

Bone marrow histopathology of differential diagnostic significance in JAK2 mutated versus MPL and CALR mutated essential thrombocythemia and myelofibrosis

Key words: essential thrombocythemia, polycythemia vera, myelofibrosis, bone marrow pathology, JAK2 mutation, MPL mutation, calreticulin mutation

Abstract

Bone marrow histology in JAK2^{V617F} mutated patients with myeloproliferative neoplasm (MPN) in essential thrombocythemia (ET) and polycythemia vera (PV) are similar and featured by clustered medium to large pleomorphic megakaryocytes and increased cellularity (60% to 90%) mainly due to increased erythropoiesis. MPL⁵¹⁵ mutated ET or 'True' ET is a distinct clonal MPN entity with large to giant large to giant megakaryocytes and hyperlobulated staghorn-like nuclei in a normocellular bone marrow without features of PV or primary megakaryocytic, granulocytic myeloproliferation (PMGM). Bone marrow histology in calreticulin (CALR) mutated hypercellular ET revealed a typical PMGM bone marrow with dysmorphic immature megakaryocytes and cloud-like nuclei, which are not seen in MPL⁵¹⁵ mutated ET and JAK2^{V627F} mutated ET and PV.

Introduction

The clinical and bone marrow histology features of "true" ET has been recognized by Thiele et al in 1988 and 2005 as clearly distinct from PV^{1,2}. The peripheral blood findings in "true" ET are featured by high platelet counts, normal values for haemoglobin, haematocrit, erythrocyte, white blood cells, LAP score, LDH and no or minor splenomegaly despite platelet counts above $1000 \times 10^9/L$ ¹. The megakaryocytes in "true" ET are larger than in PV (table 1)¹⁻³. In 2002 Michiels & Thiele have defined and distinguished normocellular "true" ET as a distinct MPN entity different from PV and hypercellular ET associated with prefibrotic primary myelofibrosis (pPMF) or primary megakaryocytic granulocytic myeloproliferation (PMGM, table 1)³. Bone marrow histology in PV is typically featured by large pleomorphic megakaryocytes with hyperploid nuclei in a hypercellular bone marrow due to increased erythropoiesis or increased erythrocytic-megakaryocytic-granulocytic (trilinear) myeloproliferation⁴. Hypercellular ET associated with prefibrotic primary myelofibrosis (pPMF³) or primary megakaryocytic granulocytic myeloproliferation (PMGM⁴) is dominated by an increase of clustered atypical dysmorphic megakaryocytes due to increases of cellular and nuclear size and bulky nuclei with clumsy lobuli and irregular roundish shaped form (so-called cloud-like nuclei), which are never described in JAK2 mutated ET and PV^{3,4}.

Bone marrow histology in MPL⁵¹⁵ mutated normocellular ET

Bone marrow histology from a patient with JAK2 wild type ET carrying the MPL^{W515L} mutation displayed clusters large megakaryocytes with a greater number of giant megakaryocytes with hyperlobulated stag-horn nuclei in a normal cellular bone marrow and no increase of erythropoiesis (figure 1). We recently described the essential differences in bone marrow histopathology features of differential diagnostic significance between patients with MPL⁵¹⁵ mutated (N=12) versus JAK2^{V617F} mutated MPN (N=12) (figure 2)⁴. **First**, The presence of clustered small and giant megakaryocytes with deeply lobulated stag-horn like nuclei (figure 1) in ET carrying the MPL⁵¹⁵ mutation are not seen in JAK2^{V617F} positive ET, prodromal PV, and classical PV (figure 2). The pleomorphic medium to large megakaryocytes in JAK2^{V617F} mutated ET and PV in bone marrow smears and bone marrow biopsy were similar in size and pleomorphy (figure 2). **Second**, there was local increase of erythropoiesis in areas of loose clustered pleiomorphic megakaryocytes in normocellular ET and prodromal PV (figure 2), which is not seen in MPL⁵¹⁵ mutated ET (figure 1). **Third**, JAK2 wild type MPL⁵¹⁵ mutated ET have no clinical, laboratory and bone marrow features of prodromal PV at diagnosis (table 1), do not evolve into PV during follow-up, and have normal LAP score, serum EPO and ferritin levels^{1,5}. Laboratory and bone marrow histology evaluations have the diagnostic potential to separate the JAK2^{V617F} mutated ET and prodromal PV with increased LAP score, low serum EPO and pleomorphic megakaryocyte morphology from MPL⁵¹⁵ mutated ET with normal LAP score and serum EPO and giant megakaryocytes with staghorn-like nuclei similar to “true” ET (table 1)^{1,3,4}.

