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ScienceDirect
Leukemia Research 31 (2007) 1031–1038
Leukemia Research
www.elsevier.com/locate/leukres

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WHO bone marrow features and European clinical, molecular, and pathological (ECMP) criteria for the diagnosis of myeloproliferative disorders

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Received 25 May 2006; received in revised form 11 January 2007; accepted 13 January 2007
Available online 23 March 2007

Abstract

The bone marrow criteria defined by the World Health Organization (WHO) are based on characteristic increase and clustering of morphologically abnormal enlarged megakaryocytes as a pathognomonic clue to describe three distinct phenotypic entities of myeloproliferative disorders (MPDs): (1) essential thrombocythemia (ET), (2) early and overt polycythemia vera (PV) and (3) prefibrotic, early fibrotic, and fibrotic chronic idiopathic myelofibrosis (CIMF-0, 1, 2 and 3). Based on established WHO bone marrow features, and the use of new molecular and laboratory markers including $JAK2^{V617F}$ mutation, endogenous erythroid colony (EEC) formation and serum erythropoietin (EPO), we present updated European clinical, molecular and pathological (ECMP) criteria for the differential diagnosis of true ET, PV and CIMF. As compared to the WHO bone marrow features, each of the laboratory and molecular markers are not sensitive enough for the diagnosis and classification of the three prefibrotic MPDs. The proposed WHO/ECMP criteria reduce the platelet count to the upper limit of normal ($>400 \times 10^9\,l^{-1}$) as inclusion criterion for the diagnosis of thrombocythemia in true ET, early stages of PV and prefibrotic CIMF. The combined use of WHO and ECMP criteria differentiate PV from congenital and acquired erythrocytosis, true ET from reactive thrombocytosis and separates true ET from CIMF-0/1 mimicking ET. Only half of the patients with true ET and CIMF carry the $JAK2^{V617F}$ mutation (sensitivity 50%). Early PV mimicking ET is featured by the presence of $JAK2^{V617F}$ mutation, EEC, low serum EPO levels, normal hematocrit, and increased bone marrow cellularity due to increased erythropoiesis ("forme fruste" PV) when WHO/ECMP criteria are applied. The combination of $JAK2^{V617F}$ PCR test and increased hematocrit is diagnostic for PV (sensitivity 95%, specificity 100%). The degree of $JAK2^{V617F}$ positivity of granulocytes is related to disease stage: heterozygous in true ET and early PV and mixed hetero/homozygous to homozygous in overt and advanced PV and CIMF. Bone marrow histology assessment should remain the gold standard criterion for the diagnosis and staging of the MPDs true ET, PV and CIMF and its differentiation from primary or secondary erythrocytosis, reactive thrombocytosis and thrombocythemias associated with atypical MPD, myelodysplastic syndromes, and chronic myeloid leukemia,. The proposed WHO/ECMP criteria allow a cross talk between clinicians, pathologists and scientists to much better characterize the nature and natural history of each of the WHO/CMP defined early and overt MPDs.

Keywords: Myeloproliferative disorders; Essential thrombocythemia; Polycythemia vera; Chronic idiopathic myelofibrosis; Erythropoietin; Endogenous erythroid colony assay; $JAK2^{V617F}$ mutation; Bone marrow pathology

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doi:10.1016/j.leukres.2007.01.021

1. Introduction

The classification of myeloproliferative disorders (MPD) according to the World Health Organisation (WHO) in 2001 [1] is an attempt to integrate bone marrow morphological criteria alongside PVSG clinical criteria for essential thrombocythemia (ET), polycythemia vera (PV) and chronic idiopathic myelofibrosis (CIMF). The WHO bone marrow features clearly distinguish three types of prefibrotic myeloproliferative disorders: true ET, early and overt PV and CIMF-0 (Fig. 1). By using platelet count in excess of $600 \times 10^9\,l^{-1}$ as criterion for the diagnosis of ET and increased red cell mass (RCM) as the main criterion for PV, the early stages of ET and PV are overlooked by the 2001 WHO classification. This comprises about 30% of early stage MPD indicating the need to lower the platelet count cut-off to $400 \times 10^9\,l^{-1}$ (upper limit of normal) for the clinical diagnosis of thrombocythemia in various MPDs (Table 1) [2,3]. In none of the PV patients is the information from RCM measurement found to be of additional diagnostic value, because in our experience all PV patients with increased red cell mass show a typical PV bone marrow histology picture [3]. Increased RCM had a sensitivity of 76% in the diagnosis of PV and a specificity of 79% in distinguishing PV and non-clonal polycythemia [4]. Spontaneous EEC along with low serum EPO levels are specific criteria for the diagnosis of early and overt PV, but have insufficient diagnostic sensitivity as isolated parameters to differentiate between PV, congenital or acquired erythrocytosis and normal controls [5–7]. About 50% of PVSG-defined ET patients show spon-

