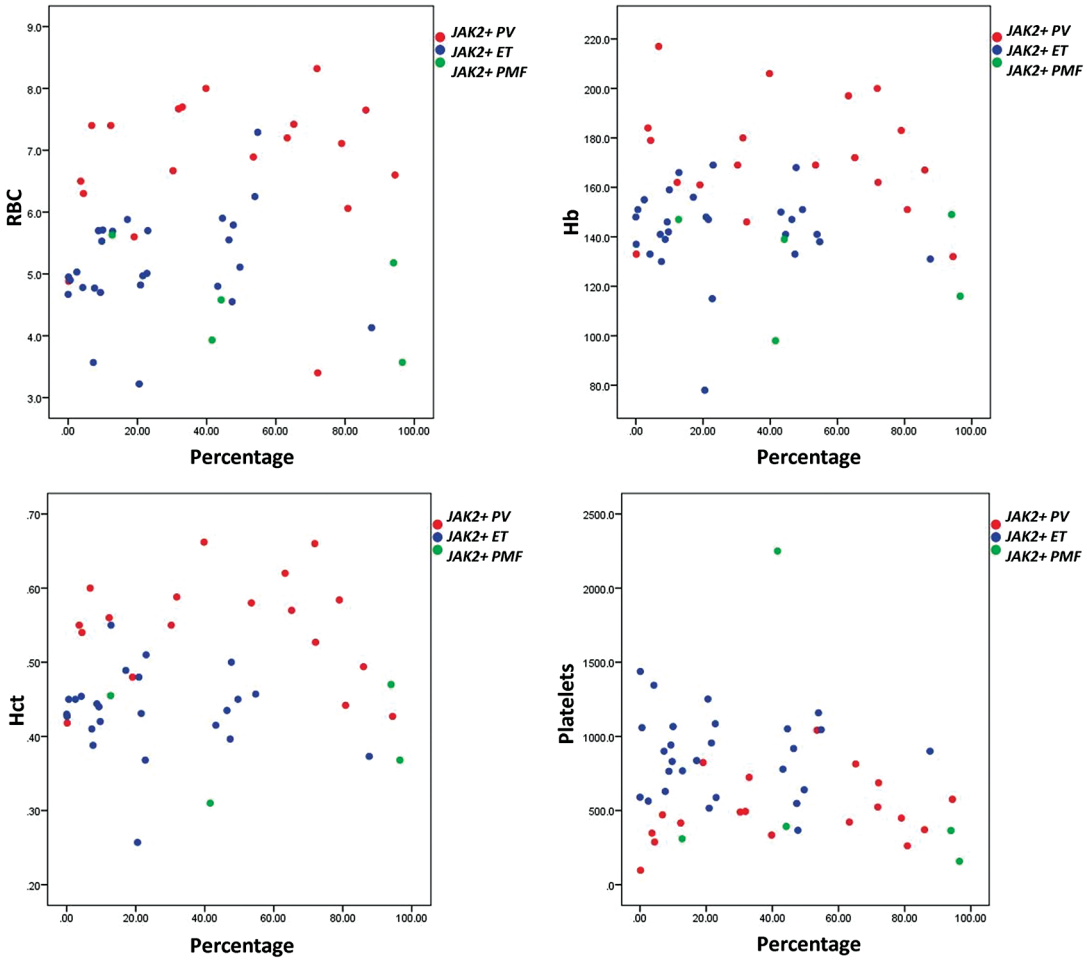

FIGURE 4. Relationship between *JAK2*^{V617F} allele burden and hematologic parameters in ET and PV patients. The mutant allele burden was directly correlated with RBC values ($p = 0.362$, $p < 0.05$), Hb level ($p = 0.140$, $p > 0.05$), and hematocrit ($p = 0.206$, $p > 0.05$), and inversely correlated with Plt count ($p = -0.12$, $p > 0.05$). *JAK2*: Janus kinase 2; ET: Essential thrombocythemia; PV: Polycythemia vera; RBC: Red blood cell; Hb: Hemoglobin; Plt: Platelet.

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 another study performed by Tefferi et al., triple negative status in PMF patients did not show additional prognostic information for OS [36].

