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Peripheral and bone marrow CD34$^+$ cell levels on chronic myeloproliferative disease

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ABSTRACT

Purpose: The aim of the present study was to examine the relationship between peripheral CD34$^+$ and bone marrow CD34$^+$ levels and the clinicopathologic characteristics and laboratory parameters of myeloproliferative disease (MPD) patients.

Patients and methods: A total of 103 MPD patients were enrolled in this study. We examined the relationship between bone marrow CD34$^+$ and peripheral CD34$^+$ levels and the patients' clinicopathologic and laboratory parameters.

Results: There were no significant correlations between the peripheral CD34$^+$ levels and the JAK-2 V617F mutation, thrombosis, white blood cells (WBC), lactate dehydrogenase (LDH), transferrin saturation (TS), ferritin, or bone marrow cellularity. In addition, there were no significant correlations between bone marrow CD34$^+$ levels and the JAK-2 V617F mutation, thrombosis, WBC, LDH, TS, ferritin, or bone marrow cellularity ($P > 0.05$). We did not identify any significant relationship between peripheral CD34$^+$ and bone marrow CD34$^+$ levels ($P > 0.05$). However, there were significant correlations between peripheral CD34$^+$ levels and bone marrow fibrosis ($P < 0.001$), between bone marrow CD34$^+$ levels and constitutional symptoms ($P < 0.05$), and between bone marrow CD34$^+$ levels and bone marrow fibrosis ($P < 0.001$).

Conclusion: We did not find any significant relationship between the clinicopathologic and laboratory characteristics and peripheral and bone marrow CD34$^+$ cells from bone marrow fibrosis patients. There was also no significant relationship between bone marrow CD34$^+$ cells and peripheral CD34$^+$ cells. Some peripheral CD34$^+$ cells may originate from the spleen rather than the bone marrow, which may given us different result of some parameters.

KEYWORDS

Peripheral CD34$^+$ cells; bone marrow CD34$^+$ cells; myeloproliferative disease; bone marrow fibrosis

Introduction

Myeloproliferative neoplasms (MPN) are characterized by stem-cell-derived clonal myeloproliferation [1,2]. According to the WHO's classification, Philadelphia translocation (BCR-ABL)-negative MPN include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [1,2].

CD34 is a small peptide that settles on the surface of hematopoietic stem cells [3]. CD34 is attached by a glycosylphosphatidylinositol bridge structure to the cell membrane. CD34 is an indicator of myeloid immaturity. CD34$^+$ cells constitute only a small part of the total number of bone marrow cells (<1%) [4,5]. Collagen collects in the bone marrow during progressive disease. This results in anemia, cytopenia, and splenomegaly (agnogenic myeloid metaplasia) [6–8]. The accumulation of collagen in the bone marrow causes CD34$^+$ cells to move into the blood, resulting in congestion in the lymph nodes, liver, and spleen [8,9]. Therefore, spleen volume increases and splenic CD34$^+$ cell counts are much higher than in bone marrow in the osteosclerotic stage [10–13]. In this stage, constitutional symptoms occur. Splenomegaly and hepatomegaly cause early satiety and swelling, and anemia causes weakness and fatigue [12]. PMF is characterized by the mobilization of hematopoietic stem cells and hematopoietic progenitor cells and the establishment of extramedullary hematopoiesis, leading to an increased number of circulating CD34$^+$ progenitors in the peripheral blood [14]. CD34$^+$ cells are found in both the peripheral blood and bone marrow. Peripheral CD34$^+$ levels are determined by cytometric study, while bone marrow CD34$^+$ levels are determined by immunohistochemical study. Studies have shown a relationship between the peripheral CD34$^+$ levels and the clinicopathologic characteristics of myeloproliferative disease (MPD) patients [4,5,15].

The aim of this study was to examine the initial values of peripheral and bone marrow CD34$^+$ levels and to determine their relationship between patients' clinicopathologic characteristics and each other.

