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The Role of Bone Marrow Biopsy Morphology and Clinical Characteristics in Facing the Challenges of Diagnosing Primary Myelofibrosis, Polycythemia Vera, and Essential Thrombocythemia at Cipto Mangunkusumo Hospital

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ABSTRACT

Background
Myeloproliferative neoplasm (MPN) is a neoplasm characterized by the proliferation of one or more myeloid cells and their derivatives. The limitations of molecular examination in Indonesia make the diagnosis of MPN based on clinical and histopathological examination very crucial. The aim evaluate the clinicopathological profile of primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET) and identify the typical morphological characteristics of bone marrow biopsy.

Methods
A retrospective study of cases diagnosed with MPN was conducted at the Department of Anatomic Pathology FMUI/RSCM in 2015-2019. Clinical data and evaluation of bone marrow morphology consisting of cellularity, erythroid myeloid ratio, cluster and megakaryocyte morphology, blast cells, fibrosis, osteosclerosis, and sinus dilatation were analyzed.

Results
A total of 172 cases were diagnosed as MPN *BCR-ABL1-negative* (PMF: 74; ET: 56; PV: 42). On routine blood examination, there was an increase in hemoglobin (Hb) and hematocrit (Ht) in PV, a decrease in Hb in PMF and an increase in platelets in ET ($p<0.001$). Splenomegaly is mostly found in PMF. Myeloid erythroid ratio was decreased in PV, normal in ET and increased in PMF ($p<0.001$). Megakaryocytes were arranged in loose clusters in 88.1% of PV cases and 96.4% of ET, dense clusters were found in 91.9% of PMF cases. Staghorn-like megakaryocytes were found in all ET cases and bulbous/cloud-like megakaryocytes were found in 97.3% of PMF cases. Most cases of PV (90.5%) and all cases of ET showed pre-fibrotic bone marrow (grade 0-1), while 77% of PMF cases showed fibrosis grade 2-3.

Conclusion
In diagnosing PV, ET, and PMF, correlation between clinical data, laboratory, and bone marrow histomorphological evaluations, especially cellularity, myeloid:erythroid ratio, cluster and megakaryocyte morphology, degree of fibrosis, osteosclerosis, and sinus dilatation is required.

Keywords: Myeloproliferative neoplasm, polycythemia vera, primary myelofibrosis, essential thrombocythemia.

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INTRODUCTION

Myeloproliferative neoplasm (MPN) is a hematological neoplasm consisting of hematopoietic cell proliferation of myeloid cell lineages (granulocytic, erythroid and megakaryocytic).[1-4]

Based on archival data from the Department of Anatomic Pathology FKUI/RSCM for 2015-2019, out of 2,270 bone marrow biopsy cases, there were 445 cases of MPN. The incidence of MPN in Europe and North America is 0.01 to 2.8 cases per 100,000 population. The incidence of MPN was reported more in men with a ratio of 1-2:1, with a median age of 60 years.[1,5]

MPN is classified into several groups based on the World Health Organization (WHO) Classification, namely: BCR-ABL1-positive chronic myeloid leukemia, chronic neutrophilic leukemia, polycythemia vera (PV), primary myelofibrosis (PMF), essential thrombocythemia (ET), chronic eosinophilic leukemia, and unclassifiable myeloproliferative neoplasm (MPNU). Myeloproliferative neoplasms with *BCR-ABL1* negative mainly consist of PMF, PV, and ET.[1]

PMF is a myeloproliferative neoplasm characterized by abnormal proliferation of megakaryocytes and granulocytes in the bone marrow with fibrosis and extramedullary hematopoiesis in further development. There are two stages of disease in PMF, namely pre-fibrotic and overt fibrotic.[1,6-8] The incidence of PMF in a year is 0.5-1.5 cases per 100,000 population and 30-50% of cases are prefibrotic/early PMF.[1]

PV is a myeloproliferative neoplasm involving erythroid, granulocytes, and megakaryocytes series, resulting in an increase in the number of hematopoietic cells of the three lineages in the bone marrow (panmyelosis). The incidence of polycythemia vera in a year is around 0.84 cases per 100,000 population.[1,7,9] There are 2 phases in PV, namely the polycythemia phase (an increase in haemoglobin, hematocrit and red blood cell levels) and the post-polycythemia myelofibrosis phase, in which cytopenia occurs (due to inadequate hematopoiesis), bone marrow fibrosis, extramedullary hematopoiesis, and hypersplenism.[1,10,11]