Table 1
WHO bone marrow features and European clinical, molecular and pathological (ECMP) criteria for the diagnosis of essential thrombocythemia (true ET)

Clinical and molecular criteria
C1. Sustained platelet count above the upper limit of normal: $>400 \times 10^9\,l^{-1}$
C2. Presence of large or giant platelets in a peripheral blood smear
C3. Normal values of hemoglobin, hematocrit, erythrocytes, white blood cell differential count
C4. Presence of $JAK2^{V617F}$ or MPL^{515} mutation
C5. Absence of the Philadelphia chromosome or any other cytogenetic fusion-gene abnormality
Pathological criteria (WHO)
P1. Increase of dispersed or loosely clustered, predominantly enlarged megakaryocytes with mature cytoplasm and hyperlobulated nuclei (Fig. 1A)
P2. No proliferation or immaturity of erythropoiesis and granulopoiesis and no or borderline increase of reticulin (myelofibrosis grade 0, see Table 2) [17]

According to WHO/ECMP criteria C1 + P1 and P2 establish the diagnosis of true ET. A typical ET bone marrow histological picture (Fig. 1A) excludes PV, CIMF, CML, MDS, RARS-T and reactive thrombocytosis.

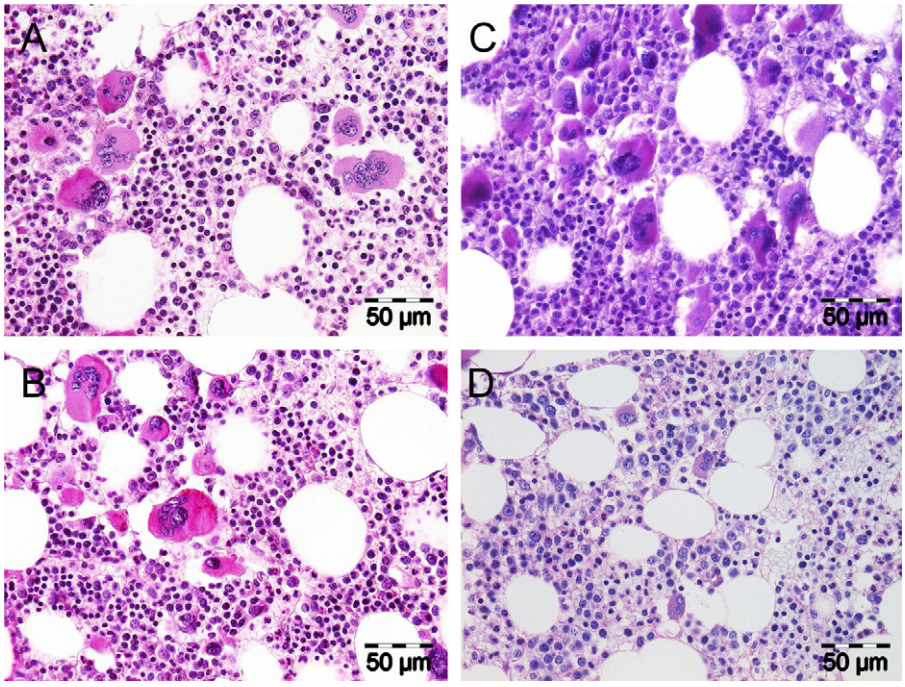

Fig. 1. Charateristic WHO bone marrow features of myeloproliferative disorders and secondary erythrocytosis in trephine biopsies stained with Periodic Acid Schiff (PAS). (A) Essential thrombocythemia (ET) or thrombocythemia vera (true ET). Increase and loose clustering of enlarged mature megakaryocytes with hyperlobulated nuclei and there is a slight increased cellularity. (B) Polycythemia vera (PV). Increase and clustering of small to enlarged or giant (pleomorphic) megakaryocytes with mature cytoplasm and hyperlobulated nuclei and increased cellularity due to increased erythropoiesis and granulopoiesis. (C) Chronic idiopathic myelofibrosis (CIMF-0/1). Increase and dense clustering of small to large (giant) immature megakaryocytes (dysmegakaryopoiesis) with dysmature hyperchromatic cloud-like nuclei and increased cellularity due to dual hyperplasia of granulopoiesis and megakaryopoiesis. (D) Secondary erythrocytosis (SE). Increased cellularity due to selective increase of erythropoiesis and megakaryocytes of normal size and morphology with normal nuclear lobulation and absence of clustering.

