WHO 2016 Prefibrotic Myelofibrosis in the Patients with WHO 2008 Essential Thrombocythemia

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ABSTRACT

According to newly defined features of bone marrow (BM) histology, some of the patients who were previously diagnosed with essential thrombocythemia (ET) were accepted as early/prefibrotic PMF in recent publications. The aim of this study was to explore actual rate of pre-PMF according to the 2016 revised WHO criteria. Demographic characteristics of 160 patients diagnosed with ET between 2000-2017; laboratory values; cytogenetic profile; the treatments using; disease-related thromboembolic complications; progression of the disease to MF and AML; mortality rates and cause were recorded retrospectively. The diagnosis of pre-PMF or ET was confirmed by BM morphology and clinical follow-up. BM biopsy samples obtained during the initial diagnosis were available in 107 cases. 53 cases with inaccessible BM biopsies were excluded from the study. The distribution of female/male in cases of ET was 46/28. The incidence of progression to AML was higher in pre-PMF patients. (Progression to AML: PMF 15.15%; ET 4.05%; p= 0.044). The mean duration of progression-free survival in patients with progression to AML or PMF in pre-PMF patients was 52.1±7.37 months; ET was 62.44±10.20 months. Approximately 30% of patients previously diagnosed with ET consisted of pre-PMF patients. Anemia, high LDH level, and splenomegaly are parameters that can be used in the differential diagnosis of PMF.

Keywords: Essential thrombocythemia, Primary myelofibrosis, Anemia, Splenomegaly, Progression, Bone marrow

INTRODUCTION

World Health Organization (WHO) revised the classification system for tumors of the hematopoietic and lymphoid tissues in 2016.[1] The current classification mainly focuses on the myeloproliferative neoplasms (MPNs) and their subgroups. A novel description, early/prefibrotic and overt primary myelofibrosis were introduced as subtypes of primary myelofibrosis (PMF).[2] According to newly defined features of bone marrow (BM) histology, some of the patients who were previously diagnosed with essential thrombocythemia (ET) were accepted as early/prefibrotic PMF in recent publications.[3-6] Diagnostic differentiation between ET and pre-PMF is not only important to distinguish characteristic morphological BM features of two distinct entity but it is also important by the different clinical behavior.[7,8] Although, the incidence of major thrombosis is comparable in both ET and pre-PMF, Pre-PMF has worse prognosis in terms of evolution to PMF, blast crisis and mortality.[7]

Increased platelet count could be detected in variety of diseases, but the accurate diagnosis is important in terms of both prognosis estimation and treatment. The presence of clonal markers, such as JAK2, CAL-R, and MPL, reliably excludes ET, polycythemia vera (PV), or PMF patients from reactive thrombocytosis and other myeloid neoplasms.[9-11] However, the key for distinguishing ET from the early stages of PMF (early/prefibrotic PMF) is detailed bone marrow (BM) histology, with focused assessment of megakaryocyte morphology and degree of myeloproliferation as defined in the current classification.[1]

In this study, we retrospectively re-evaluated our ET patients and aimed to explore actual rate of pre-PMF according to the 2016 revised WHO criteria. Also, we assessed the presence or absence of clonal markers (JAK2, CAL-R, and MPL), transformation to blastic phase and prognosis of both groups.

PATIENTS and METHODS

Institutional research ethics committees of this study were approved (Samsun Training& Research Hospital Ethical Board, GOKA/2020/5/5, February 26, 2020) and the written informed consent was obtained from all patients and/or first degree relatives in accordance with the Helsinki Declaration.

Demographic characteristics of 160 patients diagnosed with ET, who applied to Hematology Department of Health Sciences University Samsun Training and Research Hospital and Ondokuz Mayis University Faculty of Medicine between 2000-2017; laboratory values; cytogenetic profile; the treatments using; disease-related thromboembolic complications; progression of the disease to MF and AML; mortality rates and cause were recorded retrospectively. Study inclusion criteria include the presence of BM biopsies obtained at the time of diagnosis or one year after diagnosis.

