Bone Marrow Histology is a Pathognomonic Clue to Each of the JAK2^{V617F}, MPL, and Calreticulin Mutated Thrombocythemia in Myeloproliferative Neoplasms

Hendrik De Raeve¹,², Karel Fostier³, Francisca Valster⁴, Vincent Potters⁵, Yango Kim⁵,⁶, Myungshin Kim⁵,⁶, Zwi Berneman⁷, Wilfried Schroyens⁷, Jan Jacques Michiels⁷,⁸

¹Department of Pathology, OLV Hospital Aalst, OLV Ziekenhuis, Moorselbaan 164, B-9300 Aalst, Belgium, ²Department of Pathology, Belgium and University Hospital Brussels, Laarbeeklaan 101, B-1090, Brussels, Belgium, ³Department of Hematology, University Hospital Brussels, Laarbeeklaan 101, B-1090, Brussels, Belgium, ⁴Department of Internal Medicine, Bravis Hospital, Boerhaaveplein 1, 4624 VT, Bergen op Zoom, Netherlands, ⁵Department of Pathology, Bravis Hospital, Boerhaaveplein 1, 4624 VT, Bergen op Zoom, Netherlands, ⁶Department of Pathology, Bloodcoagulation and Vascular Medicine Specialist, Goodheart Institute in Nature Medicine and Health, Erasmus Tower, Veenmos 13, 3069 AT Rotterdam, Netherlands, ⁷Department of Hematology, University Hospital Antwerp, Wijirjstraat 10, B-2650, Edegem, Belgium, ⁸Dutch MPN Foundation and International Collaboration and Academic Research on Myeloproliferative Neoplasms, Goodheart Institute, Erasmus Tower, Veenmos 13, 3069 AT, Rotterdam, The Netherlands

ABSTRACT

According to the World Health Organization and Clinical Laboratory Molecular and Pathological criteria bone marrow pathology in JAK2^{V617F} mutated trilinear myeloproliferative neoplasm (MPN) patients essential thrombocythemia (ET) and polycythemia vera are indistinguishably featured by clustered medium to large pleomorphic megakaryocytes and increased cellularity (60–90%) due to increased erythropoiesis and megakaryopoiesis. MPL^{515} mutated ET is the second distinct clonal MPN characterized by thrombocythemia in a normocellular bone marrow showing clustered increased large to giant mature megakaryocytes with staghorn-like hyperlobulated nuclei. Calreticulin (CALR) mutated hypercellular thrombocythemia associated with prefibrotic megakaryocytic, granulocytic myeloproliferation (MGM) recently became the third distinct MPN characterized by thrombocytemia in a normocellular bone marrow showing clustered increased large to giant mature megakaryocytes with cloud-like nuclei. Bone marrow pathology in newly diagnosed MPN patients appears to be a pathognomonic clue for diagnostic differentiation between JAK2^{V617F} mutated trilinear MPN, MPL^{515} normocellular thrombocythemia, and CALR thrombocythemia with MGM characteristics followed by secondary reticulin fibrosis. Their natural histories clearly differ featured by an increase of erythro/granulopoiesis and cellularity in JAK2^{V617F}, decrease of erythropoiesis and cellularity in MPL^{515} and increase of dual megakaryo/granulopoiesis and cellularity in CALR mutated MPN.

Key words: Bone marrow pathology, calreticulin mutation, essential thrombocythemia, JAK2 mutation, MPL mutation, myelofibrosis, polycythemia vera, reticulin fibrosis

Address for correspondence:
Jan Jacques Michiels, Multidisciplinary Internist and Scientific Investigator, Goodheart Institute and Foundation in Nature Medicine, Rotterdam, International Collaboration and Academic Research on Myeloproliferative Neoplasms, Erasmus Tower, Veenmos 13, 3069 AT Rotterdam, The Netherlands. E-mail: goodheartcenter@outlook.com