Table 2
WHO bone marrow features and European clinical, molecular and pathological (ECMP) criteria for the diagnosis of polycythemia vera (PV) and diagnostic differentiation between PV and congenital or acquired erythrocytosis

Clinical and molecular criteria
Major
A0. Early PV: hematocrit in the upper limit of normal (Ht: 0.45–0.51 in male and 0.43–0.48 in female)
A1. Classic PV: hematocrit >0.51/>0.48 in male/female
A2. The presence of JAK2^{V617F} mutation
A3. Low serum Epo level
Minor
B1. Persistent increase of platelet count: grade I: 400–1500, grade II: >1500
B2. Granulocytes $>10 \times 10^9\,l^{-1}$ or leukocytes $>12 \times 10^9\,l^{-1}$ and/or raised LAP-score or increased PRV-1 expression in the absence of fever or infection
B3. Splenomegaly on palpation or on ultrasound echogram (>12 cm length in diameter)
B4. Spontaneous endogenous erythroid colony (EEC) formation (optional)
Pathological criteria (WHO)
P1. Bone marrow pathology: increased cellularity (according to age) due to trilinear increase of erythropoiesis, megakaryopoiesis and granulopoiesis and clustering of small to giant (pleomorph) megakaryocytes with hyperlobulated nuclei (overt PV, Fig. 1B), absence of stainable iron. No pronounced inflammatory reaction (plasmacytosis, cellular debris)
P2. Selective increase of erythropoiesis, normal granulopoiesis and megakaryocytes of normal size, morphology and no clustering in primary/secondary erythrocytosis (Fig. 1D)
P3. Grading of myelofibrosis (MF) [17]. Post-PV-MF-0, 1, 2 and 3: see Table 3

WHO/ECMP criteria for early and overt PV. A0, A2, B1 and P1 establish early PV (mimicking ET) PV stage 0, or masked PV. A1, A2, P1 and none of B establish polycythemic PV stage 1. A1, A2, P1 and one or more of B establish classic and advanced PV stages 2 and 3. A1 and P2 with normal or increased values of serum EPO is consistent with erythrocytosis. A3 confirms PV. B4 important research option.

taneous EEC, low serum EPO levels and the presence of the JAK2^{V617F} mutation may indicate a biologically distinct subgroup of early PV ("forme fruste" PV, Tables 2 and 3) [3]. Bone marrow histopathology not only differentiates between the 3 prefibrotic MPDs (true ET, early PV and CIMF-0) but has also a close to 100% sensitivity and specificity to differentiate PV (Fig. 1B) from congenital (primary) polycythemia (CP), idiopathic erythrocytosis (IE) or acquired (secondary) erythrocytosis (SE, Fig. 1D) without the need of red cell mass measurement [1,3]. In the present study we update the European clinical, molecular and pathological (ECMP) [3] criteria for the diagnosis of the 3 prefibrotic MPDs by including the new molecular markers, JAK2^{V617F} and MPL^{515} mutations [8–10] on top of the 2001 WHO bone marrow features [1,3] (Fig. 1) for more accurate diagnostic differentiation of each of the early and overt MPD phenotypes as a sound basis for prognosis assessment and treatment guidelines.