ET is a myeloproliferative neoplasm involving megakaryocytic proliferation.[1,7] This neoplasm is characterized by an increase in the number of platelets ≥450 times10^9/L in the peripheral blood. The annual incidence of essential thrombocythemia is 0.2-2.3 cases per 100,000 population.[1]

Based on WHO 2017, there are several major and minor criteria needed in diagnosing MPN in each subtype. Laboratory data, clinical data, bone marrow morphology and molecular examination are the main criteria in diagnosing MPN.[1,4,7,12,13]

Until now diagnosing BCR-ABL1-negative MPN (PV, PMF and ET) is still challenging, especially in countries where molecular testing is difficult to access. The three types of MPN also often provide overlapping clinical and laboratory features, so it is difficult to distinguish them only by relying on clinical and laboratory data. Therefore, the role of bone marrow biopsy morphology is needed in diagnosing the three types of MPN.[1,2,4,14]

In the WHO Classification of Haematopoietic and Lymphoid Tissues 2017 there are several histopathological parameters used in diagnosing *BCR-ABL1*-negative MPN, including cellularity, granulopoiesis, erythropoiesis, megakaryopoiesis, cell size and arrangement of megakaryocyte, cluster size and morphology of megakaryocyte, degree of fibrosis, and bone marrow stroma. These features often overlap among the MPN subtypes. This study aims to evaluate clinicopathological profiles and identify more specific histopathological parameters to differentiate PV, PMF, and ET.

METHODS

This study is a retrospective study of patients diagnosed with MPN. MPN case data was collected from the archives of the Department of Anatomic Pathology FMUI/RSCM from January 2015 to December 2019, then traced the histopathological preparations. The inclusion criteria used were all patients with a histopathological diagnosis of MPN (PV, PMF and ET) with slides available in the archives that had been stained with reticulin and trichrome. Exclusion criteria from this study were cases with non-representative or suboptimal histopathological slides for assessment, incomplete clinical data, cases with histopathological morphology not typical for MPN and cases with *BCR-ABL*-positive examination. Clinical data were obtained from medical records and histopathological forms, including age, sex, presence of splenomegaly, routine blood tests (hemoglobin, hematocrit,

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leukocytes, and platelets), and examination of the *Janus Kinase 2 (JAK2) V617F* genetic mutation.

Histopathological form and slides were collected, then the slides were reassessed to obtain histopathological characteristic data. There were 206 cases of PMF, PV, and ET. There were 9 cases with *BCR-ABL1*-positive and there were 9 cases with unavailable slides. After reassessing the slides, 16 cases were found with features that were not typical for MPN. Histopathological preparations (172 cases) were divided into 3 WHO classifications namely PV, PMF, and ET. Assessment of histopathological characteristics was assessed based on cellularity, myeloid erythroid ratio, arrangement, cluster size and morphology of megakaryocytes, the presence of endosteal translocation, maturation defects, increased blast cells, degree of fibrosis, osteosclerosis and sinus dilatation. Data analysis in this study used SPSS 25.0. Numerical data is presented in the form of an average if the data is normally distributed. Categorical data is presented in the form of numbers (percentages). Qualitative data were analyzed by independent sample t-test, while categorical data were analyzed by chi-square test or fisher's exact test if the Chi-Square test requirements were not fulfiled. The difference is considered significant if the p-value <0.05.

RESULTS

During 2015-2019, 172 cases of *BCR-ABL1*-negative MPN were found with representative slides and data. Clinical and histopathological characteristics data of patients were divided into 3 groups: PV (42 cases), PMF (74 cases), and ET (56 cases).

Clinical Characteristics

Clinical characteristics data of patients including age, sex, presence of splenomegaly, routine blood tests (hemoglobin, hematocrit, leukocytes, platelets) and examination for genetic mutations (*JAK2 V617F*) are described in Table 1. The age range in this study was 19-81 years with an average age of 50 years (PV), 54 years (PMF), and 50 years (ET). From the sex data, there were 27 men and 15 women (PV), 40 men and 34 women (PMF), 26 men and 30 women (ET). The age and sex data did not have statistically significant differences in this study. Out of 172 cases of *BCR-ABL1*-negative MPN, only 104 cases could be tested for JAK2 mutations. As many as 84.8% of PV cases, 67.9% of PMF cases and 46.5% of ET cases had *JAK2* mutations.

