Increased mean platelet volume (MPV) is an independent predictor of inferior survival in patients with primary and secondary myelofibrosis

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Abstract:

Background: Neoplastic megakaryopoiesis is a dominant feature of Philadelphia-chromosome-negative myeloproliferative neoplasms (Ph-MPNs), and elevated mean-platelet-volume (MPV) is a common finding in these diseases. The clinical and prognostic significance of MPV in patients with primary (PMF) and secondary myelofibrosis (SMF) have not been reported.

Methods: We retrospectively analyzed 87 patients with myelofibrosis (66 with PMF, 21 with SMF) treated at our institution. MPV was recorded in addition to other hematological and clinical parameters.

Results: MPV was elevated in both PMF and SMF patients in comparison to controls, whereas there was no statistically significant difference between PMF and SMF. Elevated MPV was associated with lower platelets (P=0.016), higher white-blood-cells (P=0.015), higher percentage of circulatory blasts (P=0.009), higher lactate-dehydrogenase (P=0.011), larger spleen size (P=0.014) and higher Dynamic-International-Prognostic-Score category (P=0.027), while there was no statistically significant association with driver mutations or degree of bone marrow fibrosis. Higher MPV was univariately associated with inferior overall survival in the whole cohort (HR=3.82, P=0.006), PMF (HR=4.35, P=0.007) and SMF patients (HR=7.22, P=0.034). These associations remained significant in multivariate analyses adjusted for DIPSS.

Conclusion: Higher MPV is associated with more aggressive disease features and exhibits powerful independent prognostic properties in both PMF and SMF setting.

Keywords: Philadelphia chromosome negative myeloproliferative neoplasm; Primary myelofibrosis; Mean platelet volume; Survival

Introduction:

Primary myelofibrosis (PMF) is a Philadelphia chromosome negative (Ph-) myeloproliferative neoplasm (MPN) [1] that develops from clonally transformed hematopoietic stem cell [2]. It is characterized by development of bone marrow fibrosis, leucoerythroblastic blood smear and extramedullary erythropoiesis in spleen and liver. Two other related Ph- MPNs, polycythemia vera (PV) and essential thrombocytosis (ET) can also develop significant degree of bone marrow fibrosis and PMF-related features during disease course when these conditions are termed secondary myelofibrosis (SMF) [3]. Patients with PMF and SMF exhibit similar disease course and are at increased risk of death due to disease progression or transformation to acute leukemia. Most of Ph- MPN patients bear a mutation in either of *Janus-kinase-2* (*JAK2*), *Calreticulin* (*CALR*) or *Myeloproliferative-leukemia-virus-oncogene* (*MPL*) genes [4] that lead to constitutive activation of JAK2 – signal-transducer-and-activator-of-transcription (STAT) signaling pathway and contribute to high inflammatory atmosphere characteristic for diseases. Chronic inflammation in Ph- MPNs accelerates development of atherosclerosis [5] and diseases of cardiovascular system are most prevalent comorbid conditions in PMF patients which has important implications on patient survival [6]. Risk of death in myelofibrosis can be estimated using the International Prognostic Scoring System (IPSS) [7] at the time of diagnosis and the Dynamic International Prognostic Scoring System (DIPSS) [8] during patient follow-up, respectively. Both prognostic systems assign score for patient's age, white blood cells (WBC), hemoglobin level, presence of circulatory blasts and presence of constitutional symptoms.

Mean platelet volume (MPV) is a platelet index routinely measured by automated cell counters. Elevation in MPV is associated with higher platelet activity [9-11] and accelerated megakaryopoiesis [12]. Elevated MPV has been recognized as a negative prognostic parameter in a variety of cardiovascular [13-15] and some solid neoplastic diseases [16, 17],

whereas lower MPV was shown to be predictive of inferior survival in majority of hematological neoplasms [18-20]. Ph- MPNs are characterized by neoplastic megakaryopoiesis and elevated MPV is a common finding in these diseases [21, 22]. Clinical and prognostic significance of MPV in patients with PMF and SMF have not been previously investigated.

In this study, we investigate clinical associations of MPV in patients with myelofibrosis, show that higher MPV is associated with more aggressive disease features and possesses powerful prognostic properties in both PMF and SMF setting.