2. Myeloproliferative disorders caused by JAK2^{V617F} and MPL^{515} mutations

The JAK2^{V617F} mutation has recently been discovered as the underlying cause of trilinear MPD and is detectable in CD34$^+$ hematopoietic bone marrow cells, erythroblasts, in EEC forming stem cells, blood platelets and granulocytes [3,8]. Applying allele-specific polymerase chain reaction (PCR) analysis in PVSG-defined MPD patients, a high frequency of the JAK2^{V617F} mutation of 95% (92–97%) in PV, and a lower frequency of 53% (49–57%) in ET and 52% (44–55%) in idiopathic myelofibrosis (IMF) are described [3]. Only 3–4% of ET, 24–27% of PV and 6–18% of IMF patients are homozygous for the JAK2^{V617F} mutation [3]. About 50% of PVSG-defined ET patients show spontaneous EEC and increased PRV-1 expression together with low serum EPO levels and the presence of the JAK2^{V617F} mutation indicating that EEC/PRV-1-positive ET comprises a biologically distinct subgroup of ET patients reflecting early PV ("forme fruste" PV) [3]. Two hypotheses have been proposed to explain why three different phenotypes of MPD are caused by the same JAK2^{V617F}mutation: the "dosage" hypothesis and the "additional events" hypothesis [8]. According to the dosage hypothesis (based on animal studies and different mutation states of JAK2^{V617F} in MPD patients), the level and duration of JAK2^{V617F} directly contribute to the phenotypic diversity of trilinear MPDs [8–11]. The thrombopoietin receptor (TPOR) MPL is expressed at high levels in megakaryocytic cells to control TPO physiologic levels. It is possible that activation of a few TPO receptors (MPL) by low levels of JAK2^{V617F} (heterozygous) is sufficient to send a signal to megakaryocytic precursor cells for enhanced growth. A slight increase in numbers of heterozygous mutated megakaryocytes and platelets (about $200 \times 10^9\,l^{-1}$) might be enough to produce platelet-mediated microvascular circulation disturbances. Conversely, the EPO-receptor (EPOR) is expressed at low levels on hematopoietic progenitor cells and therefore high percentage of heterozygous (1st genetic step) or homozygous (2nd genetic step) hematopoietic precursor cells carrying JAK2^{V617F} may be required to activate EPOR and generate PV-like phenotype [3,8]. Sustained high levels of JAK2^{V617F} during long-term follow-up subsequently may lead to a high level activation of EPOR and granulocyte colony stimulating factor receptor (G-CSFR) leading to extramedullary hematopoiesis (splenomegaly) and cytokine mediated secondary myelofibrosis [3,8]. The degree of JAK2^{V617F} positivity (increased percentage and progression from heterozygous to homozygous) is strongly correlated with polycythemia rubra vera-1 gene (PRV-1) over-expression in granulocytes, with the ability to form spontaneous EEC and with progressive post-PV myelofibrosis [9,10]. Transition from heterozygosity to homozygosity of the JAK2V617F mutation due to mitotic recombination of chromosome 9p (9pLOH) of the JAK2^{V617F} mutation represents a very important step in the progression from classic PV to post-PV myelofibrosis [9,10]. Scott et al.

Table 3
Staging of PV patients according to WHO/ECMP criteria: therapeutic implications [3]

PV, ECP stage	0	1	2	3	4	5
Clinical	Early PV	Polycythemic PV	Classic PV	Advanced PV	Post-PV myelofibrosis	Spent phase PV
LAP-score and/or PRV-1	↑[a]	↑	↑	↑/↑↑	Variable	Variable
Red cell mass	N	↑	↑	↑	Variable	N/↓
Serum EPO	N/↓	↓	↓	↓	Variable	N/↓
Leukocytes ($\times 10^9\,l^{-1}$)	<12	<12	N–>12	>15	>20	>20
Platelets ($\times 10^9\,l^{-1}$)	>400	<400	>400	>1000	Variable	Variable
Peripheral blood red cells						
Hemoglobin (g/dl–)mmol/l	<16–10	>16–10	>16–10	>16–10	Variable	N/↓
Hematocrit	<0.51	>0.51	>0.51	>0.51	Variable	N↓
Erythrocytes ($\times 10^{12}\,l^{-1}$)	<6	>6	>6	>6	Variable	N/↓
WHO bone marrow	Early PV	Early PV	Trilinear PV	Trilinear PV	Trilinear/MF	MF
Bone marrow cellularity (%)	50–80	50–80	80–100	80–100	Decreased	Decreased
Grading myelofibrosis [17]	MF 0	MF 0	MF 0/1	MF 1/2	MF 3	MF 3
Splenomegaly	Slight	No	Slight/moderate	Moderate	Large	Large
Spleen size, echogram (cm)	12–15	<12	12–15	12–20	>20	>20
Specific MPD markers EEC	+	+	+	+	+	+/?
Molecular $JAK2^{V617F}$						
Granulocytes	+	+	+/++	+/++	++	++
BFU-E colonies	+ or ++[a]	+ or ++	++	++	++	++
Therapeutic implications						
First line treatment	Aspirin	Phlebotomy, aspirin	Phlebotomy, aspirin	Interferon, hydroxyurea, aspirin	Hydroxyurea, interferon, busulfan	Supportive

[a] ↑ = increased, ↓ = decreased, N = normal, + = present or heterozygous; ++ = homozygous.