Fifty-three cases in which bone marrow biopsies were not available were excluded from the study. Bone marrow biopsies available 107 cases; Ondokuz Mayis University, Faculty of Medicine, Department of Pathology; revised according to WHO 2016 criteria. Hematoxylin & eosin, Gomori reticuline, and Giemsa stained slides of initially diagnosed as ET were reexamined by two expert pathologists. All hematopoietic series, cellularity, megakaryocyte morphology, distribution, and reticular fibrosis were reassessed. Grading of bone marrow fibrosis was done according to the European Consensus scoring system accepted by WHO.[12]

According to the new diagnostic criteria, it was aimed to determine the number and clinical features of pre-PMF.

The diagnosis of pre-PMF or ET was confirmed by BM morphology and clinical follow-up. The BM biopsy samples were graded according to the European consensus scoring system accepted by WHO.

Patients received standard therapy consisting of low-dose aspirin and cytoreductive drugs such as hydroxyurea, anagrelide, and interferon-alpha (IFN-α), according to the instructions of the relevant institutions.

Statistical Analysis

SPSS 21.0 statistical software package (IBM, NY, USA) were used. Distribution was evaluated by the Kolmogorov-Smirnov test. Mean, standard deviation and percentage values were specified. Categorical variables between ET and PMF groups were compared with Chi-square, numerical variables were compared with Independent Sample T-test. Kaplan-Meier analysis was used for predictive survival estimation. Significant p value was accepted as < 0.05.

RESULTS

According to WHO diagnostic criteria published in 2008, 160 cases diagnosed with ET were analyzed retrospectively. BM biopsy samples obtained during the initial diagnosis were available in 107 cases. 53 cases with inaccessible BM biopsies were excluded from the study. According to the revised WHO criteria, 74 of the patients (69.2%) were ET; 33 (30.8%) were diagnosed with pre-PMF.

While hypercellularity was not observed in patients diagnosed with ET, megakaryocytes were large, hyperlobuled, and loosely arranged. Reticular fibrosis rarely increased up to grade 1 (Figure 1A-B). In the reexamination, the cases we included in

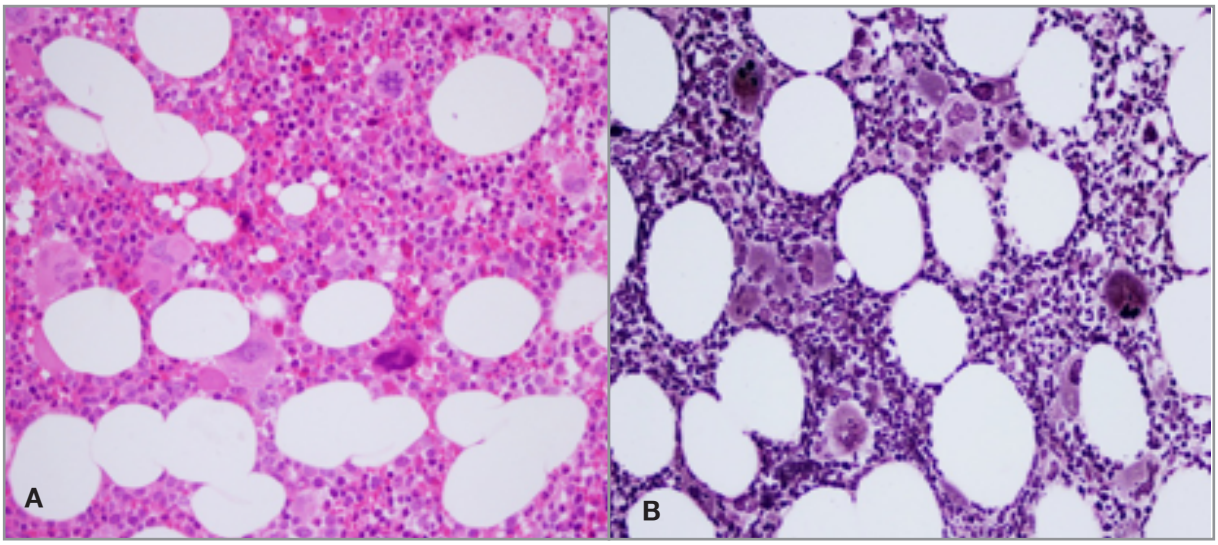

Figure 1 A, B. Morphological features of essential thrombocythemia and bone marrow fibrosis increased up to grade 1(1A: H&E, 400X, 1B: Gomori reticuline, 400X)