Patients and methods:

A total of 87 patients with myelofibrosis that were evaluated in our institution in period from 2006 to 2017 and were fulfilling 2016 WHO criteria [1] for PMF and IWG-MRT [3] criteria for SMF diagnosis were included into this study. There were 66 (75.9%) patients with PMF and 21 (24.1%) patients with SMF. A total of 73 (83.9%) patients were evaluated at the time of diagnosis and 14 (16.1%) patients were evaluated during follow-up period. A subset of 56 (64.4%) patients were treatment-naïve (newly-diagnosed PMF patients), whereas 10 (11.5%) PMF patients and 21 (24.1%) SMF patients that progressed from previously treated Ph-MPNs were exposed to hydroxyurea at the time of MPV measurement. All patients provided written informed consent for molecular analyses. The study was approved by the University Hospital Dubrava Review Board.

Patients were staged according to the DIPSS prognostic scoring system [8]. Spleen and liver size were assessed by palpation. Bone marrow fibrosis was graded according to the current European consensus [23]. MPV was determined using automated cell counter and was recorded in addition to other hematological and clinical parameters (age, gender, WBC,

circulatory blasts, hemoglobin level, mean corpuscular volume (MCV), red cell distribution width (RDW), platelets, C reactive protein (CRP), lactate dehydrogenase (LDH), albumin, erythrocyte sedimentation rate, serum iron, total iron binding capacity (TIBC), ferritin, transfusion dependency, presence of constitutional symptoms, blast phase disease, *JAK2*, *CALR* or *MPL* mutational status). MPV values of PMF and SMF patients were compared to MPV values of 30 age and gender matched healthy controls.

The normality of data distribution was tested using the Kolmogorov-Smirnov test. Numerical variables were presented as median and interquartile range (IQR), or as arithmetic mean ± standard deviation depending on normality of data distribution. Categorical variables were presented as proportions. The Mann Whitney U test, the Kruskal-Wallis test, the $\chi 2$ (Chi squared) test and the Spearman rank correlation were used where appropriate. Jonckheere-Terpstra test for trend was used to test trend of increase in MPV over DIPSS risk categories. Survival analyses [24] were performed using methods of Kaplan and Meier, the Cox-Mantel version of the log-rank test [25] and the Cox regression analysis. Receiver operating characteristic (ROC) curve analysis using survival status as classification variable was performed for determining an optimal MPV cut-off value for survival analyses. P values <0.05 were considered significant. Associations of different prognostic factors with survival were screened for using custom made MS Excel workbook [26]. Analyses were performed using MedCalc Statistical Software version 17.6 (MedCalc Software BVBA, Ostend, Belgium).

Results:

There were total of 87 myelofibrosis patients analyzed, 66 with PMF and 21 with SMF. There were 55 (63.2%) male patients, mean age was 65.3 ±10.5 years. Patients' characteristics are shown in Table 1.

MPV values were non-normally distributed. Median MPV was 9.3 fL, IQR (8.4 - 10.2) in a whole cohort of patients, median 9.3 fL, IQR (8.3 - 10.1) in PMF and median 9.3 fL, IQR (8.6 - 10.5) in SMF. In comparison to 30 healthy controls who had median MPV values of 7.95 fL, IQR (7.4 – 8.6), both PMF and SMF patients had significantly higher MPV values ($P<0.05$ for both comparisons), while there was no statistically significant difference between PMF and SMF (Figure 1) showing that both PMF and SMF are characterized by elevated MPV.

Higher MPV values were statistically significantly associated with lower platelets (Rho -0.26, $P=0.016$), higher WBC (Rho 0.26, $P=0.015$), higher percentage of circulatory blasts (Rho 0.28, $P=0.009$), higher LDH (Rho 0.28, $P=0.011$) and larger spleen size (Rho 0.27, $P=0.014$) as shown in Figure 2A-E. We also observed statistically significant trend of increase in MPV values over DIPSS risk categories ($P=0.027$) as shown in Figure 2F. We observed no statistically significant associations of MPV with age or gender, or other disease specific parameters like degree of bone marrow fibrosis, *JAK2*, *CALR* or *MPL* mutation status, presence of constitutional symptoms, transfusion dependency, blast phase disease, liver size, hemoglobin level, MCV, RDW, parameters of iron metabolism, CRP, albumin and erythrocyte sedimentation rate. Therefore, higher MPV is associated with parameters indicating more aggressive biological behavior of disease and higher risk of death, while there is no evident difference in MPV regarding driver mutations, quality of erythropoiesis, classical parameters of inflammation or severity of bone marrow fibrotic process.