recently showed that BFU-E colonies are already homozygous for the $JAK2^{V617F}$ mutation in PV patients with a heterozygous pattern of $JAK2^{V617F}$ in their peripheral blood granulocytes [10]. In contrast, the BFU-E colonies from heterozygous patients with ET did not contain a subpopulation of $JAK2^{V617F}$ homozygous cells [10]. According to the "additional events" hypothesis, alternative and/or additional molecular abnormalities modify, or precede a homozygous state deferred to by the $JAK2^{V617}$ mutation alone, combinations carrying the JAK^{V617F} and MPL^{515} mutations [12,13] or other combinations of still unknown mutations. Mechanisms other than mitotic recombination such as duplication of the mutated allele is observed in a proportion of PV and CIMF patients displaying a gain of 9p, mostly due to trisomy 9 [8]. $JAK2^{V617F}$ may be may be dependent not only on the amount of heterozygous and homozygous mutant protein, but also on the various pathway regulating JAK2 activity including MPL, JAK2, STAT, MAP kinase and P13K signalling pathway. This has led to the recent discovery of the MPL^{W515L} and MPL^{W515K} mutations as the underlying etiology in some ET and CIMF patients [12,13], and to the recent discovery of JAK2 exon 12 mutations in $JAK2^{V617F}$ negative PV by Scott and Green (European School of Hematology, MPD Conference Madeira, September 2006). Therefore, there may be an overlap between "dosage" and "additional molecular events" hypotheses. Long-term studies are warranted to delineate the chronology and impact of various putative additional molecular abnormalities on the natural history, biology and prognosis of MPD. Sex appears to be a powerful genetic background modifier in $JAK2^{V617F}$-positive MPDs as ET is more common in females and PV in males.

3. WHO bone marrow features for the differential diagnosis of ET, PV and CIMF

For the diagnosis and classification of MPD, bone marrow trephine biopsy specimens should be embedded in paraffin or plastic, both with its technical limitations [14–17]. Paraffin requires decalcification preferably with EDTA allowing reasonable DNA quality or acid electrolysis. The specimens should have at least 4 evaluable bone marrow spaces with hematopoiesis. Recommended stains include: hematoxylin and eosin (H&E), Giemsa (3 μm sections), periodic acid-Schiff (PAS); Perls for estimation of hemosiderin content; chloro-acetate esterase (Leder) for identification of granulocytic differentiation; silver stain for reticulin; and trichrome-Masson for collagen staining. Immunostains of paraffin embedded specimens should include glycophorine C for erythropoiesis, myeloperoxydase for granulopoiesis, CD42b, CD61 or FVIII-related antigen for megakaryocytes; CD34 for CD34-positive blasts and CD117 for myeloid differentiation.

Regarding MPD, clinicians want to receive from their pathologist a detailed report on:

- Cellularity of the three hematopoietic cell lines related to patient age.
- Erythropoiesis: quantity, appearance of erythroblast islands, morphology and degree of maturation.
- Granulopoiesis: quantity, morphology, maturation, location of the immature precursors.
- Megakaryopoiesis: quantity, morphological features including size, lobulation of nuclei, chromatin structure, localization, the degree and pattern (loose or tight) of clustering. Increase of clustered enlarged mature or dysmorphic megakaryocytes appear to be a pathognomonic clue to the diagnosis of a myeloproliferative disorder (Tables 1–3, Fig. 1) [1,3,14–16].
- Quantity, localization and appearance of monocytes, mast cells, pseudo-Gaucher cells, lymphocytes, and plasma cells.
- Absence or presence of reticulin fibers and grading of fine or course reticulin and some or coarse bundles of collagen using a uniform scoring system (Table 3) [17].
- Absence or presence of bone trabeculae remodelling, osteoblasts, and osteoclasts.
- Ectasia of the sinusoids, and the eventual appearance of hematopoietic precursors and megakaryocytes.
- Estimation of the microvessel density on the basis of CD34 or CD31 immunostain (optional).

According to well established WHO bone marrow criteria, increase and loose clustering of enlarged mature megakaryocytes with hyperlobulated nuclei in a normocellular bone marrow and platelet count $>400 \times 10^9\,l^{-1}$ represent the hallmark of true ET (Table 1, Fig. 1A) [1,3,14–16]. In true ET there is no proliferation or immaturity of granulopoiesis or erythropoiesis. In congenital and acquired erythrocytosis and in reactive thrombocytosis the megakaryocytes are of normal size and morphology and there is no tendency to cluster (Fig. 1D). A typical WHO histopathological ET picture of the bone marrow excludes reactive thrombocytosis (RT) and distinguishes true ET from early PV, CIMF-0, and CIMF-1 (Fig. 1) and thrombocythemia associated with atypical MPD, MDS, refractory anemia with increased ringed sideroblasts (RARS-T) or Ph^1-positive thrombocythemia in chronic myeloid leukemia (CML) [14]. In the chronic stage of Ph^{1+} thrombocythemia without or with features of CML, the megakaryocytes in bone marrow smears and biopsy material are smaller than normal with round nuclei, showing no or little lobulations [3,14–16].

