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Original contribution

Utility of *JAK2* V617F allelic burden in distinguishing chronic myelomonocytic Leukemia from Primary myelofibrosis with monocytosis ☆

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Summary The concurrent presence of *JAK2* V617F, monocytosis, and bone marrow fibrosis can be observed in both chronic myelomonocytic leukemia (CMML) and primary myelofibrosis (PMF). It can be challenging to distinguish CMML with *JAK2* mutation and fibrosis from other myeloid neoplasms, particularly PMF. To identify key features that may help distinguish these 2 entities, we retrospectively studied 21 cases diagnosed as "CMML" with *JAK2* V617F and bone marrow fibrosis that were identified from a cohort of 610 cases of CMML diagnosed in 2006 to 2016. Upon further review, we confirmed the diagnosis of CMML in 7 cases, 11 cases were reclassified as PMF, and 3 cases had features intermediate between CMML and PMF (gray zone). These 11 cases of PMF with monocytosis featured a higher *JAK2* V617F allelic burden (median, 43%; range, 20%-62%) and atypical pleomorphic megakaryocytes with hyperchromatic nuclei. Complete blood count showed more pronounced myeloid left shift. In contrast, 7 CMML cases had significantly lower *JAK2* V617F allelic burden (median, 17%; range, 5%-36%; $P < .0001$) and dysplastic megakaryocytes along with variable degree of dysplasia in other lineages. The median survival of PMF and CMML patients was 32 and 40 months, respectively. We conclude that besides morphology of megakaryocytes and other features, *JAK2* V617F allelic burden can help differentiate CMML from PMF with monocytosis. *SRSF2* and *RAS* mutations are observed in both disease categories. Rare gray-zone cases exist with hybrid features.

1. Introduction

Monocytosis (peripheral blood absolute monocyte count $\geq 1 \times 10^9$/L) is the defining criterion of chronic myelomonocytic leukemia (CMML) [1,2]. However, monocytosis is a nonspecific finding also observed in patients with other types of myeloid neoplasms, both proliferative or dysplastic. A

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variable degree of monocytosis can be observed in advanced stage of chronic myeloid leukemia, polycythemia vera, essential thrombocytosis, or myelodysplastic syndrome (MDS). Monocytosis may occur during the cellular phase or fibrotic stage of primary myelofibrosis (PMF) [2-4]. The 2016 revision of the World Health Organization classification requires that CMML be diagnosed after exclusion of myeloproliferative neoplasms (MPNs) and 10% of monocytes in peripheral blood is added to the diagnostic criteria in addition to $\geq 1 \times 10^9$/L monocyte count. However, distinguishing between CMML versus MPN with monocytosis can be challenging, particularly in cases of PMF, as patients often present with anemia or even pancytopenia with atypical megakaryocytes [2,4]. In the prefibrotic stage of PMF, the typical features are not always evident. Conversely, CMML patients may show only mild dysplasia in the so-called proliferative subtype with splenomegaly. Furthermore, occasional ring sideroblasts and mild dyserythropoiesis also can be observed in some PMF cases, further complicating the differential diagnosis [5].

JAK2 V617F mutation has been identified in MPNs, MDS/MPNs, and rarely MDS [5-11]. Most cases of MDS/MPN with *JAK2* mutation can be categorized as refractory anemia with ring sideroblasts and thrombocytosis [12-14]. Two large studies that focused mainly on patients with MPN or MPN/MDS also reported *JAK2* V617F mutation in CMML, but no further details are provided about the clinicopathological features of these cases [9,15]. The reported frequency of *JAK2* V617F mutation in CMML is 5% to 10% [11,16,17]. CMML with *JAK2* mutations has been described as having more proliferative features [18]. However, the correlation of allelic burden of the *JAK2* V617F with CMML morphologic features and myelofibrosis, as described in a subset of CMML cases with an inferior outcome [19], is unknown.

Anecdotally, we have encountered cases where a patient presented initially with monocytosis and other features that met the criteria for CMML, but follow-up showed features more consistent with PMF. Conversely, we have encountered patients who were diagnosed initially as having PMF but subsequently developed profound monocytosis mimicking CMML. In this study, we focused on this differential diagnosis and systemically assessed the potential overlap between CMML and PMF to determine features that are helpful in this differential diagnosis.