Median follow up of our patients was 51.4 months. Median overall survival was 67 months. To investigate prognostic properties of MPV, patients were divided into high MPV and low MPV groups based on ROC curve analysis using survival status as classification variable. Best survival discriminatory properties were achieved with MPV cut-off values of 8.5 fL, 8.3 fL and 9.4 fL for whole cohort, PMF and SMF cohorts, respectively. Higher MPV was univariately associated with inferior overall survival in overall population (hazard ratio (HR)=3.82, *P*=0.006), PMF (HR=4.35, *P*=0.007), and SMF patients (HR=7.22, *P*=0.034), respectively. Survival curves are shown in Figure 3.

We investigated whether prognostic properties of MPV are independent of current prognostic score and other correlated factors by performing a series of Cox regression analysis models. High MPV was significantly associated with inferior overall survival after adjusting for DIPSS in three separate models for a whole cohort of patients (High MPV HR=3.58, *P*=0.018; DIPSS HR=3.67, *P*<0.001), PMF patients (High MPV HR=3.83, *P*=0.032; DIPSS HR=3.64, *P*<0.001) and SMF patients (High MPV HR=10.6, *P*=0.042; DIPSS HR=6.09, *P*=0.058), respectively. These associations remained significant for a whole cohort and PMF patients after adjusting same models for age and gender. Due to significant association of higher MPV with lower platelet count and higher LDH which are known to be prognostic in patients with myelofibrosis [27, 28], we additionally created an age and gender adjusted model comparing MPV, DIPSS, platelets <100 $x10^9$/L and LDH where all tested variables excepting gender demonstrated independent prognostic properties (result for platelets was of borderline statistical significance), Table 2. Considering above analyses, high MPV provides additional prognostic information in patients with myelofibrosis and has a potential for improvement of current IPSS/DIPSS based prognostic scores.

Discussion:

To the best of our knowledge, our study is first to investigate associations of MPV with clinical features in patients with myelofibrosis, and first to identify MPV as a powerful independent predictor of inferior overall survival in these patients.

We identified six previous studies [21, 22, 29-32] assessing role of MPV in Ph- MPN patients (PMF patients comprised a minority of analyzed populations in two works [21, 22] and were not included in other studies). These studies investigated relationship between MPV, *JAK2* mutational status and history of thrombosis with inconsistent findings among studies. Only one work reported statistically significant result for MPV and history of thrombosis in ET [29]. Similarly, higher MPV was associated with *JAK2* mutation in two studies [31, 32], whereas result did not reach statistical significance or was not tested in other. It should be noted that these studies analyzed different populations than we did and some of them might be underpowered to detect statistically significant result. We did not observe statistically significant association of MPV with *JAK2* mutation, nor did we assess history of thrombosis in our patients. Thus, no definitive conclusion on these issues is possible at the moment.

By a literature review, it is currently not clear how and to what extent myelofibrosis specific therapies might affect MPV. Available data support the view that most of commonly used drugs are not associated with changes in MPV. Hydroxyurea [32-34] and alpha-interferon [35] typically do not affect MPV, although one of the studies suggested that Ph- MPN patients receiving cytoreductive therapies might present with higher MPV values [22]. Most of analyzed patients in that study had ET and were treated with anagrelide, a drug with a pronounced effect on MPV [22, 36] that is not commonly used in myelofibrosis. Data regarding corticosteroids and MPV are limited and difficult to interpret due to different disease contexts and concomitant therapies (changes in MPV were reported in both directions

[37, 38]) and cannot be reasonably extrapolated to myelofibrosis setting. Systematic data regarding ruxolitinib, other JAK inhibitors, and other drug classes like immunomodulatory drugs are lacking. Hence, no clear association of MPV with treatment status can be established at the moment.

In our cohort of patients, higher MPV was associated with more aggressive disease features like elevated WBC, elevated LDH and increased percentage of circulatory blasts suggesting that MPV reflects increased proliferative potential of leukemic hematopoietic stem cell. Aberrant neoplastic megakaryopoiesis that is typical for Ph- MPNs in cellular and fibrotic phases of diseases results in increased proportion of immature platelet fractions [39]. Such platelets are characterized by higher platelet volume than the mature ones [12] and were reported to be similarly associated with lower platelet count, increased WBC and increased percentage of circulatory blasts in patients with myelofibrosis [39]. Therefore, population of immature platelets probably contributes to MPV elevation in our cohort of patients as well and higher MPV is, at least in part, a consequence of more immature megakaryopoiesis.

