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REVIEW ARTICLE

Utility of bone marrow biopsy in the diagnosis of myeloproliferative neoplasm

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Abstract

A diagnostic approach of myeloproliferative neoplasms, according to the 2008 WHO classification system for hematological malignancies, has to consider clinical, molecular, and cytogenetic information as well as bone marrow histology. A diagnosis of chronic myeloid leukemia requires the presence of BCR-ABL-1, and the Philadelphia chromosome-negative (Ph-1-negative) myeloproliferative neoplasms constitute three main subtypes, including primary myelofibrosis, polycythemia rubra vera, and essential thrombocythemia. These three Ph-1-negative myeloproliferative neoplasms share many pathogenic characteristics such as JAK2 mutations; however, they differ in prognosis, progression to myelofibrosis, and risk of leukemic transformation. There are currently various major points of interest in bone marrow examination in myeloproliferative neoplasms. One is the morphology of megakaryocytes, which are the hallmark of Ph-1-negative myeloproliferative neoplasms and play a crucial role in separating the different subtypes of myeloproliferative neoplasms. Another is reticulin fibrosis or collagen fibrosis, which may only be detected on a bone marrow biopsy specimen by reticulin and trichrome stains, respectively, and immunohistochemistry and certain molecular techniques may be applied in bone marrow biopsies as supporting evidence of certain features of myeloproliferative neoplasms. (Gac Med Mex. 2016;152:366-76)

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Introduction and brief historical review

Myeloproliferative neoplasms (MPN) are a heterogeneous group of clonal-origin hematopoietic stem cells alterations, characterized by excessive production of myeloid-lineage cells, which is reflected in increased cellularity in peripheral blood and bone marrow (BM)[1,2].

Of the four classical MPNs, three, polycythemia vera (PV or Vaquez-Cabot-Osler disease), primary myelofibrosis (PMF or Heuck-Askanazy-Assmann disease) and chronic myeloid leukemia (CML) were described in 1951 by the American-nationalized Russian physician William (Ze ev) Dameshek (1900-1969) (Fig. 1). Essential thombocythemia (ET or Epstein-Goedel disease), originally known as hemorrhagic thrombocytemia, does not appear in Dameshek's original list[3,4].

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In the article entitled *Some speculations on the myeloproliferative syndromes*, which appeared in the *Blood* magazine in 1951, Dameshek indicates that: "It is possible that these various conditions –myeloproliferative disorders– are all somewhat variable manifestations of proliferative activity of the bone marrow cells, perhaps due to a hitherto undiscovered stimulus"[3]. The WHO 2008 hematopoietic neoplasms classification includes other entities in this MPN group such as chronic neutrophilic leukemia, mastocytosis, chronic eosinophilic leukemia, not otherwise specified, and MPN, unclassifiable[5]. Among MPNs, CML is the only one that has the Philadelphia 1 (Ph1) chromosome and, therefore, sometimes MPNs are classified as positive (Ph1+) (CML) and negative (PV, ET, PMF) to chromosome Ph1[5,6].

The Ph1 chromosome was discovered by Peter Nowell (1928-) from the Pennsylvania University and David Hungerford (1927-1993), from the Fox Chase Cancer Center Institute in Philadelphia, in two patients with CML. In a letter to the editor published in the *Science* magazine in 1960, they described this finding that turned out to be the first documentation of a genetic alteration in tumors[4]. The observation in these patients of a small, abnormal chromosome, similar to the Y chromosome, prompted Nowell to propose the hypothesis that this genetic variation might be what drove the cell to abnormal growth[7]. Using Giemsa, quinacrine and acridine orange dyes for chromosome banding (Q-banding), enabled for the Ph1 chromosome to be identified in chromosome 22, and by 1972, the University of Chicago geneticist Janet Rowley (1925-2003) identified that the Ph1 chromosome was the result of a reciprocal translocation between chromosomes 9 and 22 (t[9;22][q34;11]). And it was in the 80's when the v-abl (Abelson) human analog (*ABL*; 225 kb) to chromosome 9 was located and, subsequently, chromosome 22 breakpoint site in a 5.8 kb area that was named breakpoint cluster region (bcr). In 1985, Owen Witte and David Baltimore (1975 medicine Nobel Prize) identified the *BCR-ABL* fusion. Negative Ph1 chromosome-negative MPNs (PV, ET and PMF) variably show mutation of *Janus kinase (JAK) 2*, located at chromosome 9p24, which was simultaneously discovered by 4 groups of independent investigators (Gary Gilliland, William Vainchenker, Radek Skoda and Anthony Green), and this is why PV, ET and PMF are also known as *JAK2*-positive MPNs[4]. *JAK2* gene's most prevalent mutation is that characterized by point mutation (G to T-transversion), which results in the substitution of valine for phenylalanine in the JHJ2 domain at the amino acid 617 position (V617F) of Jak2 protein[8].