2. Materials and methods

2.1. Case selection

We searched our archives for cases diagnosed as CMML from January 1, 2006, to December 31, 2016. Those cases with concurrent *JAK2*V617F mutation, with or without fibrosis, were selected and reviewed. Cases of typical MPN such as polythythemia vera and essential thrombocytosis were excluded. Pathology of each case was longitudinally reviewed, in conjunction with the clinical characteristics and laboratory data. The pathological materials including histologic slides, molecular studies, and cytogenetic results were retrospectively reviewed. Patients' clinical information including treatment regimens, disease progression, and follow-up data was collected. This study was approved by the institutional review board at our institution.

Megakaryocyte morphology and their distribution were assessed. We noted whether there were pleomorphic hyperlobated forms with bulbous nuclei ("cloud-like" or "balloon-shaped" morphology versus dysmorphic or small megakaryocytes). All cases met the criteria of monocytosis $\geq 1.0 \times 10^9$/L and $\geq$10% of white blood cells (WBCs). Peripheral blood findings such as presence of leukoerythroblastosis or marked left shift with >10% of intermediate myeloid elements were considered features more in keeping with PMF. Significant dysplasia in myelomonocytic and erythroid components supports a diagnosis of CMML.

2.2. Histopathologic examination and immunophenotypic analysis

Wright-Giemsa stains were performed on peripheral blood and bone marrow aspirate smears. All bone marrow biopsy specimens were fixed in formalin and paraffin embedded. Routine hematoxylin and eosin stains were performed on these biopsy sections. Reticulin and Trichrome stains were performed on the bone marrow core biopsies and retrospectively reevaluated. The degree of myelofibrosis was graded by using European myelofibrosis network grading criteria (MF 0-3) [7].

Immunohistochemical studies were performed using formalin-fixed, paraffin-embedded tissue sections according to standard protocols. The antibodies used included CD34 (1:40; BD Biosciences, San Jose, CA), CD61 (1:100; Cell Marque, Rocklin, CA), CD117 (1:100), myeloperoxidase (1:6000), and TdT (1:50; Dako, Carpinteria, CA).

2.3. Molecular studies

Molecular studies in our department have evolved significantly during the interval of 2006 to 2016. The molecular studies were performed in the peripheral blood and/or bone marrow aspirate samples. Originally, *JAK2* mutation analysis was performed by polymerase chain reaction (PCR) and a quantitative pyrosequencing method for the presence of a mutation at codon 617 of *JAK2*. PCR-based DNA sequencing was performed to examine codons 12, 13, and 61 of the *KRAS* and *NRAS* proto-oncogenes. Quantitative real-time reverse transcriptase PCR analysis was performed for the *BCR-ABL1* fusion transcripts e13a2 (b2a2), e14a2 (b3a2), and e1a2. In the recent years, next-generation sequencing (NGS)–based analysis was performed for the detection of somatic mutations in the coding sequence of 53 or 28 genes on the DNA extracted from bone marrow aspirate samples [20].

In 53 gene panel, mutations are assessed in the hotspot sequence of 53 target genes; in 28-gene panel, mutations are assessed in the exon sequence of 28 genes (details in Supplementary Tables 1 and 2). Variant allele frequency (VAF) is the relative frequency of the mutant allele and calculated as the fraction of all chromosomes in the population that carry that allele. Quantification of *JAK2*V617F VAF obtained by NGS had been previously validated against PCR and quantitative pyrosequencing. Splicing factor *SRSF2* mutation analysis was not included in the NGS panel and was retrospectively examined by PCR and Sanger sequencing. For comparison, we also reviewed the allelic burden of 70 consecutive patients diagnosed as having PMF who did not meet the criteria monocytosis $\geq 1.0 \times 10^9$/L and $\geq$10% of WBCs from our institution.

2.4. Cytogenetic studies

Conventional chromosomal analysis was performed on G-banded metaphase cells prepared from unstimulated bone marrow aspirate cultures (24 and 48 hours) using standard methods. A total of 20 metaphase cells were analyzed for conventional cytogenetic studies. The karyotype was reported according to the current International System for Human Cytogenetic Nomenclature [21]. Fluorescence in situ hybridization (FISH) for *BCR-ABL1* rearrangement was assessed in all 25 cases. FISH analysis was performed either in 20 metaphase cells or in 200 interphase cells.

2.5. Statistical analysis

The statistical software of GraphPad Prism 7 (GraphPad Software, San Diego, CA) was used to perform univariant survival analysis. Data for continuous variables are described as median and range, and categorical variable is described as the number of patients. Significance level was determined when the P value is <.05 for all analyses.