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INTRODUCTION

The clinical and pathological features for prodromal erythrocythemia and classical polycythemic stages of polycythemia vera (PV) are variable and featured by increased erythrocytes above $6 \times 10^{12}/L$, increased leukocyte alkaline phosphatase (LAP) score (increased CD11b expression), normal or increased platelets, leukocytes, and spleen size, and by characteristic bone marrow features with increased pleomorphic large megakaryocytes and erythropoiesis [Table 1]. The peripheral blood findings in “true” essential thrombocythemia (ET) without features of ET. Any other criterion confirms MPL

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<th>Clinical criteria PV</th>
<th>Pathological criteria JAK2V617F mutated PV</th>
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<tr>
<td>A1 Erythrocyt count below and above $6 \times 10^{12}/L$ in ET and PV respectively. Levels below or above hemoglobin 18.5 g/dL male and $&gt;16.5$ g/dL female in ET and PV resp.</td>
<td>B1 Normal to increased cellularity due to increased erythropoiesis or due to trilinear myeloproliferation of megakaryopoiesis, erythropoiesis, and granulopoiesis (e.g., pannemalosis). The proliferation of small medium-sized and large (pleomorphic) megakaryocytes. The absence of stainable iron, No or slight increase of reticulon fibers.</td>
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<tr>
<td>A2 Persistent increase of platelet count Grade I between 400 and $1000 \times 10^9/L$, Grade II above ($&gt;1000 \times 10^9/L$)</td>
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<td>A3 Splenomegaly on echogram or computed tomography ($&gt;12$ cm) or splenomegaly on palpation</td>
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<td>A4 Granulocytes $&gt;10 \times 10^9/L$ or leukocytes $&gt;12 \times 10^9/L$ and raised LAP score $&gt;100$ in the absence of fever and no increase of ESR</td>
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<td>A5 Presence of JAK2V617F mutation</td>
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<td>A6 Low plasma or serum EPO level</td>
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<tr>
<th>Clinical criteria “true” normocellular ET</th>
<th>Pathological criteria “true” MPL515 mutated ET</th>
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<tr>
<td>A1 Persistent increase of platelet count Grade I between 400 and $1000 \times 10^9/L$, Grade II above $1000 \times 10^9/L$</td>
<td>B1 Predominant proliferation of enlarged to giant mature megakaryocytes wit hyperlobulated staghorn-like nuclei and mature cytoplasm, lacking conspicuous cytological abnormalities</td>
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<td>A2 Normal spleen or only minor splenomegaly on echogram</td>
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<td>A3 Normal LAP score, normal plasma or serum EPO, normal ESR and increased MPV</td>
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<td>A4 Spontaneous megakaryocyte colony formation (CFU‑Meg)</td>
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<td>A5 Presence of MPL515 mutation</td>
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<tr>
<td>A6 No preceding or allied other subtype of MPN, PV, MDS, or CML</td>
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<th>Clinical criteria PMGM</th>
<th>Pathological criteria CALR mutated PMGM</th>
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<td>A1 Thrombocythemia with no preceding or allied other subtype of MPN, PV, CML, or MDS</td>
<td>B1 Primary megakaryocytic or megakaryocytic and granulocytic myeloproliferation (PMGM) and relative or absolute reduction of erythropoiesis erythroid precursors Abnormal clustering and increase of atypical immature medium‑sized large to giant megakaryocyte containing (cloud‑like) hypolobulated nuclei and definitive maturation defects</td>
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<tr>
<td>A2 Presence of CALR mutation</td>
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<td>Early clinical‑stage: Platelets Grade I between 400 and $1000 \times 10^9$/and Grade II above $1000 \times 10^9/L$, normal hemoglobin, or anemia Grade I: hemoglobin $&gt;12$ g/dL, slight or moderate splenomegaly on palpation or $&gt;11$ cm on ultrasound or CT. Platelets around or above $1000 \times 10^9/L$</td>
<td>Staging of myelofibrosis: MF in ET, PV, and PMGM</td>
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<td>Intermediate clinical stage: Anemia Grade II, hemoglobin $&gt;10$ g/dL, definitive leukoeerythoblastic blood picture and/or tear‑drop erythrocytes. Splenomegaly on palpation, no adverse signs</td>
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<td>Advance clinical stage: Anemia Grade III, hemoglobin $&gt;10$ g/dL, significant splenomegaly, and adverse signs</td>
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PV are featured by high platelet counts, normal values for hemoglobin, hematocrit, erythrocyte and white blood cells, and normal values for LAP score, LDH and no or minor splenomegaly despite platelet counts above 1000×10⁹/L at time of first presentation.[3-5] The megakaryocytes in “true” ET are larger than in ET preceding PV and classical PV [Table 1].[3-6] The Hannover Bone Marrow Classification of the MPDs distinguished three primary prefibrotic MPDs ET, PV, and primary megakaryocytic granulocytic myeloproliferative (PMGM) from the advanced fibrotic stages of MPD.[7]