Bone marrow histology in CALR mutated JAK2 wild type ET and MF

In six consecutive newly diagnosed CALR positive ET cases in 2014 we found consistent bone marrow characteristics of hypercellular ET as the presenting feature of prefibrotic and early fibrotic stages of PMGM. Five of these six CALR mutated ET patients presented with aspirin sensitive microvascular disturbances of erythromelalgic, cerebral and ocular ischemic manifestations (Sticky Platelet Syndrome) as the specific presenting manifestations of myeloproliferative thrombocythemia. Bone marrow histology in typical prefibrotic CALR ET (figure 3) and in early fibrotic CALR myelofibrosis (MF, figure 4) show dysmorphic megakaryocytes with definite abnormalities of maturation with bulky (bulbous) hyperchromatic nuclei and some disturbances of the nuclear cytoplasmic ratio consistent with

CALR mutated PMGM⁴, which are not seen in MPL⁵¹⁵ mutated ET and also not in JAK2^{V617F} mutated ET, prodromal PV and classical PV.

Discussion

In a prospective study of 59 JAK2^{V617F} positive ET and 44 JAK2 wild ET cases, Piche *et al*⁶ described that JAK2^{V617F} mutated ET patients have PV-like morphological bone marrow changes of medium sized to large pleomorphic megakaryocytes similar to our findings in newly diagnosed JAK2^{V617F} mutated ET, prodromal PV patients and PV patients⁴. JAK2^{V617F} positive ET and prodromal PV patients usually have low serum EPO, increased LAP score, and slight to moderate increased bone marrow cellularity due to increased erythropoiesis. Increase of bone marrow erythropoiesis, granulopoiesis and serum LDH levels and spleen size are more pronounced in advanced JAK2^{V617F} mutated ET (masked PV) at higher JAK2^{V617F} mutation allele burden. Clustered large and giant megakaryocyte with hyperlobulated ‘staghorn’ nuclei are rare in JAK2 mutated MPN⁶, but typically present in MPL⁵¹⁵ mutated ET patients with no features of PV in the bone marrow consistent with the diagnosis of ‘True’ ET and normal blood values for serum EPO, ferritin levels and LAP score^{1,4}. The prevalence of MPL⁵¹⁵ mutated ET or MF patients ranges from 5 to 10% of the JAK2 wild type MPN population^{5,7}. In the large collaborative European study of 176 MPN cases with the MPL⁵¹⁵ mutations W515L and W515K were detected in 110 and in 58 respectively⁸. The overall MPL mutation allele levels in granulocytes were lower (25%) in W515L (N=106) than in W515K (37%, N=32). Of the 138 cases (ET, N=99; MF, N=36), the median W515L mutation allele levels were significantly lower (21%) in ET than those (46%) in MF⁸.

The bone marrow findings in CALR mutated ET and MF in figures 3 and 4 are consistent with prefibrotic PMF or PMGM (table 1), which clearly differs from the pleomorphic megakaryocytes in JAK2^{V617F} mutated ET and PV and from giant megakaryocytes and hyperlobulated staghorn-like nuclei in MPL⁵¹⁵ mutated ET^{3,4}. Recently, the JAK2 wild type CALR-mutated ET and MF has become the third distinct MPN entity without features of PV at time of diagnosis and during follow-up^{9,10}. The finding of CALR mutation as the driver mutation in the majority of JAK2 wild type ET has been confirmed by the MPN Study Group in Belgium in 40 of 64 JAK2 wild type MPN (ET or MF); 24 of these 64 MPN cases were JAK2/MPL/CALR triple negative (Timothy Devos and Peter Vandenberghe, University Hospital Leuven, Belgium, Annual Meeting of the Belgian

Hematology Society January 31, 2014). The blood and bone marrow features of the 40 CALR mutated MPN (ET and MF) and 24 JAK2/CALR triple negative MPN cases are under investigation and will be compared to a group of 50 previously untreated cases with JAK2^{V616F} mutated ET, prodromal PV and PV.