3. Results

3.1. Clinical characteristics

A total of 610 patients were diagnosed as having "CMML," initially at an outside hospital or at our institution in 2006 to 2016. Of these patients with CMML, 25 (4%) patients were found to carry *JAK2* mutation, variable myelofibrosis, and monocytosis $\geq 1.0 \times 10^9$/L; among them, 21 also met the threshold of $\geq$10% and were included in the study. One patient initially presented with 8% of monocytes but rose to 13% within 2 months. This case was considered to have presented at an early stage of disease and included in the study. There were 15 men and 6 women with a median age of 71 years (range, 60-80 years; Table 1). All 21 patients were diagnosed as having CMML at the outside hospitals and referred to our hospital for further evaluation and/or treatment. All patients

Table 1 Laboratory findings and clinical characteristics

	PMF (n = 11)	CMML (n = 7)	GZ (n = 3)
Age (y)	67 (60-80)	73 (62-80)	67 (65-68)
Sex (n)			
Female	3	2	1
Male	8	5	2
CBC			
WBC (K/μL)	22.8 (4.7-65.1)	28 (6-71.2)	70 (20-72)
Hb (g/dL)	12.2 (7.6-16)	10.3 (8.5-12.1)	9.2 (8.7-10.3)
Plt (K/μL)	261 (89-341)	105 (37-861)	42 (38-44)
Monocyte (%)	16 (8-27)	14 (12-20)	18 (17-26)
AMC (K/μL)	2.8 (1.2-4.3)	2.1 (1.8-5.1)	2.3 (1.8-18.2)
Blasts (%)	0-6	0-12	1-9
BM			
Cellularity (%)	85 (50-100)	80 (60-100)	95 (90-100)
Megakaryocyte morphology	MPN type	MDS type	MPN type mainly
Myeloid/erythroid dysplasia	Mild	Yes	Yes
Clinical characteristics			
LDH, median (range)	970 (572-3615)	802 (432-4064)	2594 (2045-6075)
Splenomegaly	10/11 patients	5/7 patients	3/3 patients
Dead at the last F/U	10/11 patients	6/7 patients	3/3 patients
OS (mo)	32 (3.5-109)	40 (9-74)	29 (22-52)

Abbreviations: AMC, absolute monocyte count; CBC, complete blood count; F/U, follow-up; Hb, hemoglobin; LDH, lactate dehydrogenase; OS, overall survival; Plt, platelet.

had an abnormal peripheral blood count. WBC ranged from 4.7 × 10^9/L to 72 × 10^9/L with documented leukocytosis in 19 (90%) of 21 patients. Assessment at our hospital found that 18 (86%) of 21 patients had elevated lactate dehydrogenase (median, 1052 U/L; range, 432-6075 IU/L; reference range, 313-618 IU/L) and 18 (86%) of 21 patients had documented splenomegaly by physical exam or imaging studies.

3.2. Histopathologic findings

The pathological materials including peripheral blood smears, histologic slides from the referring institutions, or in-house bone marrow biopsy/aspirate smears were extensively reviewed at our institution. Of these 21 cases with *JAK2* mutations, 11 cases had histologic features consistent with PMF, 7 cases were consistent with CMML, and 3 cases had features intermediate between CMML and PMF (gray zone; Table 1).

In the PMF group, 9 (82%) of 11 patients initially had anemia at presentation with level of hemoglobin ranging from 7.6 to 12.2 g/dL. Leukocyte count ranged from 4.7 to 65.1 × 10^9/L (median, 22.8 × 10^9/L), and 7 (64%) of 11 patients showed more pronounced myeloid left shift with variable number of nucleated red blood cells. Only 2 had documented thrombocytopenia. Circulating blasts were found in 6 (55%) of 11 patients, ranging from 1% to 6%. Review of the bone marrow biopsy revealed that 10 (91%) patients had hypercellular marrow, and all 11 cases had megakaryocytic hyperplasia with atypical morphology, including pleomorphic and hyperchromatic forms with focal clustering. Overall, no significant dysplasia was identified in the erythroid or myeloid lineages (Fig. 1A): the fibrosis was graded as MF-1 in 4, MF-2 in 5, and MF-3 in 2.

In the CMML group, peripheral blood smears of 6 (86%) of 7 patients had dysplastic features in granulocytes including hypogranular cytoplasm and pseudo–pulget-huet forms. Circulating blasts were present in 3 patients (1%, 5%, and 12%, respectively).