Routine blood tests showed increased hemoglobin and hematocrit in PV (median=17.3 g/dL, p<0.001 and median=53%, p<0.001), decreased hemoglobin in PMF (median=9 g/dL, p<0.001) and increased platelets in ET (mean=1022x10^3μL, p<0.001). Splenomegaly was most commonly found in PMF (51.4% of PMF cases).

Table 1. Clinical characteristics data of PV, PMF, and ET.

Characteristics	PV (n=42)	p value	PMF (n=74)	p value	ET (n=56)	p value	Total (n=172)
Mean age (year)	50±13	0.414	54±12.3	0.037	49.9±15.4	0.336	51.7±13.6
Sex (n,%)		0.155		0.155		0.192	
Man	27 (64.3)		40 (54.1)		26 (46.4)		93 (54.1)
Woman	15 (35.7)		34 (45.9)		30 (53.6)		79 (45.9)
Splenomegaly (n,%)	2 (4.8)	<0.001	38 (51.4)	<0.001	4 (7.1)	<0.001	44 (25.6)
JAK2 positive (n,%)	28/33 (84.8)	<0.001	19/28 (67.9)	<0.001	20/43 (46.5)	0.001	67/104 (64.4)
Routine blood test							
Hematocrit (%)	53 (26.4-64.8)	<0.001	30.4 (17.3-47.5)	<0.001	38.7 (12.6-47)	0.164	39.3 (12.6-173)
Hemoglobin (g/dL)	17.3 (9.2-19.9)	<0.001	9 (5.2-15)	<0.001	14 (7.1-15.7)	0.731	12 (5.2-22.3)
Leukocytes (10^3/uL)	17.6 (4.9-47.6)	0.845	17.6 (7-77.3)	0.719	12.9 (3.7-44)	0.024	14.8 (7-77.3)
Platelets (10^3/uL)	696 (137-2146)	0.876	316 (12-2168)	<0.001	1022 (451-2743)	<0.001	735 (12-2743)

Abbreviations: PV: *Polycythaemia Vera*; PMF: *Primary Myelofibrosis*; ET: *Essential Thrombocythaemia*.

Histopathological Characteristics

The average length of bone marrow biopsy preparations in this study was 0.9 cm. Increased cellularity of bone marrow biopsies was found in all cases. But only in the PMF and ET cases which have statistically significant values. The myeloid erythroid ratio was mostly decreased in PV, normal in ET, and increased in PMF (p<0.001). Loose cluster arrangement of megakaryocytes was found in 88.1% of PV cases and 96.4% of ET cases, whereas dense clusters were found in 91.9% of PMF cases. Most cases of PV, PMF, and ET show small clusters of megakaryocytes. Large clusters of

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megakaryocytes were found in 16.7% of PV cases, 28.4% of PMF cases, and 16.1% of ET cases. A "bulbous/cloud-like" appearance and a maturation defect in megakaryocytes were found in 97.3% of PMF cases, whereas "staghorn-like" appearance was found in all ET cases. Endosteal translocation was found in 4.8% of PV cases, 17.6% of PMF cases, and 14.3% of ET cases. Most PV cases (90.5%) and all ET cases showed pre-fibrotic bone marrow (grade 0-1), whereas most PMF cases (77%) showed grade 2-3 fibrosis. Osteosclerosis was found in 37.8% of PMF cases and 1 case of PV. Sinus dilatation was found in 47.3% of PMF cases and 4.8% of PV cases. The results of histopathological characteristic assessment are presented in Table 2.

Table 2. Histopathological characteristics data of PV, PMF, and ET.