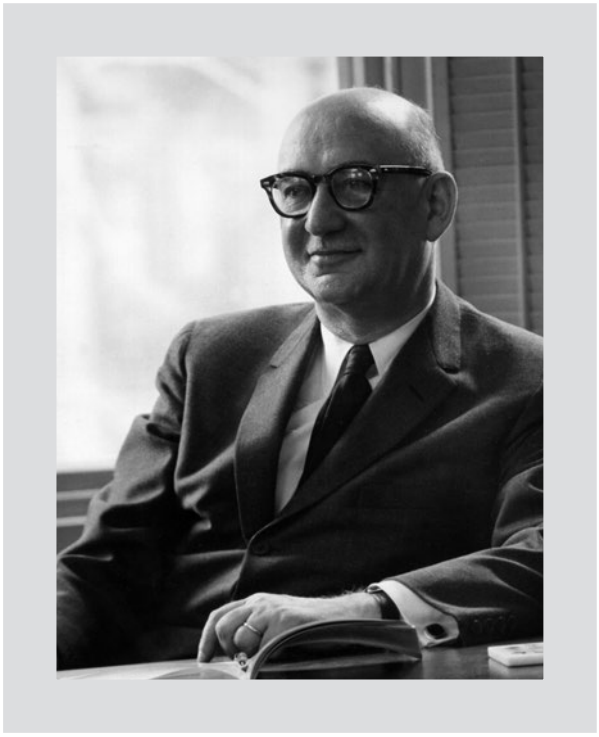


Figure 1. *William Dameshek (1900-1969) (courtesy of: Digital Collections and Archives. Tufts University).*

As a result of this mutation, the Jak2 protein activates multiple signaling pathways that affect transcription genes, which results in hematopoietic cell proliferation[6]. The determination of *JAK2* mutation is useful in the diagnosis and classification of MPNs[6].

For an adequate morphological assessment of MPNs, blood and BM components had first to be adequately identified. The first observation of red blood cells is attributed to the Dutch anatomist and zoologist Jan Swammerdam (1637-1680), and it was Antoni Leeuwenhoek (1632-1723) who described red blood cells size and shape and made the first illustration in 1695[4,9]. British physician William Hewson (1739-1774) (called the father of hematology), was who described granulocytes, and megakaryocytes were described by Johns Hopkins University North American physiologist William Henry Howell (1860-1945) in 1890[9]. The credit for the discovery of platelets is shared by the French histologist Alfred Doné (1801-1878), the German cytologist Max Schulze (1825-1874) and the Italian pathologist Giulio Bizozero (1846-1901) (who also discovered *Helicobacter pylori* and introduced Camillo Golgi to the study of histology)[10]. Blood cell morphology was specified by 1907 medicine Nobel Prize Paul Ehrlich (1854.1925), who, in 1877, used special dyes and, according to the staining affinity, he divided granulocytes

into basophils, eosinophils and neutrophils[4]. Routine BM study was started in Germany by the pathologist Ernst Christian Neumann (1747.1829) (who was Virchow's pupil and also demonstrated BM hematopoiesis, proposed that all cells originated from a precursor cell –stem cells– and also that leukemia was originated in BM abnormal cells), and in Italy, by another Virchow pupil, Giulio Bizzozero[11].

In MPNs, when observing the morphology in BM biopsies, the pathologist may tend to ask too much of him/herself by trying to arrive to an accurate diagnosis and be very conclusive; however, the WHO diagnostic criteria for each one of the MPNs are not only based in BM histological characteristics, but blood count, BM aspirate, molecular biology study and cytogenetic results are also necessary. Therefore, it is not the pathologist's obligation issuing a definitive diagnosis based exclusively on his/her observations in the bone biopsy. However, he/she should be able to recognize and identify certain histological characteristics that identify MPNs.

The purpose of this manuscript is to provide morphological, histochemical and immunohistochemical data to aid diagnosis when assessing a BM biopsy of a patient with clinical suspicion of MPN, with an emphasis on key points to facilitate BM interpretation according to the WHO criteria[5].

MPN histological diagnosis

The WHO 2008 hematopoietic neoplasms classification incorporates BM examination histological characteristics in MPNs, including: cellularity, maturation and distribution of each hematopoietic element, as well as blast location, reticular fibers and collagen fibers assessment and presence of hemosiderin[12,13].