Bone marrow pathology in PV is typically featured by large pleomorphic megakaryocytes with hyperploid nuclei in a hypercellular bone marrow due to increased erythropoiesis or increased trilinear erythrocytic, megakaryocytic, granulocytic myeloproliferation (EMGM).[6] Michiels and Thiele defined normocellular “true” ET as a distinct myeloproliferative neoplasm (MPN) entity different from classical PV and hypercellular ET associated with prefibrotic primary myelofibrosis (pPMF), which is consistent with primary megakaryocytic granulocytic myeloproliferation [PMGM, Table 1].[3-6] Georgii et al. labeled pPMF as the third MPD entity of chronic or primary MGM (CMGM/PMGM) in the absence of reticulin or collagen fibrosis in bone marrow biopsy material.[7,8] Michiels et al. replaced the term CMGM by primary MGM (PMGM) as the third JAK2 wild-type MPN without features of PV or CML.[9] Hypercellular JAK2 wild-type ET associated with PMGM 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 forms (so-called cloud-like nuclei), which are never described in JAK2 V617F mutated ET and PV [Figures 1-4].[4-6] The natural history of JAK2 V617F thrombocythemia and PV is best reflected by the increase of erythropoiesis, granulopoiesis, and cellularity followed by the degree of anemia, splenomegaly, bone marrow cellularity, and an increase of reticulin and collagen fibrosis.[7,8]

**BONE MARROW PATHOLOGY IN MPL 515 MUTATED NORMOCELLULAR THROMBOCYTHEMIA**

Bone marrow histology from a patient with thrombocythemia carrying the MPL W515L mutation displayed clusters of large megakaryocytes with a greater number of giant megakaryocytes with hyperlobulated stag-horn nuclei in normal cellular bone marrow and no increase of erythropoiesis [Figures 1-4].[9-13] We here extend our previous descriptions on the differential diagnostic significance between patients with MPL 515 mutated (n = 12) versus JAK2 V617F mutated trilinear MPN. The presence of clustered small and giant megakaryocytes with deeply lobulated staghorn like hyperlobulated nuclei [Figures 1-4] in MPL 515 mutated thrombocythemia is not seen in JAK2 V617F mutated ET and PV [Figures 5 and 6] and calreticulin (CALR) thrombocythemia. The increase of erythropoiesis is not seen in MPL 515 mutated ET [Figures 1-4].[6] MPL 515 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. JAK2\textsuperscript{V617F} mutated ET [Figure 5] show local increase of erythropoiesis in areas of loose clustered pleomorphic megakaryocytes in normocellular JAK2\textsuperscript{V617F} mutated ET [Figure 5], whereas bone marrow is hypercellular due to increased erythropoiesis and megakaryopoiesis (EM) \([12,13]\) JAK2\textsuperscript{V617F} mutated ET, prodromal PV, and classical PV have increased score for LAP stain, low serum EPO and pleomorphic medium-sized to large mature megakaryocyte morphology [Figures 5 and 6]. In contrast, MPL\textsuperscript{515} mutated normocellular ET has normal values for LAP score, serum EPO and ferritin levels.\([3,6,9-11]\) As demonstrated in Figures 2-4, the natural history of MPL\textsuperscript{515} normocellular ET is best reflected by decreased cellularity due to decreased erythropoiesis and increase of reticulin fibrosis (RF) from Grade 0 to Grades 1, 2, and 3.