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Materials and methods

Patients

Between 2000 and 2012, a total of 103 patients who were diagnosed with chronic MPN were enrolled in this study. We studied three diagnostic subgroups: PV, ET, and PMF. We evaluated clinical and laboratory data. Bone marrow material was re-evaluated after diagnosis, and immunohistochemical stains were performed for CD34. Patients were then divided into two groups according to bone marrow CD34 percentage (<%1 and ≥%1). Patients were also divided into two groups according to peripheral CD34 count ($<15\times10^6$/l vs. $\geq15\times10^6$/l). We used the 15×10^6/l level as a cut-off value because increased levels of $CD34^+$ ($\geq15\times10^6$/l) are affected the beginning of bone marrow fibrosis. We examined the relationship between bone marrow $CD34^+$ and peripheral $CD34^+$ levels and the patients' clinicopathologic features and laboratory markers.

Diagnoses of PV, ET, and PMF were made according to the WHO's 2008 criteria and based on clinical and laboratory features [16]. Risk stratification in PV and ET was used to estimate thrombotic complications. Patients were sorted into low, intermediate, and high-risk groups according to a risk assessment [17,18]. Risk assessment of PMF was performed using the International Prognostic Scoring System (IPSS), Dynamic International Prognostic Scoring System (DIPSS), and DIPSS-plus [19,20].

Bone marrow biopsies $CD34^+$ cell analysis

Immunohistochemical study was applied to biopsied bone marrow to examine $CD34^+$ cell ratios. Immunohistochemistry was performed to enumerate blasts using the antibody against CD34 on bone marrow trephine biopsy. Immunoperoxidase staining was performed using the streptavidin–biotin peroxidase method. The sections were treated with 0.3% H_2O_2 in order to suppress endogenous peroxidase activity. Antigen retrieval was performed using 0.01 M citrate buffer (pH 6.0) in a microwave, and then sections were incubated with CD34 antibody (Clone: QBEnd-10, NovoCastra, UK). 3,3′ Diaminobenzidine (DAB) chromogen was used for detecting the reaction. Determination of the blast percentage in CD34 stained bone marrow biopsy sections was performed manually. Five representative fields were selected, and CD34 positive cells were counted under the 40× objective. CD34 positive staining was determined to be granular brown cytoplasmic and membranous staining. Normal CD34 expression in BM-nucleated cells on BM histology does not exceed 1%; therefore, MPN cases were categorized into CD34-negative (CD34-positive cells 1%) and CD34-positive (CD34-positive cells 1%, including formation of aggregates) cells.

Peripheral blood sample $CD34^+$ cell analysis

Flow cytometry analysis was used to determine peripheral blood $CD34^+$ cell levels. One hundred microliter peripheral blood samples were drawn into EDTA-anticoagulated tubes to obtain complete $CD34^+$ cell count. A total of 10 µl of CD45-FITC (fluorescein isothiocyanate) and 10 µl of CD34-PE (phycoerythrin) monoclonal antibody were added. After vortexing, the samples were kept for 20 minutes in a dark room. A lysis buffer was added followed by an additional waiting period of 5 minutes. The samples were then centrifuged for 5 minutes at 1200 rpm. After the supernatant was removed, 1.5 cc of cell wash were added, and then the mixture was vortexed and centrifuged for 5 minutes at 1200 rpm again. Then, 500 µl of cell wash were added, and the cells were vortexed again. A total of 75,000 cells were counted with a BD FACSCalibur. The peripheral CD34 count and percentage were calculated with the CellQuest software program.

Patients were divided into two groups according to their peripheral blood $CD34^+$ cell count ($<15\times10^6$/l and $\geq15\times10^6$/l). We analyzed the peripheral blood $CD34^+$ cell count and clinicopathologic and laboratory parameters for 44 patients, while bone marrow $CD34^+$ cell percentages were analyzed for 103 patients.

Statistical analyses

The SPSS 15 statistical program was used, and patients' characteristics were calculated with descriptive statistics. We used a chi-square test for categorical values and the Mann–Whitney *U* test for non-categorical values. Spearman's correlation analysis was used to determine the relationship between $CD34^+$ cell count and patients' prognostic factors. A value of $P\leq0.05$ was considered to be statistically significant.