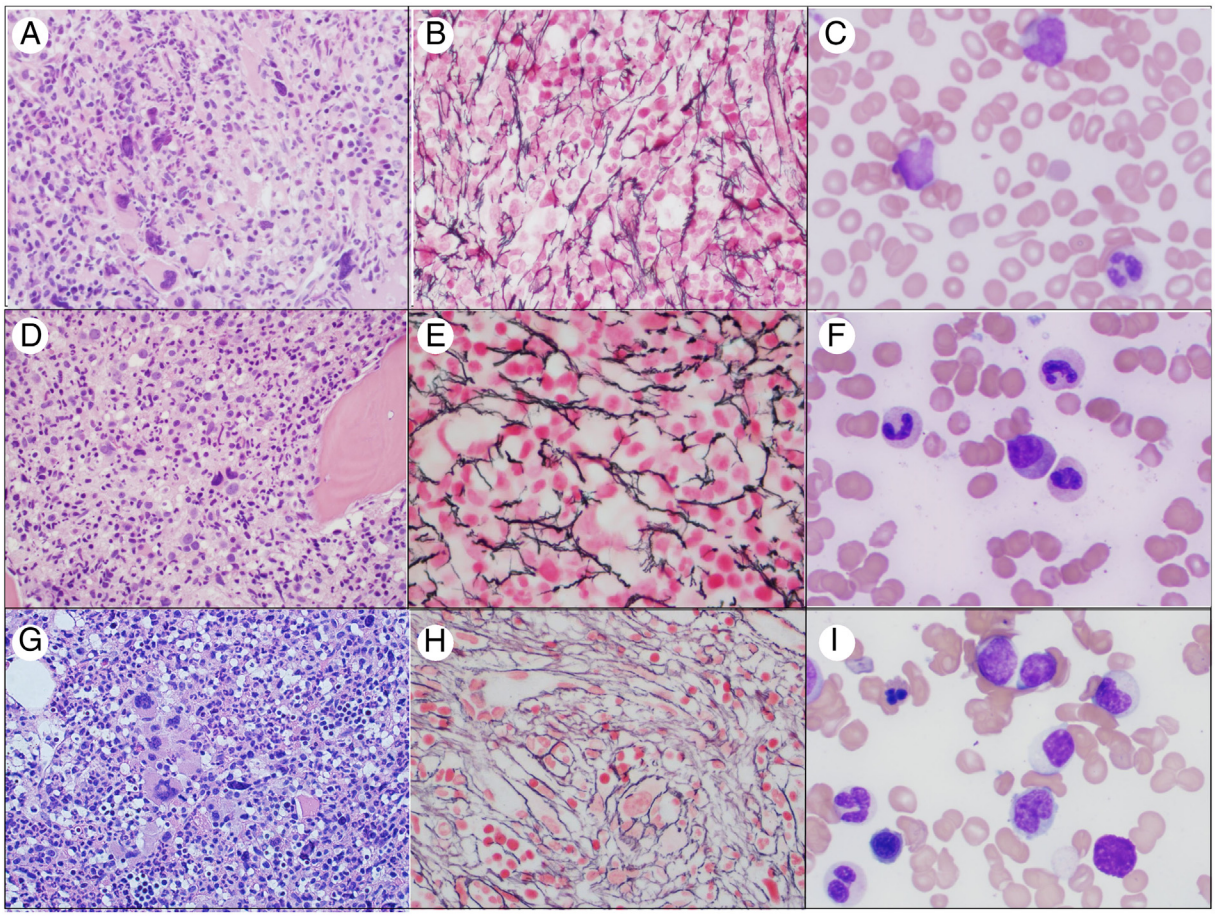

Fig. 1 The clinicopathological features in 3 groups (PMF, CMML, and gray zone). A-C, The histopathologic features in 1 PMF example. A, BM core biopsy from MF case 3 shows hypercellular marrow with increased pleomorphic megakaryocytes (hematoxylin and eosin stain, original magnification ×500). B, Reticulin stain shows dense increased reticutin fibrosis (×500). C, BM aspirate smear shows hemodiluted specimen (Wright-Giemsa stain, ×1000). D-F, CMML case 3. D, BM biopsy shows hypercellular marrow with dysplastic megakaryocytes, small hypolobated forms (hematoxylin and eosin stain, ×400). E, Reticulin stain shows increased reticulin fibrosis (×500). F, BM aspirate smear with dysplastic granulocytes (Wright-Giemsa stain, ×1000). G-I, Gray zone case 2. G, BM core biopsy shows hypercellular marrow with pleomorphic PMF-like megakaryocytes (hematoxylin and eosin stain, ×500). H, Reticulin stain shows dense reticulin fibrosis (×500). I, BM aspirate smear shows dysplastic erythroid and myeloid precursors (Wright-Giemsa stain, ×1000). BM, bone marrow.