In addition, platelet activation that is known to occur in PMF [40] also contributes to MPV elevation [41]. Spleen that is typically enlarged in myelofibrosis patients is also a place of increased platelet activation [42] and this might explain our finding of higher MPV observed with increasing spleen size. Activated platelets provide a plethora of pro-inflammatory cytokines and might promote development of tumor-protective microenvironment and more aggressive disease features observed with higher MPV. Interestingly, we observed no straightforward association of MPV with degree of bone marrow fibrosis or driver mutation status. Similarly, MPV was not evidently associated with erythropoiesis-related, iron metabolism-related and classical inflammation parameters. We acknowledge that our study might be underpowered to detect some of possibly existing associations. However, due to

weak/non-existing associations with MPV, we conclude that MPV in myelofibrosis is probably not dominantly regulated through aforementioned mechanisms.

Irrespective of mechanisms involved, higher MPV was significantly associated with shortened overall survival in our cohort of myelofibrosis patients. This is independent of a currently established prognostic scoring system in both PMF and SMF setting, confirming similar biology of these two diseases. MPV provides additional prognostic information to DIPSS, decreased platelet count and LDH. Hence, it has a potential of improvement of currently established IPSS/DIPSS based prognostic scores. Other hematologic neoplasms like aggressive Non Hodgkin lymphoma [18] multiple myeloma [19] and myelodysplastic syndrome [20] show opposite association of MPV with survival (lower MPV predicts poor survival), although this is not specific for malignant diseases in general and recent meta-analysis [43] found no firm association of MPV with survival in pooled analysis of different diseases. This is understandable, as changes in platelet volume might be mediated through different dominant processes in those diseases with different consequences on patient prognosis (inflammation, splenomegaly, bone marrow failure…). It should also be noted that megakaryopoiesis in Ph- MPNs is neoplastic and platelet morphology, size and function are intrinsically deranged, not only regulated by outside mechanisms. On the other hand, elevated MPV was shown to be predictive of adverse outcomes and shorter survival in variety of cardiovascular diseases [13-15, 44]. Cardiovascular diseases represent a substantial comorbid burden in PMF [6] and PMF patients have increased risk of cardiovascular events [45] due to accelerated atherosclerosis in presence of chronic inflammatory atmosphere [5]. Cardiovascular diseases might therefore be considered as "complications" of myeloproliferative disease and similar associations of different prognostic parameters [46, 47] (including MPV) with survival are not surprising.

Limitations of our study are single center experience, retrospective study design, small number of patients and inability to separately analyze cardiovascular mortality. Changes in MPV and their relationship with clinical features/specific therapies were not longitudinaly investigated and therefore we could not directly evaluate whether changes in MPV during disease course have specific clinical significance. In addition, information on non-driver mutations that bear adverse prognosis in myelofibrosis was available for a small subset of patients only, and due to insufficient power of such analyses, we could not adequately evaluate their potential relationship with MPV. Nevertheless, our results provide interesting new insights into clinical and prognostic significance of parameters reflecting megakaryopoiesis in Ph- MPNs.

In conclusion, higher MPV is associated with more aggressive disease features and possesses powerful independent prognostic properties in both PMF and SMF setting. Presented independent association of MPV with survival needs to be further investigated in new and larger cohorts of myelofibrosis patients as MPV has a potential for improvement of currently established IPSS/DIPSS based prognostic scores.

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Conflict of interest: The authors report no conflicts of interest.

Ethical approval: The study was approved by the University Hospital Dubrava Review Board.

Informed consent: All subjects provided written informed consent for molecular studies.

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Table 1: Patients' characteristics and their associations with mean platelet volume (MPV). Abbreviations: IQR – interquartile range; *JAK2 – Janus kinase 2*; *CALR – Calreticulin*; *MPL – myeloproliferative leukemia virus oncogene*; WBC – white blood cells; RDW – red cell distribution width; LDH – lactate dehydrogenase; CRP – C reactive protein; POS – positive; NEG – negative.