Results

Patients and baseline laboratory parameters

The study included 103 BCR-ABL negative MPD patients. Fifty-six patients were PV (54.4%), 32 were ET (31.1%), and 15 were PMF (14.6%). The median age of the MPN patients was 50.52 ± 15.14 (PV: 52.50 ± 14.52; ET: 46.50 ± 17.74; PMF: 51.73 ± 9.45). Fifty-eight (56.3%) of the 103 patients were female and 45 (43.7%) were male. Twenty-seven (48.2%) of the 56 PV patients were female and 29 (51.8%) were male; 22 of 32 (68.8%) ET patients were female and 10 (31.3%) were male; and 9 of 15 (60%) PMF patients were female and 6 (40%) were male. The patients' general characteristics are given in Table 1. We did not find any significant differences between PV, ET, and PMF patients in regard to sex, constitutional symptoms, smoking, hypertension, diabetes mellitus,

atherosclerotic heart disease, hyperlipidemia, or cerebrovascular events (CVE) ($P > 0.05$).

The risk scores for PV patients at diagnosis showed that 25 (46.3%) had low risk, 12 (22.2%) had intermediate risk, and 17 (31.5%) had high risk. For ET patients, eight (23.5%) patients had low risk, 16 (47.1%) had intermediate risk, and 10 (29.4%) had high risk. We used DIPPS-plus risk scores for PMF patients and found that nine (60%) had low risk, two (13.3%) had intermediate 1 risk, four (26.7%) had intermediate 2 risk, and none of the patients had high risk.

Sixteen (15.5%) patients had thrombosis: five (4.9%) before diagnosis, six (5.8%) at diagnosis, and five (4.9%) after diagnosis. Eight (7.8%) patients had arterial thrombosis, two (1.9%) had CVE, five (4.9%) had myocardial infarction (MI), and one had subclavian arterial thrombosis. Half of the 16 patients had venous thrombosis, three (2.9%) had deep vein thrombosis (DVT), four (3.9%) had pulmonary thromboembolism (PTE), and one had jugular venous thrombosis. In the subgroups, 11 (19.6%) of the PV patients and five (15.6%) of the ET patients had thrombosis while none of the PMF patients had thrombosis. Three (27.3%) of the 11 PV patients had thrombosis before diagnosis, four (36.4%) had thrombosis concurrent with the diagnosis, and four (36.4%) had thrombosis after diagnosis. Five (45.5%) PV patients had arterial thrombosis, two (18.2%) had CVE, and three (27.3%) had MI. Six (54.5%) PV patients had venous thrombosis, three (27.3%) had DVT, two (18.2%) had PTE, and one (9%) had jugular venous thrombosis. Two (40%) out of five ET patients had thrombosis before diagnosis, two (40%) had thrombosis concurrent with diagnosis, and one (20%) had thrombosis after diagnosis. Three (60%) of the ET patients had arterial thrombosis while two (40%) had venous thrombosis (two of them with MI, one of them with subclavian arterial thrombosis, and two of them with PTE).

Six patients had a history of bleeding: one (16.7%) before diagnosis, one (16.7%) concurrent with diagnosis, and four during disease follow-up. All six patients had mucosal bleeding (two of them gastrointestinal bleeding, four of them epistaxis). In the subgroups, four (66.7%) PV patients and two (33.3%) ET patients had a history of bleeding while none of the PMF patients had such a history. One (25%) PV patient had a history of bleeding before diagnosis and three (75%) had bleeding concurrent with diagnosis; two (50%) of these patients experienced gastrointestinal bleeding while two (50%) had epistaxis. One (50%) ET patient had a history of bleeding concurrent with diagnosis, and the other patient (50%) experienced bleeding during disease follow-up. Both patients had epistaxis (100%).

Eighty-five out of 103 patients showed JAK2 V617F mutation results in our data, 63 (61.2%) of which were positive. JAK2 V617F mutations were 91.1% positive in PV, 62% positive in ET, and 36.4% positive in PMF.

Table 1 Patients general characteristics.