Table 2 Molecular and cytogenetics findings

No.		Karyotype	Molecular findings	
PMF	*JAK2*V617F (%)			*SRSF2*
1	38	46,XY,del(20)(q11.2q13.3)[13]/46,XY[8]		c.284C>T p.P95H
2[a]	22	46,XY,del(4)(q21q34)[1]/46,XY[19]		NA
3	46	46,XX[20]		Wild type
4[b]	46	46,XX[20]	*NRAS*	NA
5	62	46,XY[20]		c.284C>T p.P95L.
6[c]	58	46,XY,del(13)(q12q14)[4]/46,XY[16]	*PTPN11*	c.284C>T p.P95H
7	41	46,XY[20]		NA
8[d]	20 → 43	46,XY[20]		NA
9[e]	43	46,XX,r(7)(p22q11.2)[20]	*IDH2, NRAS, BRAF*	NA
10	53	46,XY,dup(1)(q25q44)[9]		Wild type
11[f]	48	46,XX[20]	*ASXL1*	NA
CMML				
1	5	46,XY,add(7)(q36)[20]		Wild type
2[g]	6	46,XX[20]	*ASXL1, WT1*	c.284C>T p.P95R
3[h]	18	47,XY,+8[5]/46,XY[15]	*RUNX1,ASXL1, TET2*	Wild type
4	17	47,XY,+19[16]/48,XY,+8,+19[3]/46,XY[1]		c.284C>T p.P95H
5	18	45,X,−Y[19]		NA
6	14	45-48,XY,5q−,+19,add(19q)x2,+0−5mar[cp5]/46,XY[5]		Wild type
7	36	45,XX,t(6;8)(q27;q22),−7[20]		c.284C>T p.P95H
Gray zone				
1	47	46,XY,−6,del(7)(q22q34),+r[20]		Wild type
2	30	47,i(X)(p10),+13,del(13)(q12q14)x2[20]		c.284C>T p.P95H
3[i]	39 → 46	47,XY,+8[9]/45,XY,add(4)(q27),−12,add(17)(p11.2)[4]/46,XY[7]		NA

Abbreviation: NA, not available.

[a] *JAK2* V obtained after hydroxyurea V617 VAF treatment.

[b] *NRAS* c.35G>A p.G12D.

[c] PMF case 6: *PTPN11 c.*76A>C p.G76A (16%).

[d] *JAK2*V617F level increased from 20% to 43% at follow-up 6 months later.

[e] PMF case 9: *IDH2* c.419G>A p.R140Q, (43%) *NRAS* c.38G>A p.G13D (9.3%), *NRAS*, c.35G>A p.G12D (2.8%), *BRAF* c.1801A>G p.K601E (3%).

[f] PMF case 11: *ASXL1* c.2230_2231insCG p.G744 fs*29 (48%).

[g] CMML case 2: *ASXL*c.2355del p.R786fs*32; (41%) and *WT* c.1131_1137delins GAC p.L378 fs. (35%).

[h] CMML case 3: *ASXL1* c.1935dupT p.G646fs*11 (39.5%); *TET2* c.3023dupA p.Q1009fs*9 (47%*RUNX1c*.877_880dupTCTC p.P294fs*307 (20.8%); *RUNX1c*.443_444insTCAGAAATGCT AC p.A149fs*15 (14.1%).

[i] *JAK2*V617F level increased from 39% to 46% at follow-up 1 month later.

All patients had anemia; hemoglobin level ranged from 8.5 to 12.1 g/dL, with a median of 10.3 g/dL. The median platelet count was 105 K/μL. In all 7 patients, the bone marrow biopsy showed increased cellularity with dysplastic megakaryocytes morphologically distinct from those observed in PMF cases. Dysmegakaryopoiesis is more pronounced with many hypolobated or monolobated or hyperchromatic nuclei, but less pleomorphic than those seen in the PMF subgroup (Fig. 1D). Loose clusters of dysplastic megakaryocytes were occasionally seen. Fibrosis was graded as MF-1 in 4, MF-2 in 1, and MF-3 in 1 of 6 cases assessed. These cases were classified as CMML-0 in 2, CMML-1 in 3, and CMML 2 in 2 cases, respectively.