**BONE MARROW HISTOLOGY IN CALR MUTATED ET AND MF**

We found consistent bone marrow characteristics of hypercellular ET as the presenting feature of prefibrotic and early fibrotic or even advanced stages of PMGM in 13 consecutive newly diagnosed CALR positive ET cases (manuscript in preparation). 13 CALR mutated PMGM patients do not present with ET complicated by aspirin-sensitive microvascular disturbances of erythromelalgic, cerebral and ocular ischemic manifestations (Sticky Platelet Syndrome) as the specific presenting manifestations of JAK2\textsuperscript{V617F} and MPL\textsuperscript{515} mutated myeloproliferative thrombocythemia. Bone marrow histology in prefibrotic CALR Thrombocythemia in Figure 7 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 PMGM, which are not seen in MPL mutated ET [Figures 1-4] and also not in JAK2/MPL wild-type hypercellular essential thrombocythemia in 2006/2008: Platelets 1205×10^9/L, Hb 12.5 g/dl, leukocytes 18×10^9/L, borderline LDH, and spleen size 13 cm on echogram (normal value <12 cm) as the presenting of primary megakaryocytic granulocytic myeloproliferative with reticulin fibrosis Grade 2 and diagnosed according to World Health Organization – Clinical Laboratory Molecular and Pathological criteria as CALR mutated thrombocythemia with secondary myelofibrosis in 2014 [Table 1]. Case described by Michiels et al. 2015 [6].

Figure 9: (a-d) Standardized set of bone marrow histology in a case of calreticulin mutated thrombocythemia and myelofibrosis (formerly diagnosed as primary megakaryocytic granulocytic myeloproliferation and myelofibrosis) with symptomatic anemia and splenomegaly featured by dense clustered immature large megakaryocytes with immature cloud-like nuclei and increased reticulin fibrosis reticuline fibers Grade 2 with CALR mutated PMGM, which are not seen in MPL mutated ET [Figures 1-4] and also not in JAK2/MPL wild-type hypercellular essential thrombocythemia in 2006/2008: Platelets 1205×10^9/L, Hb 12.5 g/dl, leukocytes 18×10^9/L, borderline LDH, and spleen size 13 cm on echogram (normal value <12 cm) as the presenting of primary megakaryocytic granulocytic myeloproliferative with reticulin fibrosis Grade 2 and diagnosed according to World Health Organization – Clinical Laboratory Molecular and Pathological criteria as CALR mutated thrombocythemia with secondary myelofibrosis in 2014 [Table 1]. Case described by Michiels et al. 2015 [6].

Figure 8: (a-f) Clinical case of calreticulin (CALR) positive thrombocythemia in a 37-year-old woman (asymptomatic except fatigue) with JAK2/MPL wild-type hypercellular essential thrombocythemia in 2006/2008: Platelets 1205×10^9/L, Hb 12.5 g/dl, leukocytes 18×10^9/L, borderline LDH, and spleen size 13 cm on echogram (normal value <12 cm) as the presenting of primary megakaryocytic granulocytic myeloproliferative with reticulin fibrosis Grade 2 and diagnosed according to World Health Organization – Clinical Laboratory Molecular and Pathological criteria as CALR mutated thrombocythemia with secondary myelofibrosis in 2014 [Table 1]. Case described by Michiels et al. 2015 [6].