Variable	**All patients (N=87)**	**Direction and significance of association with MPV**
MPV	9.3, IQR (8.4 - 10.2)	-
Platelets (x10^9/L)	336, IQR (179 - 540)	NEG, *P*=0.016 *
Age (years)	65.3 ±10.5	POS, *P*=0.131
Male gender	55/87 (63.2%)	NEG, *P*=0.071
Fibrosis grade 0 and 1 Fibrosis grade 2 and 3	34/87 (39.1%) 53/87 (60.9%)	POS, *P*=0.106
JAK2 pos.	54/84 (64.3%)	POS, *P*=0.487
CALR pos.	8/65 (12.3%)	NEG, *P*=0.742
MPL pos.	2/65 (3.1%)	NEG, *P*=0.190
Constitutional symptoms	31/87 (36%)	POS, *P*=0.746
Spleen size (cm)	4, IQR (1 - 10)	POS, *P*=0.014 *
Blast phase disease	8/87 (9.2%)	POS, *P*=0.058
WBC (x10^9/L)	10.9, IQR (6.9 - 16.6)	POS, *P*=0.015 *
Circulatory blasts (%)	0, IQR (0 - 0)	POS, *P*=0.009 *
Hemoglobin level (g/L)	114 ±25.7	NEG, *P*=0.341
RDW (%)	19.6, IQR (18.2 - 21)	NEG, *P*=0.517
LDH (U/L)	540, IQR (345 - 811)	POS, *P*=0.011 *
CRP (mg/L)	5.3, IQR (2 - 14.2)	POS, *P*=0.318

* statistically significant at *P*<0.05

Table 2: Cox regression model demonstrating independent prognostic properties of mean platelet volume (MPV) in a whole cohort of patients after adjusting for the Dynamic International Prognostic System (DIPSS), platelets <100 x10^9/L, lactate dehydrogenase (LDH), age and gender. Abbreviations: HR – hazard ratio, C.I. – confidence interval.

Variable	**HR**	**95% C.I. for HR**	***P* value**
High MPV	7.13	[1.64 - 31.03]	0.009 *
DIPSS	2.61	[1.39 - 4.9]	0.003 *
Platelets <100 x10^9/L	3.3	[0.88 - 12.32]	0.076
LDH	1.00	[1.00 – 1.00]	<0.001 *
Age	1.07	[1.02 - 1.12]	0.010 *
Male gender	1.29	[0.55 - 3.01]	0.563

* statistically significant at *P*<0.05

Figure 1: Mean platelet volume (MPV) was significantly elevated in both primary (PMF) and secondary myelofibrosis (SMF) in comparison to healthy controls, but did not differ between PMF and SMF.