	PV		ET		PMF	
Variables	*n*	%	*n*	%	*n*	%
Sex						
Female (*n*:58)	27	46.6	22	37.9	9	15.5
Male (*n*:45)	29	64.4	10	22.2	6	13.3
Constitutional symptoms						
Negative (*n*:72)	36	64.3	24	75	12	80
Positive (*n*:31)	20	35.7	8	25	3	20
Smoking						
None (*n*:88)	45	51.1	29	33	14	15.9
Smoker (*n*:5)	4	80			1	20
Exsmoker (*n*:10)	7	70	3	30		
Hypertension						
Negative (*n*:64)	32	50.0	21	32.8	11	17.2
Positive (*n*:39)	24	61.5	11	28.2	4	10.3
Type 2 DM						
Negative (*n*:95)	51	53.7	30	31.6	14	14.7
Positive (*n*:8)	5	62.5	2	25.0	1	12.5
AHD						
Negative (*n*:96)	52	54.2	31	32.3	13	13.5
Positive (*n*:7)	4	57.1	1	14.3	2	28.6
Hyperlipidemia						
Negative (*n*:94)	51	54.3	29	30.9	14	14.9
Positive (*n*:9)	5	55.6	3	33.3	1	11.1
CVE						
Negative (*n*:99)	53	53.5	31	31.3	15	15.2
Positive (*n*:4)	3	75.0	1	25.0		
Fibrosis						
Grade 0	16	28.6	20	62.5		
Grade 1	22	39.3	12	37.5		
Grade 2	14	25			3	20
Grade 3	4	7.1			12	80

n: number; %: percentage; DM: diabetes mellitus; AHD: atherosclerotic heart disease; CVE: cerebrovascular events; PV: polistemia vera; ET: essential thrombocytosis; PMF: primary myelofibrosis.

Table 2 Median value of laboratory parameters.

	Subgroups		
	PV	ET	PMF
Hemoglobin (g/dl)	17.03	13.60	10.66
Hematocrit (%)	52.46	40.10	31.70
Leukocyte (per u)	13 293	9739	8846
Platelets (per ul)	611 937	841 987	338 820
TS (%)	14.89	18.40	17.97
Ferritin (ng/ml)	24	53	100
LDH (U/l)	324	281	621

TS: transferrin saturation; LDH: lactate dehydrogenase; PV: polistemia vera; ET: essential thrombocytosis; PMF: primary myelofibrosis.

We examined the patients' bone marrow biopsies: 75 (72.8%) of the 103 patients had grade 1–3 fibrosis while 28 (27.2%) had grade 0 fibrosis. Table 1 shows bone marrow biopsy fibrosis in the subgroups at diagnosis. Table 2 lists the laboratory parameters that were used to calculate the relationship between bone marrow and peripheral CD34 levels.

Peripheral CD34$^+$

Sixteen PV, 12 PMF, and 16 ET patients had peripheral CD34$^+$ values at diagnosis. The cut-off value of peripheral CD34$^+$ was 15×10^6/l. Thirty patients had peripheral CD34$^+$ values was $<15 \times 10^6$/l, and 13 patients had peripheral CD34$^+$ values was $\geq 15 \times 10^6$/l. The peripheral CD34$^+$ group and median values for each to subgroup are shown in Table 3.

There were no significant correlations between peripheral CD34$^+$ levels and constitutional symptoms, JAK-2 V617F mutation, thrombosis, white blood cells (WBC), lactate dehydrogenase (LDH), transferrin saturation (TS), ferritin, bone marrow cellularity, or the risk of transformation to acute leukemia ($P > 0.05$), while a significant correlation was found with bone marrow fibrosis in PMF ($P < 0.001$). The peripheral CD34$^+$ cell level was high in PMF patients when the bone marrow fibrosis grade was also high. We did find a significant correlation between peripheral CD34$^+$ levels and bone marrow fibrosis in ET and PV patients ($P > 0.05$). In addition, we examined peripheral CD34$^+$ levels in prefibrotic and fibrotic bone marrow for all (ET, PV, and PMF) patients. We divided patients into two groups based on peripheral CD34$^+$ levels: less than 15×10^6 and over 15×10^6. Fibrotic bone marrow could be seen when the peripheral CD34$^+$ level was over 15×10^6 ($P < 0.05$). Table 4 shows the relationship between the peripheral CD34$^+$ levels and bone marrow fibrosis stage.

Table 3 Peripheral and bone marrow CD34$^+$ group and median value.

	PV	ET	PMF
Peripheral CD34$^+$ group			
$<15 \times 10^6$	15	13	3
$\geq 15 \times 10^6$	1	3	9
Median value	$2.3 \pm 4.3 \times 10^6$/l	$3.8 \pm 60.3 \times 10^6$/l	$30.8 \pm 122.2 \times 10^6$/l
Bone marrow CD34$^+$ group			
<1%	23	10	6
≥1%	33	22	9
Median value	1 ± 0.9%	1 ± 0.9%	1 ± 1.1%

PV: polistemia vera; ET: essential thrombocytosis; PMF: primary myelofibrosis.