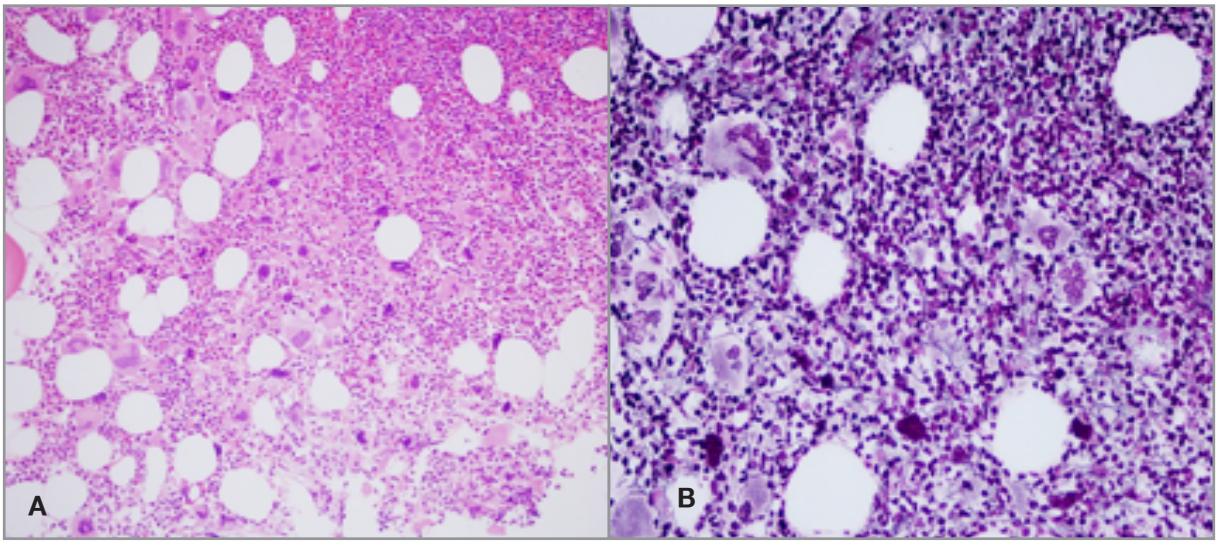

Figure 2 A, B. Morphological features of primary myelofibrosis and the grade 3 fibrosis in bone marrow (2A: H&E, 200X, 2B: Gomori reticuline, 400X)

the Pre-PMF group had hypercellularity, increased myeloid/erythroid ratio, increased reticular fibrosis up to grade 1.

Variable sized megakaryocytes are hypolobe and have increased nuclear-cytoplasmic rate and intense clusters. (Figure 2A, B) No case with blast increase was observed with CD34 staining.

The demographic characteristics of the patients are shown in Table 1.

The distribution of female/male in cases of ET was 46/28. The mean age was 55.58 ± 16.27. In the ET cases, 59.46% (44/74) JAK-2 positive; 5.41% (4/74) CAL-R positive; 2.70% (2/74) MPL positive; 13.51% (10/74) triple-negative were seen.

The distribution of female/male in pre-PMF cases was 18/15. The mean age was 59.00±16.24. In the PMF cases, 54.55% (18/33) JAK-2 positive; 3.03% (1/33) CAL-R positive; 6.06% (2/33) MPL positive; 12.12% (4/33) triple-negative were seen.

Table 1. Patient Characteristics

	n= 107	Mean±SD	Min-Max
Age		56.64±16.26	18-96
Gender	Female	64 (59.8%)	
	Male	43 (40.2%)	
Hb		12.75±2.29	5.3-17.00
WBC		10 800±4740	2 000-27530
PLT		998593±315758	899000-1922000
LDH		449.44±279.00	110-1933
Spleen Size		143.10±34.55	70-266
JAK2		62 (57.9%)	
MPL		4 (3.74%)	
CAL-R		5 (4.7%)	
Triple Negative		14 (13.6%)	
Arterial thrombosis risk		1 (0.9%)	
Venous thrombosis risk		20 (18.6%)	
ASA Usage		74 (69.2%)	
Progression to MF		11 (10.3%)	
Progression to AML		8 (7.5%)	

There was no significant difference between the mean age of ET and pre-PMF patients. (p= 0.317). While Hb and WBC values are within normal limits in the ET group (Hb: 13.12 ± 2.04, WBC: 10917.03 ± 5 026.07), the mean Hb in the pre-PMF group is 11.91 ± 2.61; WBC mean was seen as 10 542.42 ± 4 069.32 (p= 0.011 for Hb, p= 0.707 for WBC). In accordance with the literature, platelet counts did not differ in both groups (1028497.28 ± 366929.17 vs. 931536.36 ± 309875.50; p= 0.189) (Table 2).