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References

1. Thiele J, Zankovich R, Schneider G, Kremer B, Fischer R, Diehl V. Primary (essential) thrombocythemia versus polycythemia rubra vera. A histomorphometric analysis of bone marrow features in trephine biopsies. *Analyt Quat Cytol Histol* 1988;10:375-382.
2. Thiele J, Kvasnicka HM, Diehl V. Initial (latent) polycythemia vera with thrombocytosis mimicking essential thrombocythemia. *Acta Haematol* 2005;113:213-219.
3. Michiels JJ, Thiele J. Clinical and pathological criteria for the diagnosis of essential thrombocythemia, polycythemia vera and idiopathic myelofibrosis (agnogenic myeloid metaplasia). *Int J Hematol* 2002;76:133-145.
4. Michiels JJ, De Raeve H. Changing concepts on the diagnostic criteria of myeloproliferative disorders and the molecular etiology and classification of myeloproliferative neoplasms. From Dameshek 1950 to Vainchenker 2005 and beyond. *Acta Haematol* 2014, published online.
5. Vannucchi AM, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, Barosi G, Ruggeri M, Specchia G, Lo-Coco F, Delaini F, Villani L, Finotto S, Ammatuna E, Alterini R, Carrai V, Capaccioli G, Di Lollo S, Liso V, Rambaldi R, Bosi A, Barbui T. Characteristics and clinical correlates of MPL515W>L/K mutation in essential thrombocythemia. *Blood* 2008;112:844-847.
6. Beer PA, Campbell PJ, Scott LM. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. *Blood* 2008;112:141-149.
7. Jones AV, Campbell PJ, Beer PA, Schnittger, Vannucchi AM, Zoi K, Percy MJ, McMullin MF, Scott LM, Tapper W, Silver RT, Oscier D, Harrison CN, Grallert H, Kisialiou A, Strike

- P, Chase AJ, Green AR, Cross NCP. The JAK2 46/1 haplotype predisposes to MPL-mutated myeloproliferative neoplasms. *Blood* 2010;115:4517-4523.
8. Pich A, Riera L, Beggiato E, Nicolino B, Godio L, Campisi P, Sismondi S, di Cello PF. JAK2V617F mutation and allele burden are associated with distinct clinical and morphological subtypes in patients with essential thrombocythemia. *J Clin Pathol* 2012;65:953-955.
 9. Rumi E, Pietra D, Ferretti V, Klampfl T, Harutyunyan AS, Milosevic JD, Nicole, Them NCC, Berg T, Elena C, Casetti IC, Milanesi C, Sant'Antonio E, Bellini M, Fugazza E, Renna MC, Boveri E, Astori C, Pascutto C, Kralovics R, Cazzola M. Jak2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcome. *Blood* 2014;123(10):1544-1551.
 10. Rumi E, Pietra D, Pascutto C, Guglielmelli P, Martinez-Trillos A, Cassetti I, Colomer D, Pieri L, Pratcorona M, Rotunno C, Mannarelli C, Milanesi C, Boveri E, Ferretti V, Astori C, rosti V, Cervantes F, Barosi G, Vannucchi AM, Cazzola M. Clinical effects of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. *Blood* 2014;124(7):1062-1069.

Table 1
The European Clinical and Pathological (ECP) criteria for True ET (Michiels & Thiele, 2002³)

Clinical Criteria	Pathological Criteria
A1 Persistent increase of platelet count: grade I: 400-1500, grade II: $>1500 \times 10^9/L$	B1 Predominant proliferation of enlarged megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous cytological abnormalities
A2 Normal spleen or only minor splenomegaly on echogram	B2 No proliferation or immaturity of granulopoiesis or erythropoiesis
A3 Normal LAP score, normal ESR, and increased MPV	B3 No or only borderline increase in reticulins
A4 Spontaneous megakaryocyte colony formation (CFU-Meg)	
A5 No signs or cause of RT	
A6 No preceding or allied other subtype of MPD, CML, or MDS	
A7 Absence of the Philadelphia chromosome	

* The combinations of A1 and B1 + B2 establish (true) ET (thrombocythemia vera). Any other criterion confirms ET. LAP indicates leukocyte alkaline phosphatase; ESR, erythrocyte sedimentation rate; MPV, mean platelet volume; CFU-Meg, colony-forming unit-megakaryocyte; RT, reactive thrombocytosis; MPD, myeloproliferative disorder; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome.