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SUPPLEMENTAL DATA

TABLE S1. Clinical characteristics and mutational status of 138 MPN patients at diagnosis in Bosnia and Herzegovina

Characteristics	MPN (n=138)	PV (n=41)	ET (n=56)	PMF (n=10)	MPN-U (n=31)
Male/Female (% Male)	67/71 (49%)	22/19 (54%)	19/37 (34%)	6/4 (60%)	20/11 (65%)
Age, Years	66 (22–89)	69 (29–84)	66 (28–87)	59.5 (25–78)	61.50 (22–89)
RBC*	5.40 (1.89–8.44)	6.34 (2.87–8.44)	4.88 (2.68–7.94)	4.46 (1.89–5.63)	5.69 (2.12–8.32)
Hemoglobin*	150 (35–217)	170 (118–217)	145 (78–194)	121.5 (35–149)	150 (76–184)
Hematocrit*	0.45 (0.25–0.66)	0.54 (0.36–0.66)	0.43 (0.25–0.59)	0.37 (0.29–0.47)	0.47 (0.31–0.62)
MCV*	86.5 (56.8–124)	83 (56.8–124)	88.9 (73.4–114)	80.8 (77–103)	85.5 (59–106)
WBC*	10.00 (1.63–40.8)	8.96 (5.13–16.9)	10.95 (3.3–19.4)	9.85 (1.63–40.8)	9.99 (2.8–26.6)
Neutrophils*	66.00 (42–90.9)	65.5 (42–82)	67 (43.2–90.9)	75.9 (62.6–84)	64.57 (47.7–82.2)
Eosinophils*	1.7 (0.129–6.15)	1.85 (0.3–6.15)	1.8 (0.129–5.3)	1.71 (1.42–3.43)	1.50 (1–3.23)
Basophils *	0.87 (0.04–3.57)	0.91 (0.1–3.01)	0.90 (0.04–3.57)	1.8 (1.18–3.0)	0.60 (0.37–1.2)
Plt*	563.5 (76–2250)	371 (97.3–1650)	918 (367–1643)	342 (140–2250)	407.5 (76–887)
Splenomegaly	26/78 (33%)	9/24 (38%)	7/34 (21%)	6/6 (100%)	4/14 (29%)
Hepatomegaly	11/66 (17%)	2/21 (10%)	4/29 (14%)	4/5 (80%)	1/11 (9%)
LDH*	341 (155–1876)	356 (155–1876)	317 (161–613)	855.5 (254–1697)	312.5 (171–815)
Bilirubin*	16.30 (7.2–41.9)	16.3 (7.6–33.1)	8.7 (7.2–38.8)	31.95 (22–41.9)	16.4 (10.2–28.5)
Therapy	Hydroxyurea, allopurinol, controloc, aspirin protect	Hydroxyurea, litalir, allopurinol, controloc, aspirin protect	Hydroxyurea, allopurinol, controloc, aspirin protect	Surea, folacin aspirin protect	Hydroxiurea, allopurinol, controloc, aspirin protect
JAK2^{V617F}	82/117 (71%)	25/32 (78%)	31/45 (69%)	6/10 (60%)	20/30 (67%)
CALR+	11/85 (13%)	/	8/45 (18%)	2/10 (20%)	1/30 (3%)
MPL+	3/85 (4%)	/	2/45 (4%)	1/10 (10%)	0/30 (0%)
Triple negative	7/85 (8%)	/	4/45 (9%)	1/10 (10%)	2/30 (7%)
NA	21/138 (15%)	9/41 (22%)	11/56 (20%)	0/10 (0%)	1/31 (3%)
Hypercellular bone marrow	32/46 (70%)	3/5 (60%)	19/26 (73%)	6/7 (86%)	4/8 (50%)
Follow-up months	33 (1–218)	50 (2–218)	45.5 (4–170)	69 (1–109)	20 (3–145)
Deceased	18/138 (13%)	8/41 (20%)	3/56 (5%)	0/10 (0%)	7/31 (23%)