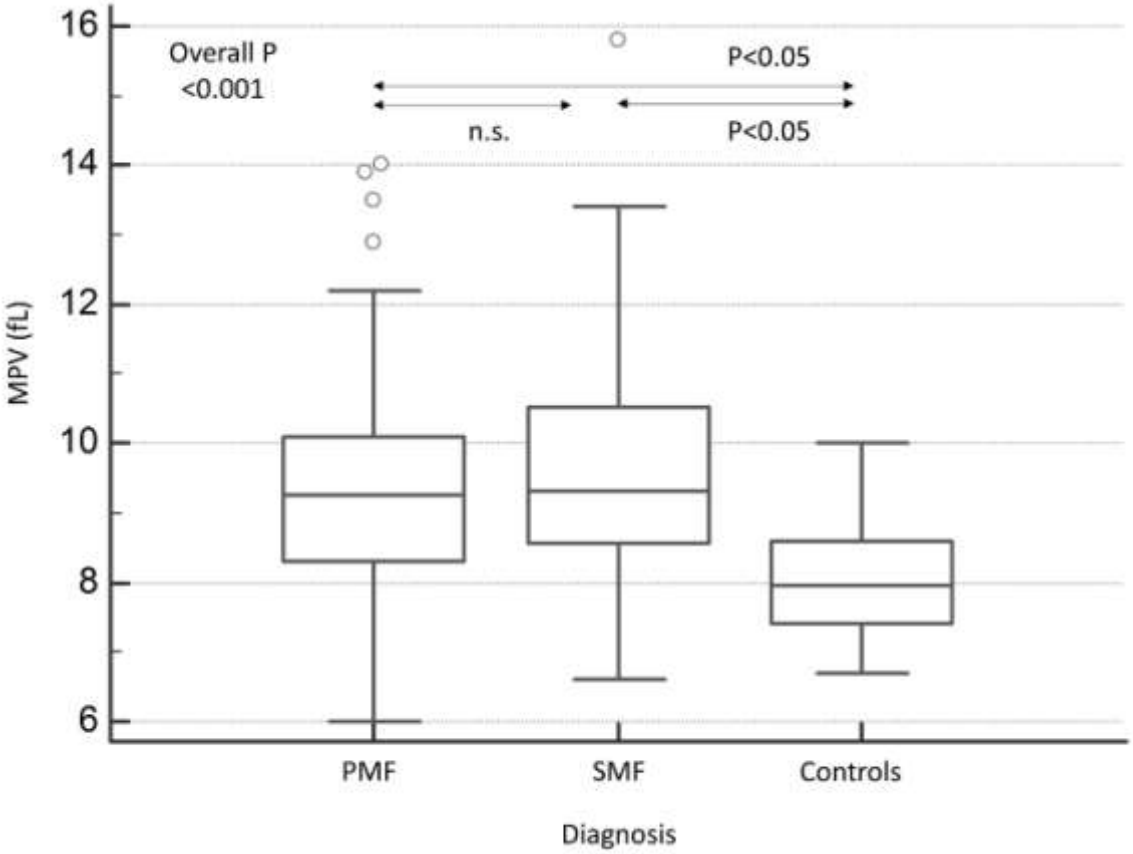

Figure 2: Higher mean platelet volume (MPV) was significantly associated with **A)** lower platelets, **B)** higher white blood cells (WBC), **C)** higher percentage of circulatory blasts, **D)** higher lactate dehydrogenase (LDH), **E)** larger spleen, and **F)** higher Dynamic Prognostic Scoring System (DIPSS) risk category. Abbreviations: 1 – Low risk, 2 – Intermediate-1 risk, 3 – Intermediate-2 risk, 4 – High risk.

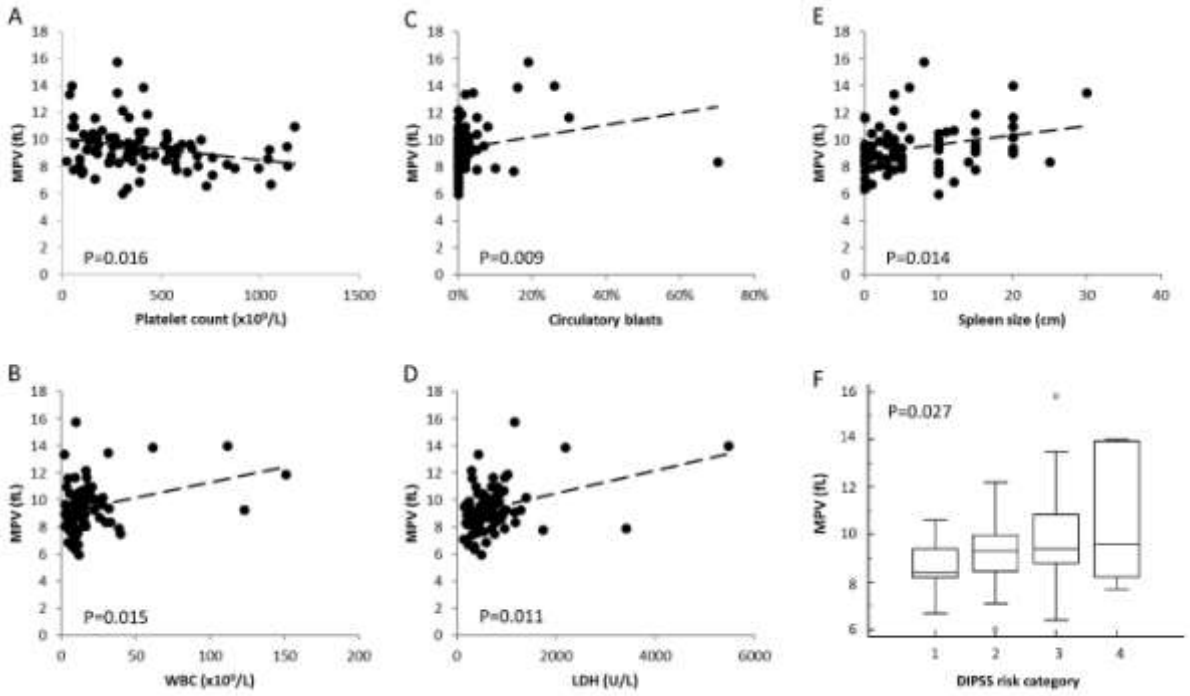

Figure 3: Overall survival (OS) stratified by mean platelet volume (MPV) in **A)** all patients, **B)** primary myelofibrosis (PMF) patients and **C)** secondary myelofibrosis (SMF) patients. Abbreviations: HR – hazard ratio.