The characteristic increase and clustering of small and enlarged pleomorphic megakaryocytes and increased erythropoiesis with increased granulopoiesis and increased

Table 4
WHO bone marrow features and European clinical, molecular and pathological (ECMP) criteria for diagnosis and staging of chronic idiopathic myelofibrosis (CIMF)

Clinical and molecular criteria
C1. Usually associated with or preceded by thrombocythemia and no preceding true ET, PV, CML, CMML, or MDS. Absence of Philadelphia chromosome. Presence of $JAK2^{V617F}$ or MPL^{515} mutation
Clinical staging (ECMP)
Early clinical stage
Platelet count $>400 \times 10^9\,l^{-1}$, usually pronounced around $1000 \times 10^9\,l^{-1}$. No leuko-erythroblastosis, no anemia. No or slight splenomegaly on echogram CIMF-0 or CIMF-1
Intermediate clinical stage
Definitive leuko-erythroblastosis. Anemia grade 1: Hb <12 to >10 g/dl or <7.5 to >6.25 mmol/l. Splenomegaly on palpation CIMF-1 and 2
Advanced clinical stage
Pronounced leuko-erythroblastosis. Anemia grade 2: Hb <10 g/dl or Hb >10 g/dl with the presence of adverse signs*. Pronounced splenomegaly. Leukocotosis, leukopenia. Normal or decreased platelet count CIMF-2 and 3
Pathological criteria (WHO)
P1. Increased cellularity (according to age) due to chronic megakaryocytic and granulocytic myeloproliferation and relative reduction of erythroid precursors
P2. Dense clustering and increase in immature medium sized to giant megakaryocytes containing bulbous (cloud-like) hypolobulated nuclei and definitive maturation defects (Fig. 1C)
Grading myelofibrosis (MF) [17]
MF 0: prefibrotic CIMF-0: scattered linear reticulin with no intersections (cross-over) corresponding to normal bone marrow
MF 1: early fibrotic CIMF-1: loose network of reticulin with many intersections, especially in peripheral areas, no collagenization
MF 2: fibrotic CIMF-2: diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis
MF 3: classic CIMF-3: diffuse and dense increased in reticulin with extensive interactions with course bundles of collagen often associated with significant osteosclerosis
MF >3: endstage hypocellular with extensive osteomyelosclerosis

Adverse signs* age > 70 years, hemoglobin < 10 g/dl, myeloblasts PB > 2%, erythro-normoblasts PB > 2%, leukocytosis $> 20 \times 10^9\,l^{-1}$, thrombocytopenia $< 300 \times 10^9\,l^{-1}$, severe constitutional symptoms, massive splenomegaly, cytogenetic abnormalities. According to WHO/ECMP criteria, C1 and P1 plus P2 establish CIMF—any other peripheral blood criterion and grading of secondary myelofibrosis (MF) contribute to ECMP staging of WHO defined CIMF.

cellularity (according to age) are the diagnostic characteristics of WHO-defined classic PV (Fig. 1B) with increased hematocrit >0.51, low serum EPO and/or JAK2^{V617F} (Tables 2 and 3) distinguishing it from congenital and secondary erythrocytosis (Fig. 1D). A typical WHO PV picture with moderately increased cellularity (according to age) is seen in patients with early PV mimicking ET or "forme fruste" PV as documented by increased platelet count ($>400 \times 10^9\,l^{-1}$), normal hematocrit (<0.51 male, <0.48 female), low serum EPO and/or the presence of the JAK2^{V617F} mutation (Tables 2 and 3). A typical WHO PV bone marrow picture is also seen in the erythrocythemic PV stage 1 featured by increased hematocrit (>0.51 male, >0.48 female), normal platelet count ($<400 \times 10^9\,l^{-1}$), normal spleen, low serum EPO and the presence of the JAK2^{V617F} mutation (Tables 2 and 3).

According to established WHO criteria, prefibrotic CIMF-0 is characterized by hypercellularity of the bone marrow (according to age) due to increased granulopoiesis, relative decrease of erythropoiesis and the presence of dense clusters of immature megakaryocytes with maturation defects (nucleo-cytoplasmic asynchrony) with bulky nuclei showing clumpy lobuli and irregular roundish shaped forms (so-called cloud-like nuclei), which are almost never seen in ET and PV (Fig. 1C, Table 4) [1,3,14–17]. The degree of dysmorphic megakaryopoiesis in CIMF-0 may range from slight maturation defect of megakaryocytes with no cloud-like nuclei to manifest maturation defects of megakaryocytes with typical cloud-like nuclei or a mixture of both. The risk of CIMF-0 to transform into early CIMF-1 and subsequent CIMF-2/3 with extramedullary hematopoiesis is clearly dependent on the degree of hypercellularity and the degree of maturation defects of megakaryopoiesis [14–16].