*RBC is given in 10^{12}/L; Hemoglobin in g/L; Hematocrit in percentages; MCV in fL; WBC as 10^9/L; Neutrophils, eosinophils, and basophils are given in percentages; Plt in 10^9/L; LDH in U/L; bilirubin in µmol/L. MPN: Myeloproliferative neoplasm; ET: Essential thrombocythemia; PV: Polycythemia vera; PMF: Primary myelofibrosis; CEL: Chronic eosinophilic leukemia; WBC: White blood cell; RBC: Red blood cell; Hb: Hemoglobin; *JAK2*: Janus kinase 2; *CALR*: Calreticulin; *MPL*: Myeloproliferative leukemia virus oncogene; LDH: Lactate dehydrogenase; MPN-U: Unclassifiable MPNs; MCV: Mean corpuscular volume; Plt: Platelet; NA: Not available (mutational status unknown)

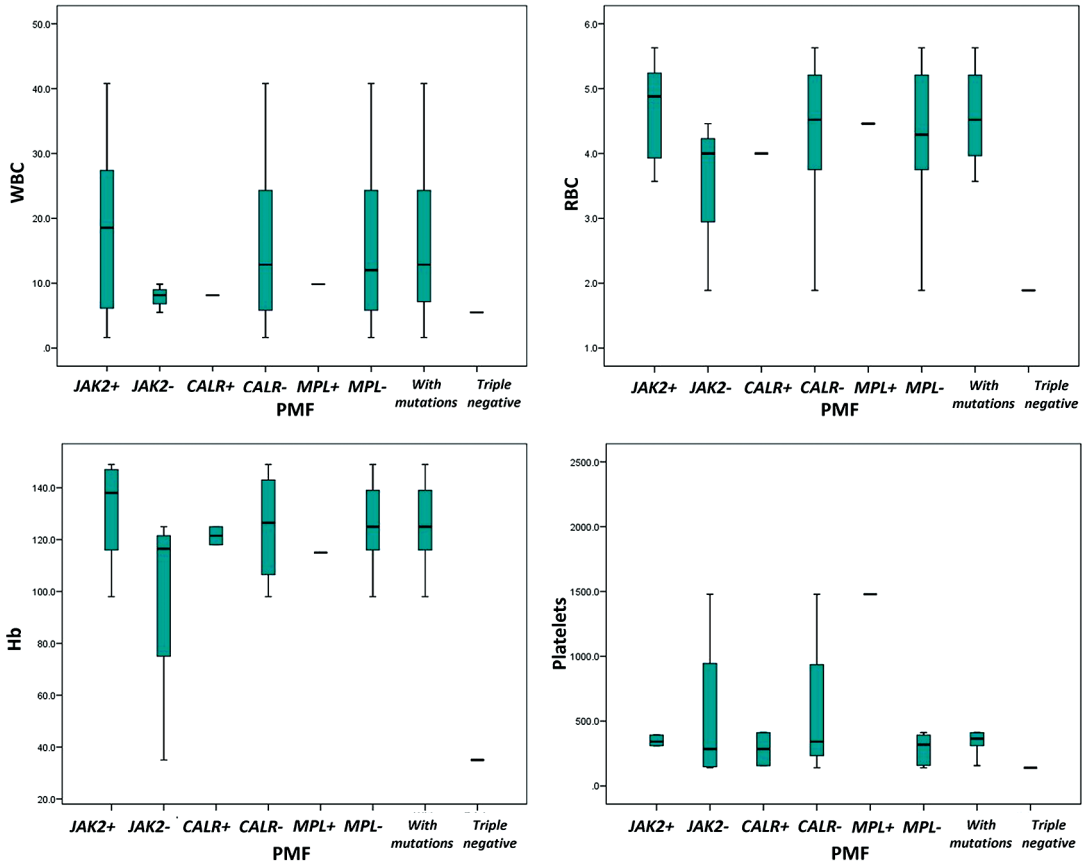

FIGURE S1. Hematologic parameters in *JAK2*^{V617F}, *JAK2*^{WT}, *CALR*-mutated, *CALR*^{WT}, *MPL*-mutated, *MPL*^{WT}, triple-negative PMF patients, and PMF patients with driver mutation. Data are presented in a box and whisker plot showing the upper and lower values (highest and lowest horizontal 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, even though the difference was not statistically different ($p > 0.05$). *JAK2*: Janus kinase 2; *CALR*: Calreticulin; *MPL:* Myeloproliferative leukemia virus oncogene; PMF: Primary myelofibrosis; RBC: Red blood cell; WBC: White blood cell; Hb: Hemoglobin.