In the 3 gray-zone cases, the morphologic features were intermediate between PMF and CMML. Although the megakaryocytes were pleomorphic and hyperchromatic forms similar to those in PMF, the background myelomonocytic and erythroid components were significantly dysplastic (Fig. 1G). Notably, the level of left shift with circulating intermediate myeloid components was beyond usual for CMML. All 3 patients had circulating blasts (1%-9%), and fibrosis was graded as MF-1 in 1 and MF-2 in 2.

3.3. Cytogenetic findings

All 21 patients had available cytogenetic results (Table 2) and were negative for t(9;22) by conventional cytogenetics and FISH analysis. A normal karyotype was identified in 8 (38%) of 21 patients. The remaining 13 patients had an abnormal karyotype. There was no specific pattern, although the PMF group had 2 cases with deletion of chromosomes 13, whereas the CMML and gray-zone groups had 6 cases with abnormal chromosomes 7 or 8, including −7 and +8.

3.4. Molecular findings

JAK2 V617F mutation was identified in all 21 cases. Interestingly, in the PMF group with monocytosis, the median

allele burden of *JAK2* V617F was 43% in 11 patients (range, 22%-62%), significantly higher than the CMML group ($P < .0001$). All but 2 patients in the PMF group had a level <30%. One of these 2 patients had received hydroxyurea before the *JAK2* study. In the other patient, *JAK2* V617F allelic burden rose to 43% during follow-up 6 months later, indicating that the lower reading at the initial workup was likely due to patient being at an early stage of disease. The median allele burden was 17% (range, 5-36%) in the CMML group and 39% (range, 30%-47%) in the gray-zone group (Fig. 2).

Mutations of other genes were detected in 11 (73%) of 15 patients analyzed and included *RAS*, *PTPN11*, *IDH2*, *BRAF*, *ASXL1*, *WT1*, *RUNX1*, *TET2*, and *SRSF2*. Specifically, *SRSF2* mutation was detected in 7 (54%) of 13 patients' samples analyzed, including 3 PMF, 3 CMML, and 1 gray-zone cases, respectively (Table 2). *RAS* mutation was detected in 2 PMF patients at a low level, and *PTPN11* mutation was detected in another PMF patient at a low level. *ASXL1* mutation was detected in 1 PMF patient and 2 CMML patients. *TP53* gene mutation was not detected.

3.5. Treatment and prognosis

In the PMF group, 7 patients received hydroxyurea, with or without JAK2 inhibitor. The JAK2 inhibitor was administered in 4 patients in total, and among them, 2 were in combination with hypomethylating agents (HMAs). Three had thalidomide. One patient was treated with decitabine, clofarabine, and simtuxumab. One patient had splenctomy, and 1 patient was also treated with stem cell transplant. Two patients were observed only. Despite therapy, 10 (91%) of 11 patients died, with a median overall survival of 32 months (range, 3.5-109 months).

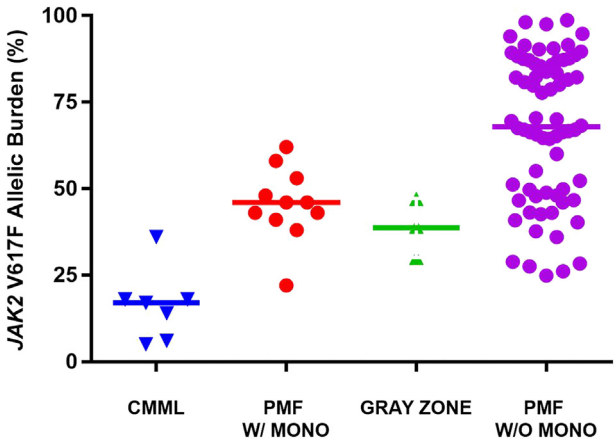

Fig. 2 Comparison of *JAK2* V617F allelic burdens in 3 study subgroups. There was significant difference of *JAK2* allelic burden between patients reclassified as PMF compared with the CMML patients ($P < .0001$). In the PMF group, the median VAF was 43% (range, 22%-62%). In the CMML group, the median VAF was 17% (range, 5%-36%). In the gray-zone group, the median VAF was 39% (range, 30%-47%). In the control group of 70 PMF patients without significant monocytosis, the median VAF was 67% (range, 25%-99%).

Only one patient was alive at 4.5-month follow-up. In the CMML group, 4 patients were treated with HMA, 2 with hydroxyurea in addition, and 1 with erythropoietin. One patient developed acute myeloid leukemia and was treated with Ara-C and clofarabine. Six patients died, with a median overall survival of 40 months (range, 9-74 months). Only 1 patient was still alive at follow-up of 48 months. All 3 gray-zone cases were treated with HMA and 1 also with rigosertib. All 3 patients died 22, 29, and 52 months after diagnosis, respectively.