High quality histological bone marrow preparations in the hands of experienced hematopathologists are required to distinguish CIMF-0 from true ET and PV in about 85–90% of the cases when applying the WHO bone marrow criteria. There are no studies that have examined the concordance between a number of pathologists who have used WHO histological features to assign cases to the prefibrotic stages of true ET, PV and CIMF-0 without knowledge of the clinical findings and biological MPD markers except age. The risk of progression of the early stages of PV into post-PV myelofibrosis remains to be demonstrated. Alike, the hypothesis that true ET never progress to CIMF [16] needs further confirmation. The bone marrow histology of CIMF-0 with slightly dysmorphic megakaryopiesis may appear to overlap with early PV (hematocrit <0.51) presenting with a trilinear hypercellular bone marrow with a relatively increased ratio of granulopoiesis as compared to erythropoiesis and a similar degree of thrombocythemia, leukocytosis, increased LAP-score and slight splenomegaly. The histology of CIMF-0 may overlap with that of true ET in cases with a mild increased of granulopoiesis and/or a mixture of mildly dysmorphic megakaryocytes and mature enlarged megakaryocytes with hyperploid nuclei. Data on the very long-term natural history of WHO/CMP defined true ET, early PV and CIMF-0 derived from large prospective studies are lacking.

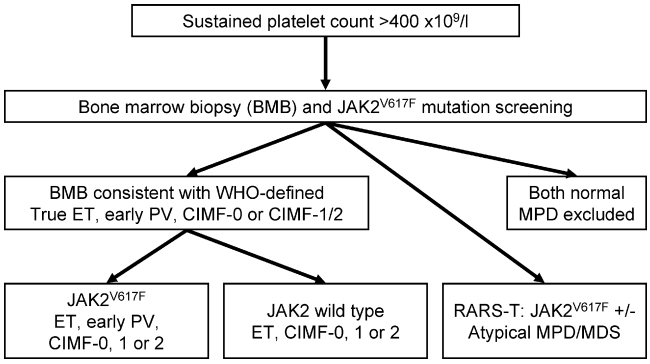

Fig. 2. Algorithm for diagnostic work-up for patients with suspected thrombocythemia as the presenting feature of ET, early PV, prefibrotic CIMF-0, early fibrotic CIMF-1 or refractory anemia with increased ringed sideroblasts (RARS-T).

4. Diagnostic work-up of patients with suspected thrombocythemia in various MPDs

Clinicians and pathologists should realize that PVSG-defined ET includes true ET, early PV mimicking ET, and thrombocythemia associated with CIMF-0 or CIMF-1 when WHO/ECMP criteria are applied. The diagnostic work-up of thrombocythemia in various MPDs (Fig. 2) according to updated WHO/ECMP criteria [3] is based on positive findings in peripheral blood and bone marrow (Tables 1, 2 and 4, Fig. 1). First, a sustained increased platelet count ($>400 \times 10^9\,l^{-1}$) and the presence of enlarged platelets in a peripheral blood smear in the complete absence of any underlying disorder associated with reactive thrombocytosis is highly suspicious for the diagnosis of MPD with thrombocythemia. Second, a subsequent pre-treatment bone marrow biopsy will reveal each of the early stages of prefibrotic MPD entities with thrombocythemia (both in JAK2^{V617F} or MPL^{515} mutated and JAK2 wild type patients) thrombocythemia when the WHO/ECMP criteria are applied: true ET (Table 1); early PV mimicking ET (Tables 2 and 3); early stage CIMF-0 or CIMF-1 without features of leukoerythrocytosis and extramedullary hematopoiesis (Table 4) [14–17]. Naturally, histopathology ascertains CIMF grades 1, 2 and 3 usually with features of leukoerythroblastosis (Table 4). The prognostic importance of the WHO bone marrow features is demonstrated in a large retrospective study of 865 thrombocythemia patients: the relative 10 years survival rates of $99 \pm 7.8\%$ for true ET, $81 \pm 11.7\%$ for CIMF-0, and $67 \pm 17.8\%$ for CIMF-1 patients, were significantly different due to progression into end-stage disease during long-term follow-up [16]. Third, the screening for JAK2^{V617F} as a first intention diagnostic test is extremely helpful in the diagnostic work-up of patients with suspected MPD with thrombocythemia to ascertain the clonal nature of the disease. Only half of ET and CIMF patients and nearly all patients with