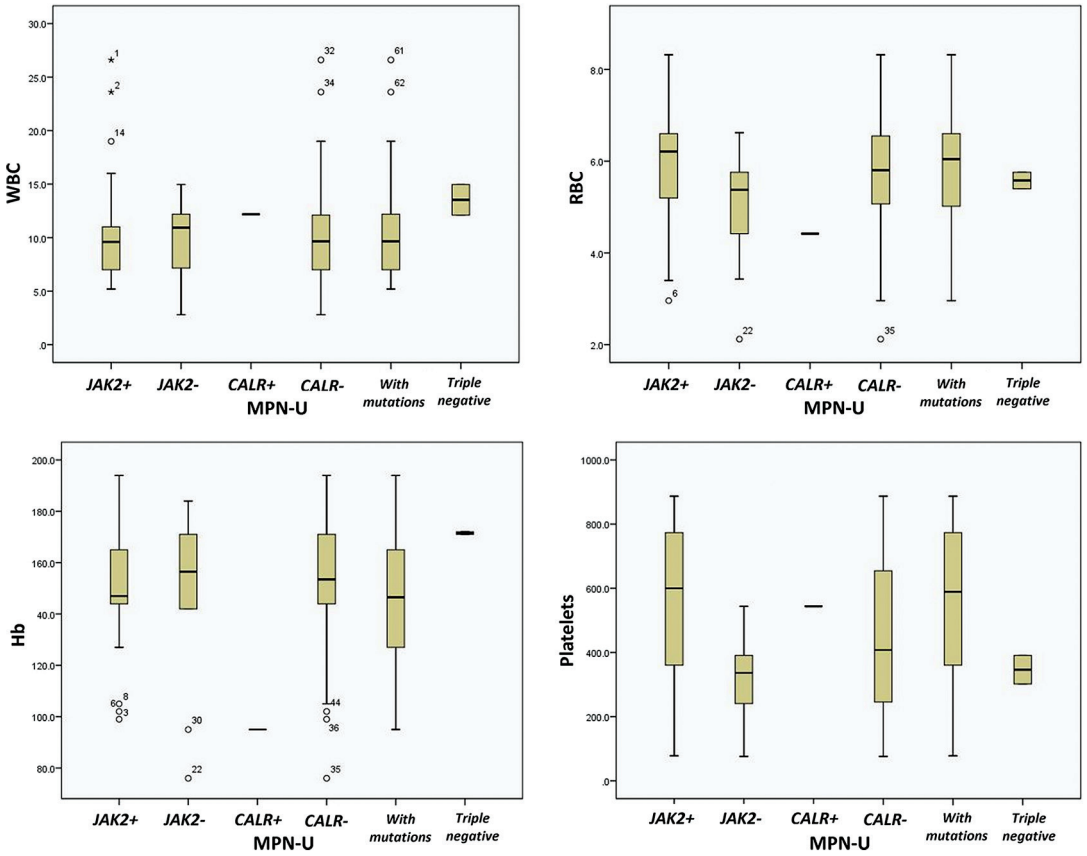

FIGURE S2. Hematologic parameters in *JAK2*^{V617F}, *JAK2*^{WT}, *CALR*-mutated, *CALR*^{WT}, triple-negative MPN-U patients, and MPN-U patients with driver mutation. Data are presented in a box and whisker plot showing the upper and lower values (highest and lowest horizontal line, respectively) and 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 the difference was not statistically different ($p > 0.05$). *JAK2*: Janus kinase 2; *CALR*: Calreticulin; MPN-U: Unclassifiable MPNs; RBC: Red blood cell.