There was no difference between the two groups in terms of ASA use (p= 0.324). According to the literature, pre-PMF patients had larger spleen sizes (137.82 ± 31.95 vs. 155.16 ± 37.67; p= 0.017). Serum LDH levels were higher than ET patients (396.62 ± 190.30 vs. 567.88 ± 393.51; p= 0.003). There was no significant difference in terms of the risk of arterial thrombosis (p= 0.502). The risk of venous thrombosis was higher in the pre-PMF group (p= 0.009) (Table 2).

The incidence of progression to AML was higher in pre-PMF patients. (Progression to AML: PMF 15.15%; ET 4.05%; p= 0.044). Progression rates to MF were similar between two groups (p= 0.268). The mean duration of progression-free survival in patients with progression to AML or PMF in pre-PMF patients was 52.1 ± 7.37 months; ET was 62.44 ± 10.20 months.

The causes of death of the patients were due to progression to AML in 5 cases and cerebrovascu-

Table 2. Comparison between ET and PMF patients

	ET (n= 74)	PMF (n= 33)	p
Age	55.58±16.27	59.00±16.24	0.317
Gender (F/M)	46/28	18/15	0.458
Hb	13.12±2.04	11.91±2.61	**0.011**
WBC	10917.03±5 026.07	10542.02±4 069.32	0.707
PLT	1028497.28±366 929.17	931536.36±309 875.50	0.189
LDH	396.62±190.30	567.88±393.51	**0.003**
Spleen Size	137.82±31.95	155.16±37.67	**0.017**
JAK2	44 (59.46%)	18 (54.55%)	0.634
MPL	2 (2.70%)	2 (6.06%)	0.286
CAL-R	4 (5.41%)	1 (3.03%)	0.708
Triple Negative	10 (13.51%)	4 (12.12%)	0.975
Arterial Thrombosis Risk	1 (1.35%)	0	0.502
Venous Thrombosis Risk	9 (12.16%)	11 (33.33%)	**0.009**
ASA Usage	49 (66.22%)	25 (75.76%)	0.324
Progression to MF	6 (8.11%)	5 (15.15%)	0.268
Progression to AML	3 (4.05%)	5 (15.15%)	**0.044**

lar events in 2 cases. Three of the patients whose progression to AML was observed according to the newly revised criteria were pre-PMF, two were diagnosed with ET.

DISCUSSION

As a result of this study, it was determined that Hb value was low, LDH levels were high, splenomegaly was seen more and the risk of venous thrombosis was increased in PMF patients. In addition, progress to AML was more common than ET patients.

The new edition of the 2016 World Health Organization (WHO) classification system for hematopoietic and lymphoid tissue tumors was published in September 2017.[2] In this review, myeloproliferative neoplasms (MPNs) were revised and divided into seven subgroups. These are chronic myeloid leukemia, chronic neutrophilic leukemia, PV, PMF, ET, chronic eosinophilic leukemia, and unclassifiable MPN (MPN-U). Moreover, according to this revised classification, myelofibrosis (MF) is divided into two categories. These are: Prefibrotic / early-stage fibrotic MF (PMF) and overt MF.[13]

For ET diagnosis according to WHO 2008 criteria and 2016 update; It was stipulated that a permanently increased platelet count was 450 x 10^9/L, no compliance with other myeloid cancer criteria, bone marrow morphology criteria, and the display of JAK2 V617F or other clonal markers. All 4 criteria in WHO 2008 are required for diagnosis in ET. Later, WHO updated the MPN criteria again in 2016. Accordingly, he added the condition of reticulin fibrosis to be grade one and lower and the CALR mutation, except for JAK2 V617F mutation, as the major criteria for bone marrow morphology. The absence of reactive thrombosis or the presence of a clonal marker remained as minor criteria.[14]

Bone marrow morphologies, especially ET, PV, and PMF can transform into each other, and may not be strictly differentiated. Reasons such as not considering the relationship with age in the evaluation of hypercellularity, and the lack of suitable biopsy sample may decrease the rate of reproducible diagnosis among pathologists. Pre-PMF may appear like an ET-like phenotype and progress to over-PMF 3. The reticulin fiber increase mentioned in PMF classification in 2008 was determined as grade 0-1 for pre-PMF and grade 2-3 for overt PMF in the 2016 revision. Conditions such as infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasms, metastatic malignancy, or bone marrow fibrosis secondary to toxic (chronic) myelopathies can cause an increase in fibrosis. It is important to consider all histomorphological findings together while evaluating these causes. In a patient who is considered to have myeloproliferative neoplasia, only the most appropriate diagnosis is made with the combination of clinical, laboratory findings, and molecular tests.