Bone marrow CD34$^+$

We examined bone marrow CD34$^+$ cell levels in 103 patients. The cut-off value was 1%. Thirty-nine patients had bone marrow CD34$^+$ values <1% while 64 patients had values ≥1%. Table 3 shows the bone marrow CD34$^+$ group and the median values of the subgroups.

No significant correlations were found between bone marrow CD34$^+$ cell levels and the JAK-2 V617F mutation, thrombosis, WBC, LDH, TS, ferritin, or bone marrow cellularity subgroups ($P > 0.05$), whereas a significant correlation was found the between bone marrow CD34$^+$ levels and bone marrow fibrosis and constitutional symptoms. If the bone marrow CD34$^+$ level was ≥1%, constitutional symptoms were more likely to be observed ($P < 0.05$). As the bone marrow CD34$^+$ level increased, the bone marrow fibrosis grade also increased ($P < 0.01$). The bone marrow fibrosis grade was significantly higher in ET patients when the bone marrow CD34$^+$ levels were ≥1% ($P < 0.01$). We divided patients into two groups based on bone marrow CD34$^+$ levels: less than 1% and over 1%. There was no significant correlation between bone marrow CD34$^+$ level and bone marrow fibrosis ($P > 0.05$). There was no statistical correlation between the peripheral CD34$^+$ and bone marrow CD34$^+$ percentage level ($P > 0.05$).

Discussion

In this study, we identified a significant correlation between peripheral CD34$^+$ levels and bone marrow fibrosis in PMF patients, and we also found a significant correlation between bone marrow CD34$^+$ levels and bone marrow fibrosis and constitutional symptoms.

A number of studies have focused on the peripheral CD34$^+$ levels and the clinicopathologic characteristics of MPD patients [5,15,21], whereas very few studies

Table 4 Relationship between peripheral CD34$^+$ level and bone marrow fibrosis stage.

	Fibrosis grade							*P*
Peripheral CD34	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Pre-fibrosis	Fibrosis	
$<15 \times 10^6$	6	13	15	2	0	19	17	0.024
$\geq 15 \times 10^6$	0	2	2	10	0	2	12	
Total	6	15	17	12	0	21	29	

have examined the bone marrow $CD34^+$ levels and the clinicopathologic characteristics of MPD patients [22]. We evaluated bone marrow $CD34^+$ levels at diagnosis and examined the relationship between circulating $CD34^+$ levels and clinicopathologic characteristics.

Two clinicopathologic characteristics were of particular importance for the present study: constitutional symptoms and thrombosis. Thirty percent of MPD patients, 35.7% of the PV patients, 25% of the ET patients, and 20% of the PMF patients had constitutional symptoms. Emanuel *et al.* found that >50% of MPD patients demonstrate constitutional symptoms initially [23].

In the literature, 30% of PV patients, 21% of ET patients, and 14% of PMF patients were shown to have thrombosis [24]. In a study of 100 ET patients, 10% had thrombosis at diagnosis and 23% had thrombosis during follow-up [25]. This result is similar to our findings, where three-fourths (3 out of 4) of ET patients were asymptomatic during follow-up. Contrary to the literature, we found that venous thrombosis was more prevalent than arterial thrombosis in our study [26,27]. This result could be explained by the fact that we diagnosed small arterial vessel thrombosis less frequently than any size of venous vessel thrombosis and because there were few patients in our study.

Bleeding disorders usually occur at platelet levels over 1000×10^9/l in ET patients and between the 400 and 1000×10^9/l platelet level in PV patients [24]. This study was too small to discuss bleeding disorders, but as in the literature, the most common bleeding sites were the mucosa of the gastrointestinal system and nose [24].