The European Clinical and Pathological (ECP) criteria for prefibrotic chronic idiopathic myelofibrosis (CIMF) or primary megakaryocytic granulocytic myeloproliferation (PMGM, Michiels & Thiele, 2002^{3,4})

Updated Clinicopathological Criteria for the Diagnosis of Idiopathic Myelofibrosis (IMF) or Agnogenic Myeloid Metaplasia (AMM)*

Clinical Criteria	Pathological Criteria
A1 No preceding or allied other subtype of MPD, CML, or MDS	B1 Megakaryocytic and granulocytic myeloproliferation and relative reduction of erythroid precursors.
A2 Early clinical stages	Abnormal clustering and increase in atypical giant to medium-sized megakaryocytes containing bulbous (cloud-like) hypolobulated nuclei and definitive maturation defects.
Normal hemoglobin or anemia, grade I: hemoglobin ≥ 12 g/dL	
Slight or moderate splenomegaly on palpation or >11 cm on ultrasound scan or CT	
Thrombocythemia, platelets $>400 \times 10^9/L$	
A3 Intermediate clinical stage	
Anemia grade II: hemoglobin ≥ 10 g/dL	
Definitive leuko-erythroblastic blood picture and/or tear-drop erythrocytes	
Splenomegaly	
No adverse sign†	
A4 Advanced clinical stage	
Anemia grade III: hemoglobin <10 g/L	
One or more adverse sign†	

Legend to the figures

Figure 1. Bone marrow histology in “True” ET (table 1) consistent with WHO normocellular ET carrying the MPL^{W515L} mutation showing giant megakaryocytes with hyperlobulated stag-horn like nuclei characteristic for ET the MPLW515L/K mutation. Courtesy of Dr Schwarz, University Hospital Prague, Czech Republic.

Figure 2. Bone marrow features of JAK2V617F heterozygous positive ET (left) and JAK2V617F homozygous acute onset PV (right). Contribution of Drs Michiels and De Raeve, University Hospital Antwerp, Belgium.

Left. Presence of normal platelets in a peripheral blood smear (a) and normal size and morphology of megakaryocytes in a smear from an aspirated bone marrow (middle left) in a control person. Increase of platelets aggregates with the presence of large platelets in a peripheral blood smear (b) and the presence of large megakaryocytes with hyperlobulated nuclei (inset) in a smear of and aspirated bone marrow (middle right) of a case with ET featured by platelet count of $435 \times 10^9/L$ and slight splenomegaly complicated by portal vein thrombosis. Bone marrow histology showing increased cellularity (70%, c left), due to increased erythropoiesis and pronounced increase of large pleomorphic megakaryocytes with hyperlobulated nuclei and no increase of bone marrow reticulinn (c right) pathognomonic diagnostic for heterozygous mutated JAK2^{V617F} ET (JAK2^{V617F} mutation load 26%).

Right. Hypercellular bone marrow due to increased megakaryopoiesis, erythropoiesis and granulopoiesis in a case with rapid onset trilinear JAK2^{V617F} homozygous positive PV.

Figure 3. Clinical case of JAK2/MPL negative and calcireticulin (CALR) positive ET who present with aspirin responsive platelet thrombophilia, normal values for hemoglobin. Hematocrit and erythrocytes, platelet count of $1832 \times 10^9/L$ and slight splenomegaly (16 cm length diameter on echogram). Bone marrow histology is hypercellular with relative decrease of erythropoiesis, dense cluster of immature megakaryocytes with hyperlobulated nuclei consistent, and no increase of reticulinn fibrosis consistent with a typical PMGM (table 1) bone marrow. Contribution of Drs Valster and Schelfhout, Lievensberg Hospital Bergen op Zoom, The Netherlands

Figure 4. Clinical case of CALR positive myelofibrosis (MF): hemoglobin 11.2 g/dL, hematocrit 0.33, leukocytes $9.2 \times 10^9/L$, platelets $347 \times 10^9/L$, LDH 1393 U/L, and the presence of tear drop erythrocytes, poikilocytosis and polychromasia in a peripheral blood smear, and hypercellular bone marrow with relative decrease of erythropoiesis, dense cluster of immature megakaryocytes with hyperlobulated nuclei consistent, and reticulinn fibrosis grade 2 consistent with bone marrow histology features similar to WHO-defined primary myelofibrosis (PMF), but distinct from JAK2^{V617F} mutated ET and PV, and distinct from MPL^{W515L} mutated ET. Contribution of Drs Forstier and De Raeve, University Hospital, Brussels, Belgium