4. Discussion

In this study, we retrospectively studied 21 cases with an initial pathology diagnosis of CMML with fibrosis and *JAK2* V617F mutation. The World Health Organization classification requires that the diagnosis of CMML be established by excluding other MPN with monocytosis. In reality, to distinguish CMML with *JAK2* V617F and fibrosis from other MPN with fibrosis can be challenging, even for experienced hematopathologists. The typical morphology of megakaryocytes in PMF is described as pleomorphic with altered nuclear-to-cytoplasmic ratio and abnormal patterns of chromatin clumping, the so-called cloud-like or balloon-shaped nuclei [2,7]. However, there are significant variations, with minimal atypia in the early stage of disease and loss of characteristic features after therapeutic intervention. At referral centers, the original diagnostic materials before therapy may not be always available. Although monocytosis has been described in patients with advanced PMF [4], our present study confirms that monocytes can be part of initial disease presentation, as reported in the literature, posing diagnostic challenge.

JAK2 V617F mutation is the most common genetic aberrations in PMF but is also found in a minority of CMML. The overall incidence of *JAK2* V617F mutation in CMML is around 5% to 10% [11,22,23]. Although all patients in this study shared common features of absolute monocytosis and *JAK2* mutation and splenomegaly was also seen in different subgroups, other features, however, indicate that they are heterogeneous. For patients with a high *JAK2* mutant burden, the morphologic features, particularly the megakaryocytes and clinical features, resemble those of PMF and should be classified as such, despite profound monocytosis.

To distinguish PMF from CMML has clinical implications because their management and prognosis differ. The natural evolution of PMF is more prone to bone marrow failure as a result of full-blown fibrosis than to an acute leukemia. For CMML patients, HMAs such as 5-azacitidine and decitabine are associated with an overall response rate of ~30% to 40%, with a complete remission rate of ~15% [22]. Allogeneic hematopoetic stem cell transplant remains the treatment of choice for young patients with higher-risk disease [1,16,17,22]. In contrast, for PMF patients, the National Comprehensive Cancer Network guidelines recommend that the goal of treatment is to reduce symptom burden and minimize the risk of leukemic transformation. The asymptomatic

patients with low-risk or intermediate-1–risk MF should be observed. Ruxolitinib or interferon is included as options for symptomatic patients [24-27]. Therefore, an accurate diagnosis is essential for patient management.

A review of 70 PMF patients without significant monocytosis in our institution revealed that only 5 (7%) patients had a *JAK2* V617F level below 40%, and none were below 25%. The allelic burdens of *JAK2* V617F of the PMF patients in our current study were comparable, ranging from 22% to 62% with a median of 43%. In contrast, the allelic burdens of CMML cases were significantly lower with a median of 17%, and none were above 40%. This feature may help distinguish PMF with monocytosis from CMML. Megakaryocyte morphology and distribution (clustering) are also key clues to the differential diagnosis. Although some CMML cases also showed loose clusters, the megakaryocyte morphology differed.

SRSF2 mutation is seen in a subset of CMML [16] as well as a subset of PMF patients. In PMF, *SRSF2* mutation is correlated with a worse clinical outcome than those without the mutation [28,29]. We identified *SRSF2* mutation across all 3 subgroups of studied patients, supporting that this mutation is not diagnostic of any particular disease category. Similarly, although point mutations of *RAS* gene are common in CMML, they are also seen in PMF and therefore not evidence to exclude a diagnosis of PMF [5,9,30]. In contrast to juvenile myelomonocytic leukemia, *PTPN11* mutations are distinctively uncommon in adult patients with CMML [31]. We also noted rare cases of *RAS* mutation in our study series, but they did not change the classification.