We found a relationship between peripheral $CD34^+$ levels and bone marrow fibrosis in MPDs patients, and there was a significant correlation between bone marrow fibrosis and PMF. In the PMF patients (for ET and PV), bone marrow fibrosis increased proportionally to peripheral $CD34^+$ levels ($<15 \times 10^6$), so we can determine the fibrosis stage (prefibrotic or fibrotic) of bone marrow without using an invasive procedure such as bone marrow biopsy. We did not find any other relation between peripheral $CD34^+$ and other laboratory and clinical parameters in ET, PV, or PMF patients. Arora *et al.* also found no relationships between peripheral $CD34^+$ levels and age, sex, disease progress, hemoglobin and thrombosis level, bone marrow fibrosis, osteosclerosis, or angiogenesis in patients with PMF, although there were relationships with WBC level, peripheral blast, immature myeloid cell ratio, and splenomegaly [5]. Barosi *et al.* found relationships between peripheral $CD34^+$ levels and leukocytosis, thrombocytosis, and splenomegaly [15]. Moreover, it is believed that anemia, high LDH levels, and splenomegaly have strong relationships with peripheral $CD34^+$ levels [6–8]. Initial disease cell turnover was very fast, so LDH levels were high but anemia was not obvious; thus, TS may have been higher than at advanced stages. PMF patients' peripheral $CD34^+$ levels were higher than those of ET and PV patients in the case described above (agnogenic myeloid metaplasia). The relationship between peripheral $CD34^+$ and bone marrow fibrosis is an interesting finding because advanced stage PMF patients were not included in this study. In addition, our data were unusual in that no relationships were found between peripheral $CD34^+$ levels and WBC levels, peripheral blasts, LDH levels, anemia, leukocytosis, thrombocytosis, or splenomegaly. Though this could be explained by the small number of patients in the different subgroups.

The present study revealed relationships between bone marrow $CD34^+$ levels and constitutional symptoms, TS, and bone marrow fibrosis. When bone marrow $CD34^+$ levels were $\geq$1%, constitutional symptoms and TS were higher compared to patients with bone marrow $CD34^+$ levels < 1%. Moreover, bone marrow $CD34^+$ levels and bone marrow fibrosis were positively correlated in ET patients. Bone marrow $CD34^+$ levels are related to proliferative activity, which occurs in the prefibrotic and fibrotic stages of PMF [12,28]. Bone marrow $CD34^+$ cells were responsible for a homing phenomenon and the regulation of hematopoietic cells [28]. There was a strong relationship between bone marrow $CD34^+$ level and the blastic phase of chronic myelocytic leukemia (CML) [28]. High bone marrow $CD34^+$ levels resulted in a worse prognosis than lower bone marrow $CD34^+$ levels in CML patients [28]. This result was the opposite of that in PV and PMF, where a high $CD34^+$ levels led to a good prognosis and OS in PMF and PV patients. When $CD34^+$ cells moved into the blood, the fibrotic stage of PMF and PV begun [28]. This was an indicator of future bone marrow failure. When the peripheral $CD34^+$ levels increased, splenic red pulp began to remove $CD34^+$ cells from the blood [12]. This resulted in an increase in $CD34^+$ levels in the spleen rather than in the bone marrow [12]. Splenectomy may help cure the constitutional symptoms, but the hepatic volume may increase after splenectomy because $CD34^+$ cells would only be able to collect hepatic tissue [12]. Thiele *et al.* studied PMF patients and found relationships between bone marrow $CD34^+$ levels and splenomegaly, peripheral blood blasts, myeloblasts, erythroblasts, LDH levels, hemoglobin level, thrombocytosis, and leukocytosis [22].

In our study, we did not find any relationship between bone marrow and peripheral $CD34^+$ levels, and the number of bone marrow $CD34^+$ cells did not differ in PV and ET. Thus, peripheral $CD34^+$ cells may not originate from the bone marrow but rather from the spleen due to extramedullary hematopoiesis. Hibbin *et al.* measured the number of myeloid progenitor cells in the circulation of patients with MF and other MPDs [9]. They showed that progenitor cell numbers were higher in the circulation of patients with MF

compared to controls. They also showed that splenectomized patients generally had reduced numbers of circulating progenitor cells. Wang *et al.* further demonstrated that the spleens of PMF patients contain malignant hematopoietic stem cells [29].

Our study found that unlike peripheral $CD34^+$ cells, bone marrow $CD34^+$ cells cannot be used to diagnose PMF or other MPD. The splenic microenvironment is different from the bone marrow microenvironment, and some of the peripheral hematopoietic stem cells may originate from the splenic microenvironment [14,30]. We hypothesize that the peripheral $CD34^+$ cells found in our study may have originated from splenic extramedullary hematopoietic tissue, thus we did not find any significant relationship between peripheral (which were mobilized from spleen) and bone marrow $CD34^+$ cells.

Disclosure statement

No potential conflict of interest was reported by the authors.

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