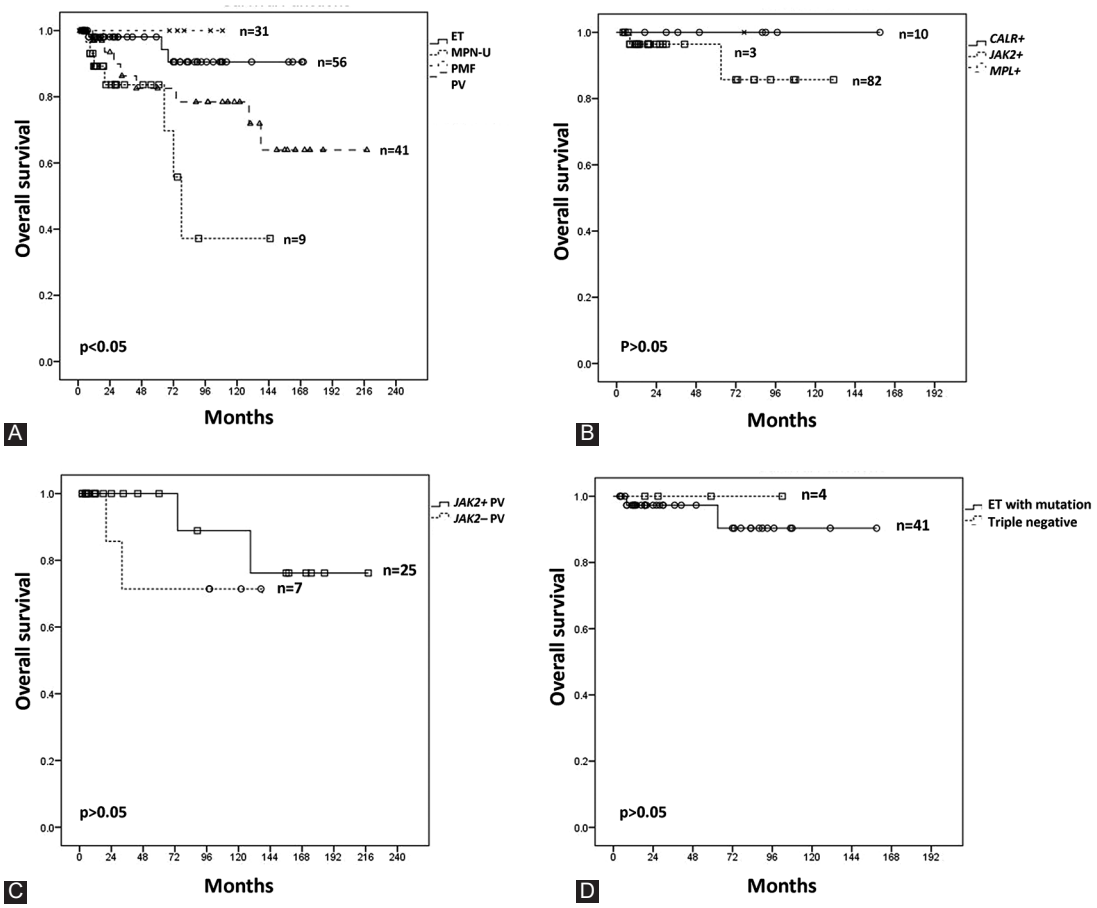

FIGURE S3. Kaplan–Meier estimates of overall survival rate in (A) MPN subtypes; (B) PV, ET, PMF and MPN-U patients categorized according to presence of driver mutation (*JAK2*^{V617F}, *CALR*-mutated, and *MPL*-mutated); (C) PV patients with and without *JAK2* mutation; and (D) triple-negative and ET patients with detected driver mutation (*JAK2*^{V617F}, *CALR*-mutated, and *MPL*-mutated). Statistically significant values regarding survival rate were found only for comparison among different MPN subtypes ($p < 0.05$). MPN: Myeloproliferative neoplasm; PV: Polycythemia vera; ET: Essential thrombocythemia; PMF: Primary myelofibrosis; MPN-U: Unclassifiable MPNs; *JAK2*: Janus kinase 2; *CALR*: Calreticulin; *MPL:* Myeloproliferative leukemia virus oncogene.