Characteristics	PV (n=42)	p value	PMF (n=74)	p value	ET (n=56)	p value	Total (n=172)
Cellularity (n,%)		0.203		<0.001		<0.001	
Not increased	3 (7.1)		1 (1.4)		19 (33.9)		23 (13.4)
Increased	39 (92.9)		73 (98.6)		37 (66.1)		149 (86.6)
Myeloid:erythroid ratio (n,%)		<0.001		<0.001		<0.001	
Decreased	34 (81)		2 (2.7)		4 (7.1)		40 (23.3)
Normal	1 (2.4)		11 (14.9)		31 (55.4)		43 (25)
Increased	7(16.6)		61 (82.4)		21 (37.5)		89 (51.7)
Megakaryocyte cluster (n,%)		<0.001		<0.001		<0.001	
Loose	37 (88.1)		5 (6.8)		54 (96.4)		96 (55.8)
No cluster	0 (0)		1 (1.4)		2 (3.6)		3 (1.7)
Dense	5 (11.9)		68 (91.9)		0 (0)		73 (42.4)
Megakaryocyte cluster size (n,%)		0.379		0.057		0.228	
Small cluster	35 (83.3)		53 (71.6)		47 (83.9)		135 (78.5)
Big cluster	7 (16.7)		21 (28.4)		9 (16.1)		37 (21.5)
Megakaryocyte morphology (n,%)							
Staghorn-like	0 (0)	<0.001	2 (2.7)	<0.001	56 (100)	<0.001	58 (33.7)
Bulbous/cloud-like	3 (7.1)	<0.001	72 (97.3)	<0.001	4 (7.1)	<0.001	79 (45.9)
Maturation defect (n,%)	3 (7.1)	<0.001	72 (97.3)	<0.001	4 (7.1)	<0.001	79 (45.9)
Endosteal translocation (n,%)	2 (4.8)	0.059	13 (17.6)	0.160	8 (14.3)	0.807	23 (13.4)
Blast cells (n,%)		1.000		0.501		0.720	
Not increased	40 (95.2)		69 (93.2)		54 (96.4)		163 (94.8)
Slightly increased	2 (4.8)		5 (6.8)		2 (3.6)		9 (5.2)
Degree of fibrosis (n,%)		<0.001		<0.001		<0.001	
0-1	38 (90.5)		17 (23)		56 (100)		111 (64.5)
2-3	4 (9.5)		57 (77)		0 (0)		61 (35.5)
Osteosclerosis (n,%)	1 (2.4)	0.004	28 (37.8)	<0.001	0 (0)	<0.001	29 (16.9)
Sinus dilatation (n,%)	2 (4.8)	0.002	35 (47.3)	<0.001	0 (0)	<0.001	37 (21.5)

Abbreviations: PV: *Polycythaemia Vera*; PMF: *Primary Myelofibrosis*; ET: *Essential Thrombocythaemia*.

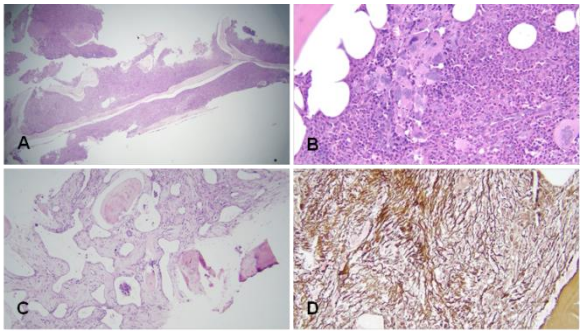

Figure 1. Histopathological features of PMF. (A) Hypercellular bone marrow (H&E, 40 times). (B) Megakaryocytes with dense cluster arrangement, large clusters, cloud-like/bulbous appearance and maturation defects (H&E, 400 times). (C) Osteosclerosis and sinus dilatation (H&E 40 times). (D) Increased reticulin fibers equivalent to degree 2-3 fibrosis (Reticulin, 400 times).

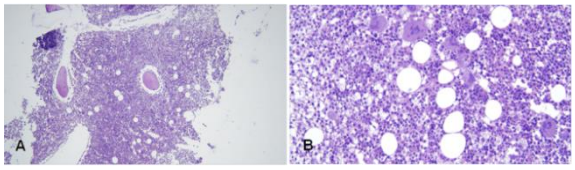

Figure 2. Histopathological features of PV. (A) Hypercellular bone marrow (H&E, 100 times). (B) Megakaryocytes with loose cluster arrangement, small clusters and megakaryocyte morphology with hypersegmented nuclei (H&E, 400 times).