Figure 7: (a-d) Clinical case of a 24 years man diagnosed in 2014 as calreticulin (CALR) mutated essential thrombocythemia (ET), who presented with aspirin responsive platelet thrombophilia (transient microvascular ischemic disturbances), normal values for hemoglobin, hematocrit and erythrocytes, increased platelet count of 1832×10^9/L and slight splenomegaly (16 cm length diameter on echogram). Bone marrow histology showed increased cellularity (65%) due to megakaryocytic granulocytic myeloproliferation with normal erythropoiesis, dense cluster of immature megakaryocytes with hypolobulated nuclei and no increase of reticulin fibrosis (reticuline fibers Grade 0) consistent with hypercellular ET associated with a typical PMGM bone marrow [Table 1]. Case described by Dr. Valster and Dr. Potters, Bravis Hospital Bergen op Zoom, Netherlands [20].

Figure 6: (a-d) Homozygous mutated JAK2^{V617F} positive polycythemia vera (PV) featured by hemoglobin 11.8 mmol/L, hematocrit 0.58, erythrocytes 6.9×10^{12}/L (normal value <5.8×10^{12}/L), leukocytes 9.1×10^{9}/L, platelets 1023×10^{9}/L, no splenomegaly at time of diagnosis in 2001 showed a typical PV bone marrow picture with increased cellularity (90%) due to increased erythropoiesis and megakaryopoiesis and reticulin fibrosis reticuline fibers Grade 1. This case has been successfully treated by hydroxyurea followed by pegylated interferon for >15 years (manuscript in preparation). Case described by Dr. Michiels and Dr. De Raeve, University Hospital Brussels, Belgium [13].

Figure 9: (a-d) Standardized set of bone marrow histology in a case of calreticulin mutated thrombocythemia and myelofibrosis (formerly diagnosed as primary megakaryocytic granulocytic myeloproliferation and myelofibrosis) with symptomatic anemia and splenomegaly featured by dense clustered immature large megakaryocytes with immature cloud-like nuclei and increased reticulin fibrosis reticuline fibers Grade 2 with CALR mutated PMGM, which are not seen in MPL mutated ET [Figures 1-4] and also not in JAK2^{V617F} mutated ET [Figure 5], prodromal PV and classical PV [Figure 5]. A 37-year-old woman (asymptomatic except fatigue) presented in 2004 with JAK2/MPL wild-type hypercellular...
De Raeve, et al.: Bone Marrow Pathology in Myeloproliferative Neoplasms

ET associated with PMGM featured by platelets $1205 \times 10^9/L$, Hb 12.5 g/dl, leukocytes $18 \times 10^9/L$, borderline LDH, spleen size 13 cm on echogram (normal value <12 cm) and PMGM bone marrow features with RF Grade 1/2 diagnosed according to the WHO - European clinical, molecular, and pathological criteria\[^{[6]}\] [Figure 8 and Table 1]. 10 years later, this case was diagnosed as end-stage CALR mutated MF and treated by allogenic bone marrow transplantation in 2014.

The three PMGM cases in Figures 7-9 clearly demonstrate that the natural history of CALR mutated PMGM, as the third distinct MPN entity, is featured by progressive anemia related to myeloid metaplasia of the spleen with splenomegaly, reduction of bone marrow erythropoiesis and progressive increase of RF Grade 1 to Grade 4.\[^{[14-18]}\] The natural history of CALR thrombocytopenia and MF is best reflected by the degree of anemia, splenomegaly, bone marrow cellularity, and an increase of RF [Figures 7-10].