This study confirmed monocytosis and *JAK2* mutation in variants of MPN/MDS other than refractory anemia with ring sideroblasts and thrombocytosis. The 3 gray-zone cases where the morphologic features bordered on CMML and PMF resemble those recently reported by Chapman and colleagues [32]. The molecular findings of the gray-zone cases suggest that there can be a spectrum of morphology that are likely related to underlying molecular makeup of the disease. It is reasonable to presume that these gray-zone cases may have a borderline genetic or molecular profile between CMML and PMF. As mentioned above, *RAS*, *SRSF2*, and *ASXL1* mutations, though common in CMML, are also reported in PMF and thus not by themselves the ultimate criteria for CMML. Perhaps the relative allelic burden of these mutations may help explain the clinical variations. In our study, 1 case classified as PMF had *JAK2*V617 VAF of 43%, while the 2 *NRAS* comutations each had <10% of VAF. In contrast, in 1 case of CMML with *JAK2*V617 VAF of 18%, the VAFs of comutated *ASXL1* and *TET2* were 39.5% and 47.2%, respectively, suggesting that *JAK2*V617 was a subclone. Because most myeloid neoplasms are composed multiple subclones, the clinical and pathology features are variable depending on the relative predominance of the driver clone or clones. Consequently, designation of disease category should be more refined to reflect such variations.

Similar to the patients who had aggressive clinical behaviors in the study by Chapman et al. [32], all 3 gray-zone cases in our cohort died within 5 years of diagnosis. Unlike their study patients, all 3 of our gray-zone patients had a relatively high *JAK2* V617 allelic burden and an abnormal karyotype, which may have in part contributed to the hybrid features (PMF-like megakaryocytes plus myeloid/erythroid dysplasia) and aggressive course. We recommend designating these cases as MPN/MDS with monocytosis to distinguish them from the typical CMML with nonspecific fibrosis. Such a distinction would allow for a more tailored clinical therapy, such as combined JAK2 inhibitors and HMA, and for future studies aimed at better elucidation of their biology.

Overall, our study highlights the critical role of comprehensive assessment and incorporating *JAK2* V617F mutant allele frequency in distinguishing PMF from CMML with fibrosis. Leukoerythroblastosis, significant left shift, and absence of granulocytic dysplasia favor PMF. In the bone marrow,

Table 3 Comparison of PMF with monocytosis and CMML with *JAK2*V617F mutation

Features	PMF	CMML
Peripheral blood		
Leukocytosis	Yes	Yes
Left shift	Yes, more pronounced, leukoerythroblastosis	Yes, less, <10% of intermediate myeloid
Monocytosis	Variable, may <10%	≥10% usually
Cytopenia (anemia)	Yes	Yes
Thrombocytopenia	Less frequent	More frequent
Bone marrow		
Dysplasia of erythroid and myeloid lineages	Yes (mild, late stage)	Yes (can be mild in the proliferative type)
Fibrosis	Yes	Yes
Genes mutations	*RAS*, *ASXL1*, *TET2*, *CBL*, *SRSF2*, *SF3B1*	*RAS*, *ASXL1*, *TET2*, *SRSF2*, *TET2*, *RUNX1*
JAK2 V617F (Frequency)	50%	<10%
JAK2 VAF (median)	40%-50%	<25%, likely subclone
Clinical findings		
Hepatosplenomegaly	Yes	Yes
Prognosis	Unfavorable	Variable

Abbreviation: BM, bone marrow.

atypical pleomorphic megakaryocytes with hyperchromatic nuclei and clustering are other clues. Finally, molecular studies could provide confirmatory information. Here, a higher allele frequency is more compatible with a MPN clone, whereas a lower *JAK2* allelic burden suggests secondary genetic changes or even background clonal hematopoiesis, not a driver mutation that is more compatible with CMML. Table 3 summarizes comparison of PMF with monocytosis and CMML. Finally, recognition of gray-zone cases is essential for better clinical management.

In summary, a subset of cases designated as CMML that carry *JAK2* V617F mutation and are associated with marrow fibrosis is, in fact, PMF. Complete blood count, megakaryocyte morphology/distribution, and *JAK2* allelic frequency are useful for distinction in these difficult cases. However, the gray-zone cases with borderline features truly exist, and they most likely result from the dual impact of *JAK2* and other genetic hits.

CRediT authorship contribution statement

Zhihong Hu: Conceptualization, Data curation, Formal analysis, Writing - original draft. **Carlos E. Bueso Ramos:** Data curation, Formal analysis. **L. Jeffrey Medeiros:** Writing - review & editing. **Chong Zhao:** Data curation, Formal analysis. **C. Cameron Yin:** Writing - review & editing, Data curation, Formal analysis. **Shaoying Li:** Writing - review & editing. **Shimin Hu:** Writing - review & editing. **Wei Wang:** Writing - review & editing. **Beenu Thakral:** Data curation. **Jie Xu:** Writing - review & editing. **Srdan Verstovsek:** Data curation, Formal analysis. **Pei Lin:** Conceptualization, Data curation, Formal analysis, Writing - review & editing.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.10.026.

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