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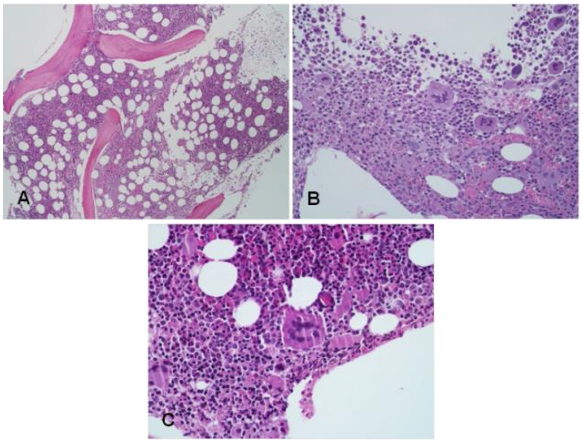

Figure 3. Histopathological features of ET. (A) Cellularity of ET (H&E, 100 times). (B) Megakaryocytes with loose cluster arrangement (H&E, 400 times). (C) Megakaryocytes with staghorn-like appearance (H&E, 400 times).

DISCUSSION

Myeloproliferative neoplasm (MPN) is a haematological neoplasm that phenotypically shows overproduction of myeloid cells and is a chronic disease lasting many years. MPN *BCR-ABL1* negative indicated by the presence of mutations in the *JAK2* gene. *JAK2* mutations are non-specific and cannot be used to differentiate one MPN subtype from another. Therefore, apart from genetic mutations, clinical and morphological correlates of bone marrow biopsies are also required.[3.16] Based on archival data from the Department of Anatomical Pathology FMUI-RSCM there were 172 cases of *BCR-ABL1* negative MPN during the study period (January 2015-December 2019). Of all the *BCR-ABL1*-negative MPNs, PMF was the most common subtype (43%). Likewise, in a study conducted by Dixith et al,[17] the incidence of PMF was higher than other MPN subtypes. According to the results of a study by Spivak et al,[10] PV is the most common subtype of MPN, with the most common *JAK2* mutation rate and the highest incidence of thromboembolic complications. The incidence of PV in our study was not high because not all PV cases were biopsied at Cipto Mangunkusumo Hospital. PV may present with isolated thrombocytosis, isolated leukocytosis, or myelofibrosis. Therefore, PV is an MPN with the highest morbidity and mortality due to the high incidence of thrombosis.[10]

Clinical characteristics observed in this study included age, sex, presence of splenomegaly, routine blood tests (hemoglobin, hematocrit, leukocytes and platelets) and *JAK2* mutation examination. In this study the incidence of *BCR-ABL1* negative MPN was found in the age range of 19-81 years, with an average age of 50 years (PV), 54 years (PMF), and 50 years (ET). PV is more common in males, with a male to female ratio of 1.8:1. PMF is more common in men (male to female ratio 1.2:1). ET is more common in women with a male to female ratio of 1:1.2. Age and gender data did not have statistically significant value in this study. Based on some literature, the incidence of PV, PMF and ET was found more in male patients than in female patients.[4,16,17] In other literature, ET is most frequently found in patients aged 50-60 years with a female predilection. Although rare, ET can also be found in children.[1,18] PV and PMF are most frequently found in the 6th to 7th decades of life.[1,14]

In this study, JAK2 mutation examination could only be performed in 104 cases. *JAK2* mutations are more common in PV cases and male sex. A total of 84.8% of PV cases, 67.9% of PMF cases and 46.5% of ET cases found *JAK2* mutations. The mutation rate has statistically significant value in this study. According to WHO 2017 and other literature, *JAK2 V617F* mutations are found in >90% of PV cases, 50-65% of PMF cases and 50-70% of ET cases.[1,3,4,13,16-19] *JAK2* is part of a functioning tyrosine kinase as a cytokine receptor, mediates signal transduction for immune response, cell growth and differentiation as well as hematopoiesis.[11,20,21] PV is the main phenotypic expression of *JAK2 V617F* because all hematopoietic stem cells (HSC) and their derivatives use tyrosine kinase as receptors for hematopoietic developmental factors. In contrast to *JAK2 V617F* which can cause ET or PMF, mutations in *JAK2 exon 12* can only cause PV and often only cause erythrocytosis. Apart from *JAK2* mutations, calreticulin mutations (CALR) and myeloproliferative leukemia (MPL) mutations can also cause MPN, but *CALR* mutations are rarely found in PV and *MPL* mutations are not found in PV. We cannot examine *CALR* and *MPL* mutations because of the testing facilities are not available.