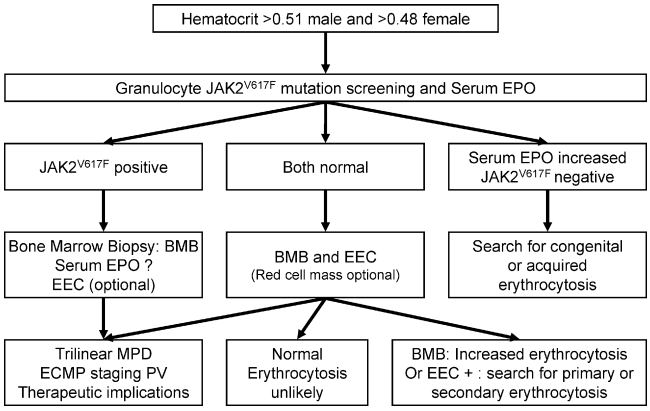

Fig. 3. Algorithm for diagnostic work-up of patients with suspected polycythemia vera or erythrocytosis.

early PV carry the JAK2^{V617F} mutation (Figs. 2 and 3). In contrast, MPL^{515} mutations are found in a few cases of ET and CIMF and not in PV. Comparing the laboratory features of JAK2^{V617F} positive versus JAK2 wild type MPD patients with thrombocythemia showed that JAK2^{V617F} positive ET was characterized by higher values for hemoglobin, hematocrit, neutrophil counts, LAP score, by lower values for serum EPO levels, serum ferritin and MCV, and by increased cellularity of the bone marrow in biopsy material [18]. These observations argue for the concept that JAK2^{V617F} and EEC positive ET patients may represent an early PV mimicking ET ("forme fruste" PV, stage 1 PV, Table 3) [3]. In contrast, JAK2 wild type PVSG-defined ET patients had significantly higher platelet counts, normal serum EPO levels, a WHO bone marrow picture of true ET, no features of PV, and were at lower risk for the development of thrombotic complications [18]. These data are in line with the hypothesis that JAK2^{V617} positive and JAK2 wild type ET patients at diagnosis represent two distinct entities with a related pathophysiology (utilizing the JAK-2/STAT signalling pathway) but with different molecular etiology. This could be confirmed by the discovery of MPL^{515} mutations in some ET and CIMF patients [12,13]. Finally, the established WHO bone marrow criteria also distinguish thrombocythemia in various MPDs from thrombocythemia associated with Ph^1-chromosome and *bcr/abl* positive chronic myeloid leukemia (CML) or myelodysplastic syndromes (MDS) [14] including the so-called 5q-syndrome. Peripheral blood and bone marrow features of the 5q-minus syndrome clearly differs from refractory anemia with increased ringed sideroblasts and significant thrombocytosis (RARS-T) [3,14]. Among 9 RARS-T patients in a recent study, 6 showed the presence of JAK2^{V617F} mutation [19].

5. Diagnostic work-up of patients with suspected PV

The presence of JAK2^{V617F} mutation has a sensitivity of about 95% and positive predictive value of 100% for the diagnosis of PV in the context of absolute erythrocytosis (hematocrit >0.51 in males and >0.48 in females) (Fig. 3). Subsequent red cell mass measurement will distinguish apparent from absolute erythrocytosis but does not differentiate between PV and congenital or secondary erythrocytosis. Bone marrow histology clearly differentiates trilinear hypercellularity in PV (Fig. 1B) from isolated increase of erythropoiesis (Fig. 1D) in congenital polycythemia and secondary erythrocytosis (Table 2). Spontaneous EEC and low serum EPO as stand alone tests combined with increased hematocrit are specific but not sensitive enough for the diagnosis of PV. The detection of JAK2^{V617F} in granulocytes with sensitive PCR techniques as a first intention diagnostic test for erythrocytosis (hematocrit >0.51) simplifies the diagnostic work-up of PV (Fig. 3) [20]. The presence of the JAK2^{V617F} mutation combined with increased hematocrit (>0.51) is diagnostic for PV without the need of red cell mass measurement, but not enough to define the broad spectrum of PV phenotypes (Table 2) [20]. Additional investigations including bone marrow histopathology and low serum EPO levels are warranted to diagnose both early and overt stage PV by applying WHO/ECMP criteria (Tables 2 and 3, Fig. 3) [3]. The EEC assay is time consuming and difficult to introduce in non-specialized laboratories in a standardized manner. The widely available bone marrow histology assessment should remain the gold standard criterion for the diagnosis and staging of PV and its differentiation from the WHO-defined true ET and prefibrotic or early fibrotic CIMF (Fig. 1) [1,3].

Acknowledgements

We are grateful to Jean Brière, Department of Hematology, Hôpital Beaujon, Clichy, France, and to Hans Kreipe, Institute of Pathology, Medizinische Hochschule, Hannover, for critical reading of the manuscript.

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