DISCUSSION

WHO-defined 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 patients.\[^{[6,12,13]}\] 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 EMG is the hallmark of classical prefibrotic PV with no or minor splenomegaly, whereas serum LDH levels, CD34\(^+\) circulating cells and spleen size are more pronounced in advanced JAK2\(^{V617F}\) mutated ET (masked PV) or post-PV MF at high JAK2\(^{V617F}\) mutation allele burden up to 90–100%\[^{[6,13]}\]. Clustered large and giant megakaryocytes with hyperlobulated “staghorn” nuclei are rare in JAK2\(^{V617F}\) mutated MPN, but typically present in MPL\[^{515}\] mutated ET patients with no features of PV in the bone marrow and normal values for serum EPO, ferritin levels and LAP score.\[^{[9,10]}\] The prevalence of MPL\[^{515}\] mutated ET or MF patients ranges from 5% to 10% of the JAK2 wild-type MPN population.\[^{[9-11]}\]

Dr. Kralovics and his team in Vienna Austria first discovered the occurrence of CALR mutation in 78 of 311 (25%) ET patients and in 72 of 203 (35%) MF patients and none of 382 PV patients.\[^{[14]}\] Since 2013 CALR mutated thrombocytopenia and MF became the third distinct MPN entity with no features of PV, which was rapidly confirmed by Nangalia et\[^{[15]}\] and by Rumi et\[^{[16,17]}\]. Nangalia et\[^{[14]}\] found somatic CALR mutations in 110 of 158 JAK2/MPL wild-type MPN, including 80 of 112 (70%) ET patients and 18 of 32 (56%) MF patients.\[^{[15]}\] CALR mutations in the study of Nangalia et\[^{[15]}\] were identified in 10 of 120 (8%) MDS patients (RA in 5 of 53, RARS in 3 of 27, and RAEB-T in 2 of 27), and in one patient each with CMMML and atypical CML.\[^{[15]}\] The bone marrow pathological findings in the present study of CALR mutated thrombocytopenia and MF in Figures 7-10 show dense clusters of large immature megakaryocytes with immature cloud-like nuclei, which clearly differ from the pleomorphic megakaryocytes in JAK2\(^{V617F}\) mutated ET and PV, which indeed are clearly distinct from the giant megakaryocytes with hyperlobulated staghorn-like nuclei in MPL\[^{515}\] mutated ET [Figures 1-4].\[^{[6]}\] Since 2014 Michiels et\[^{[14]}\] demonstrated that cases of hypercellular ET associated with PMGM belong to the third MPN of CALR-mutated thrombocytopenia and MF without features of PV at time.
of diagnosis and during follow-up.\textsuperscript{[6,14-20]} The updated 2018 WHO - Clinical Laboratory Molecular and Pathological (CLMP) criteria define five clonal mutually exclusive MPNs and transitional states at the laboratory and bone marrow level [Figure 11]: JAK2\textsuperscript{V617F} mutated ET, prodromal PV, classical and advanced PV; JAK2 exon 12 mutated idiopathic erythroid dyscrasia and PV; MPL\textsuperscript{515} mutated thrombocythemia and MF; and CALR mutated thrombocythemia and MF and triple negative ET and MF.\textsuperscript{[6,14-20]} The JAK2\textsuperscript{V617F} positive trilinear MPDs, MPL\textsuperscript{515} normocellular thrombocythemia, and CALR mutated thrombocythemia and MF mutually exclude each other [Table 1]. The natural history and MPN disease burden of each of the clonal MPNs is best reflected by the degree of anemia and splenomegaly on top of mutation allele burden, bone marrow cellularity and an increase of RF.\textsuperscript{[6,15-20]} Prospective validation studies are warranted to confirm and amend the proposed WHO-CLMP classification.

REFERENCES


How to cite this article: De Raeve H, Fostier K, Valster F, Potters V, Kim Y, Kim M, Berneman Z, Schroyens W, Michiels JJ. Bone Marrow Histology is a Pathognomonic Clue to Each of the JAK2\textsuperscript{V617F}, MPL\textsuperscript{515} and Calreticulin Mutated Thrombocythemias in Myeloproliferative Neoplasms. Clin Res Hematol 2018;1(2):1-7.