Routine blood tests in this study showed increased hemoglobin and hematocrit in PV (median=17.3 g/dL, $p<0.001$ and median=53%,

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p<0.001), decreased Hb in PMF (median=9 g/dl, p<0.001) and increased platelets in ET (mean=1022x103μL, p<0.001). In routine blood tests of PV patients, increased hemoglobin levels (>16.5 g/dl in men; >16 g/dl in women) and increased hematocrit (>49% in men; >48% in women). [1,3,18,22] Clinically PMF shows anemia, thrombocytosis, leukocytosis ≥11x109 or increased lactate dehydrogenase (LDH).[1,14,23] In ET, there was an increased number of platelets (≥450 times10^9/L) in the peripheral blood and an increased number of large mature megakaryocytes in the bone marrow.[1,22] Splenomegaly was mostly found in PMF (51.4% of PMF cases), similar to the study conducted by Dixith et al.[17] In the study conducted by Lin et al,[4] and Soyer et al,[18] splenomegaly was more common in PV.

Previous studies have demonstrated the relative incidence of different morphological features of bone marrow biopsies in *BCR-ABL1*-negative MPN subtypes based on WHO. The incidence rates are listed in Table 3.[1] The magnitude of interobserver variability in the morphological assessment of bone marrow biopsies, then the morphological assessment of granulopoiesis, erythropoiesis and megakaryopoiesis are better seen in peripheral blood smear preparations, and other additional examinations are needed in assessing some of the diagnostic criteria for MPN *BCR-ABL1*-negative and the absence of a specific standard in assessing megakaryocyte size, are the reasons not all histopathological characteristics were assessed in this study. In this study, the histopathological characteristics of bone marrow biopsies were only assessed based on cellularity, myeloid and erythroid ratio, megakaryocyte clusters, megakaryocyte nuclear morphology, increased blast cells, degree of fibrosis and sinus dilatation.

Increased bone marrow cellularity was found in all cases, but only in cases of PMF and ET which had statistical significance. The study by Dixith et al,[17] showed an increase in bone marrow cellularity in all cases of PV, PMF and ET. According to some other literature, a bone marrow biopsy in ET shows normal cellularity in most cases, but in a small number of cases it can also show hypercellular bone marrow.[1,2,14]

The myeloid erythroid ratio was mostly decreased in PV, normal in ET, and increased in PMF (p<0.001). The results of this study are in accordance with the literature and other studies.[1,17] Panmyelosis was found in the histopathological morphology of the PV bone marrow, especially an increase in the number of erythroid and megakaryocytes. In PMF, an increase in the myeloid and erythroid ratio was found. The myeloid and erythroid ratio in ET was normal.[1,2,11,14,17] In some cases, histochemistryl or immunohistochemistry staining is required to differentiate myeloid and erythroid.

Loose cluster arrangement of megakaryocytes was found in 88.1% of PV cases and 96.4% of ET cases, whereas dense clusters were found in 91.9% of PMF cases. This is in accordance with the literature. Megakaryocytes in PV often form loose cluster arrangement, located at the margins of bony trabeculae and often show marked pleomorphism and vary in size. Likewise with ET, megakaryocytes are mostly found with a loose cluster arrangement. In PMF, megakaryocytes were found to form dense clusters with usually enlarged sizes and showed an increased nuclear cytoplasmic ratio.[1,2,14] Most of PV, PMF, and ET cases in this study showed small megakaryocyte clusters, that is around 3 to 7 megakaryocytes in one cluster, whereas large clusters of megakaryocytes are more commonly found in PMF cases. According to studies conducted by Thiele J and Kvasnicka HM, large clusters of megakaryocytes are found in 20-49% of PMF cases.[1]

In this study, a bulbous/cloud-like appearance and a maturation defect in megakaryocytes were found in 97.3% of PMF cases and a staghorn-like appearance was found in all ET cases. According to the literature, megakaryocytes in PMF give a hypolobated/bulbous/cloud-like/balloon-shape appearance, whereas in ET megakaryocytes show a hypersegmented/deeply lobated (staghorn-like) nucleus. In PV cases in this study, megakaryocytes tended to be normal to hypersegmented nuclei, and there were 3 cases that had a cloud-like appearance. According to the literature, most megakaryocytes in PV show normal to hypersegmented nuclei with minimal atypia, but in some cases megakaryocytes can also give a bulbous/atypical nucleus appearance when associated with increased reticulin fibers.[1,2,17]

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Table 3. The relative incidence of different morphological features of bone marrow biopsy according to WHO. Modified and adapted from Thiele J. and Kvasnicka HM.[1]