In clinical practice, in patients presenting with thrombocytosis, it was tried to evaluate whether laboratory or clinical parameters would help distinguish ET and PMF before performing a BM biopsy.[15-17] To identify cases of pre-PMF mimicking ET, hemoglobin (Hb), white blood cell (WBC) count, and serum lactate dehydrogenase (LDH) levels were used separately to generate the algorithm[3]. Also, the left shift (the presence of normoblasts or myelocytes, metamyelocytes, promyelocytes, or myeloblasts) in the peripheral smear was hypothetically tested. Splenomegaly is also included in this algorithm as a clinical parameter. As a result, 2016 revised WHO classification requires at least one of the clinical criteria for PMF criteria, anemia, leukocytosis, high LDH, and splenomegaly. A study in Japan found that high LDH levels and splenomegaly were associated with early / prefibrotic PMF patients.[5] In our study, pre-PMF patients were found to have low Hb value, high serum LDH levels, and significantly larger spleen size.

Molecular tests are needed to support the diagnosis in a patient who is considered to have ET or PMF with clinical and laboratory findings. JAK2 V617F mutation research is recommended first[18]. The JAK2V617F mutation is 50-60% positive in ET patients.[19] If the JAK2V617F mutation is negative and clinical findings indicate ET, it is recommended to investigate CAL-R, MPL mutations. Approximately 20-25% of patients diagnosed with ET have CAL-R gene mutation and 4-8% MPL gene mutation.[20,21] However, JAK-2 exon-12 (3-5%) mutation, which is detected in a small number of patients with PV, is not observed in ET cases.[19]

In addition, all three mutations are not seen in 20% of ET patients and this group is called "triple-negative".[22] The most common mutations in this group called 'triple-negative' are ASXL1, EZH2, TET2, IDH1 / IDH2, SRSF2, SF3B1 in the 2016 WHO classification.[1] Regarding MPNs, the 2016 WHO revised classification specifically focused on the diagnostic criteria of MPNs (PV, ET, and MF) associated with the JAK2 / CAL-R / MPL mutation. In this context, it is aimed to facilitate the distinction between JAK2 / CAL-R / MPL positive ET and PMF with the 2016 WHO revised criteria. In our study, it was found that there was no significant difference between the groups, although gene positivity rates were compatible with the literature.

In selected patients, PMF or other MPNs are likely to indicate a high risk of venous thrombosis, such as age > 60 years, general cardiovascular risk factors (hypertension, diabetes, smoking), leukocytosis, and JAK2V617F mutation.[23] When microvascular symptoms (e.g. erythromelalgia) occur, a low dose of aspirin (100 mg per day) may be considered, provided that the high risk of bleeding is excluded. In patients who have had a previous vascular event, low-dose ASA is recommended for venous thrombosis.[24] In our study, the risk of venous thrombosis was found to be significantly higher in PMF patients.

Compared to ET, survival is shorter, progress to MF, and conversion to the blastic phase are more common in pre-PMF.[15] Pre-PMF patients tend to have a significantly lower overall survival, a higher risk of evolution, and a higher risk of leukemic evolution to overcome myelofibrosis compared to ET patients.[4] In our cohort, there was no significant difference for progression to MF, whereas progression to AML was higher in the pre-PMF group. In addition, progression-free survival times were shorter in the pre-PMF group.

The study has some limitations. The first is that the study was obtained from retrospective data. In addition, low number of cases and biopsy metarials is another limitation.

Conclusion

In conclusion, approximately 30% of patients previously diagnosed with ET consisted of pre-PMF patients. Anemia, high LDH level, and splenomegaly are parameters that can be used in the differential diagnosis of PMF. Due to the difference in progression and survival times, differential diagnosis of ET and pre-PMF is very important and should be confirmed by experienced clinicians

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