Bone Marrow Morphology Features		Relative Frequency of Morphological Features			
		PV (%)	ET (%)	Pre-PMF (%)	Overt PMF (%)
Cellularity	Age-related increase	>80	10-19	>80	10-19
Granulopoiesis	Increase in quantity	>80	<10	50-80	0
	Left-shifted	<10	<10	20-49	10-19
Erythropoiesis	Increase in quantity	>80	<10	<10	0
	Left-shifted	>80	<10	10-19	<10
Megakaryopoiesis	Increase in quantity	50-80	50-80	50-80	20-49
Cell size	Small	20-49	<5	20-49	20-49
	Medium	20-49	10-19	10-19	10-19
	Large	20-49	20-49	20-49	10-19
	Giant	10-19	20-49	10-19	<10
Histotopography	Endosteal Translocation	10-19	10-19	20-49	20-49
Cluster formation	Small cluster (>3)	10-19	10-19	50-80	50-80
	Large cluster (>7)	<10	0	20-49	20-49
	Dense cluster	<10	<5	20-49	50-80
	Loose cluster	20-49	20-49	50-80	10-19
Nuclear features	Hypolobulation (bulbous)	10-19	<10	50-80	50-80
	Hyperlobulation (staghorn-like)	50-80	50-80	<10	0
	Maturation defects	0	0	50-80	>80
	Naked nuclei	20-49	20-49	50-80	>80
Fibrosis	Increased reticulin	10-19	<5	20-49	>80
	Increased collagen	0	0	0	50-80
	Osteosclerosis	0	0	0	20-49
Stroma	Iron deposits	0	20-49	10-19	<10
	Lymphoid nodules	10-19	<5	10-19	<10

The morphology features of bone marrow must be distinguished between ET, pre-fibrotic PMF and myelodysplastic syndrome (MDS) because of the differences in outcomes and therapy.[24] In ET, a significant increase in bone marrow cellularity is usually not found. In pre-fibrotic PMF, megakaryocytes with atypical/bulbous nuclei appear and form dense clusters. When megakaryocytes are found to be small then the possibility of an MDS or MDS/MPN should be considered.[2]

Most PV cases (90.5%) and all ET cases showed pre-fibrotic bone marrow (grade 0-1), whereas most PMF cases (77%) showed grade 2-3 fibrosis. Based on the literature, the reticulin fibers in ET are usually normal or mildly increased (grade 1 fibrosis). In PMF, increased reticulin fibers are associated with disease progression. Pre-fibrotic PMF shows degree 1-2 bone marrow fibrosis, while overt PMF shows degree 2-3 fibrosis. In dense bone marrow collagen fibrosis, a decrease in the number of hematopoietic cells is seen.[1,2] Osteosclerosis in this study was found in 37.8% of PMF cases. Osteosclerosis is more common in PMF than other types of MPN. Osteosclerosis in PMF patients is a complication that can cause bone marrow failure.[2]

In addition to the types of MPN previously described, there is one entity, namely Myelo-proliferative neoplasm, unclassifiable (MPNU). MPNU is a neoplasm with clinical, laboratory, molecular and bone marrow morphology features suggesting MPN but does not meet the criteria for a specific entity type of MPN or the presence of overlapping features between two or more types of MPN. There are 10-15% of MPNU cases of all MPNs. The clinical features in MPNU are very diverse. Splenomegaly may not be found until massive splenomegaly. Hematological values vary greatly from mild leukocytosis to thrombocytosis. The presence of *BCR-ABL1* gene fusion excludes the diagnosis of MPNU.[1]

CONCLUSION

In conditions where examination of genetic mutations is not always accessible, a correlation between clinical data, laboratory data, and histomorphological evaluation of the bone marrow is required. Evaluation of bone marrow morphology that is important to assess in PV is panmyelosis, decreased myeloid: erythroid ratio, loose cluster arrangement of megakaryocytes and degree of fibrosis. Evaluation of bone marrow morphology that is important to assess in PMF is increased bone marrow cellularity, increased

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myeloid: erythroid ratio, dense cluster arrangement of megakaryocytes with bulbous/cloud-like nuclei and presence of maturation defects, degree of fibrosis, presence of osteosclerosis, and sinus dilatation. Evaluation of bone marrow morphology that is important to assess in ET is bone marrow cellularity, normal myeloid:erythroid ratio, loose clusters of megakaryocytes with staghorn-like nuclei, and degree of fibrosis 0-1.

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