BM biopsy has to be longer than 1.5 cm for adequate assessment[14]. Normally, the first 3 intertrabecular spaces beneath the cortical bone are hypocellular and, therefore, the presence of hypercellularity, similar to that in other intertrabecular spaces, indicates a problem in BM that might be the first suspicion of MPN. At least 4 intertrabecular spaces should be available (beneath the first 3 subcortical spaces) to adequately assess hematopoiesis. The use of hematoxylin and eosin stain is recommended together with one for reticular fibers (vide infra). Some additional dyes include periodic acid-Schiff (PAS), which can be used if megakaryocyte morphology has to be further assessed and visualize dysplastic changes in different hematopoietic series. Giemsa staining is useful to assess different cell lineages (especially the erythroid series) and the Leder technique (naphthol-AS-C-chloroacetate esterase) is used to assess the granulocytic series. Hemosiderin assessment can be made with Perls or Prussian blue (*Berliner Blau*) staining, but it is better making it in BM aspirate smear rather than in slices, since the decalcification process can alter the hemosiderin content. Immunostaining, according to diagnostic impression, may include glycophorin A, spectrin or CD71 (transferrin receptor) for erythropoiesis; myeloperoxidase, CD33 (Siglec-3), CD15 (LeuM1) or CD13 (aminopeptidase N) for granulopoiesis; CD61 (Integrin beta-3), CD42b (platelet glycoprotein Ib), factor VIII (FVIII)-related antigen for megakaryocytes and for myeloid-differentiation blasts, CD34 and CD117 (c-kit) can be used[15].

In MPNs, the pathology report must include: cellularity, myeloid:erythroid ratio, erythropoiesis (quantity and distribution, hematopoietic islands appearance, morphology and degree of maturation), granulopoiesis (morphology and distribution, maturation and localization of precursors), megakaryopoiesis (quantity, morphology, localization and degree of grouping, as well as presence of abnormal nuclear lobulations and degree of nuclear atypia/dysplasia), presence of mast cells, pseudo-Gaucher cells, lymphocytes and plasma cells, sinusoidal dilatation and presence of hematopoietic precursors and megakaryocytes within. The amount of reticulin fibrosis should be assessed using the Thiele scale[16] (Table 1). Some protocols include blood vessel count (angiogenesis) with CD31 (PECAM-1 – Platelet Endothelial Cell Adhesion Molecule-1) or CD34[17].

PV (Vaquez-Cabot-Osler disease)

PV or primary polycythemia, erythremia or Vaquez-Cabot-Osler disease is a MPN where there is an increase mainly in erythrocytes (red blood cells), but there can be also leukocytosis and thrombocytosis[18]. It occurs mainly in adults at between 50 and 60 years of age, it is more common in males and rarely affects adolescents. Clinical signs and symptoms are related to hyperviscosity, coagulopathy and excessive hematopoiesis, and include arterial and venous thromboembolism, vascular disease, neurological deficit and mild hepatosplenomegaly. Many patients can be asymptomatic. PV can occur as *de novo* disease, treatment-related disease, or secondary to ET, PMF and CML transformation. Clinical course is usually indolent, with possible transformation to fibrosis and osteosclerosis, or other

Table 1. Criteria for medullary fibrosis grading according to WHO 2008 criteria (Thiele's classification)

Grade	Description
Grade 0 myelofibrosis	Linear, disperse reticular fibers without intersections (cross-over), corresponding to normal bone marrow
Grade 1 myelofibrosis	Reticular fiber loose network with many intersections, especially in perivascular areas.
Grade 2 myelofibrosis	Reticular fiber diffuse and dense increase with broad intersections, occasionally with collagen fibers and/or focal osteosclerosis.
Grade 3 myelofibrosis	Diffuse and dense reticular fibers with broad intersections and thick collagen bands, often associated with osteosclerosis

MPN, myelodysplastic syndrome (MDS) and/or acute leukemia in approximately 1-5%[18]. *JAK2 V617F* mutation occurs in up to 95% of patients with PV[8].

In BM aspirate smears, the erythroid series is predominant. Megakaryocytes are abundant and mature, and large forms with nuclear hyperlobulation can be observed. BM biopsy is a minor criterion in PV diagnosis[5,6]. In classical cases, BM is hypercellular with panmyelosis with erythrocyte, granulocyte and megakaryocyte proliferation (Fig. 2A). There is predominant increase of the erythroid series that can be made evident by means of glycopherin A, spectrin and/or CD71 immunostaining (Fig. 2B). Characteristically, megakaryocytes are markedly increased, with variable pleomorphism and can

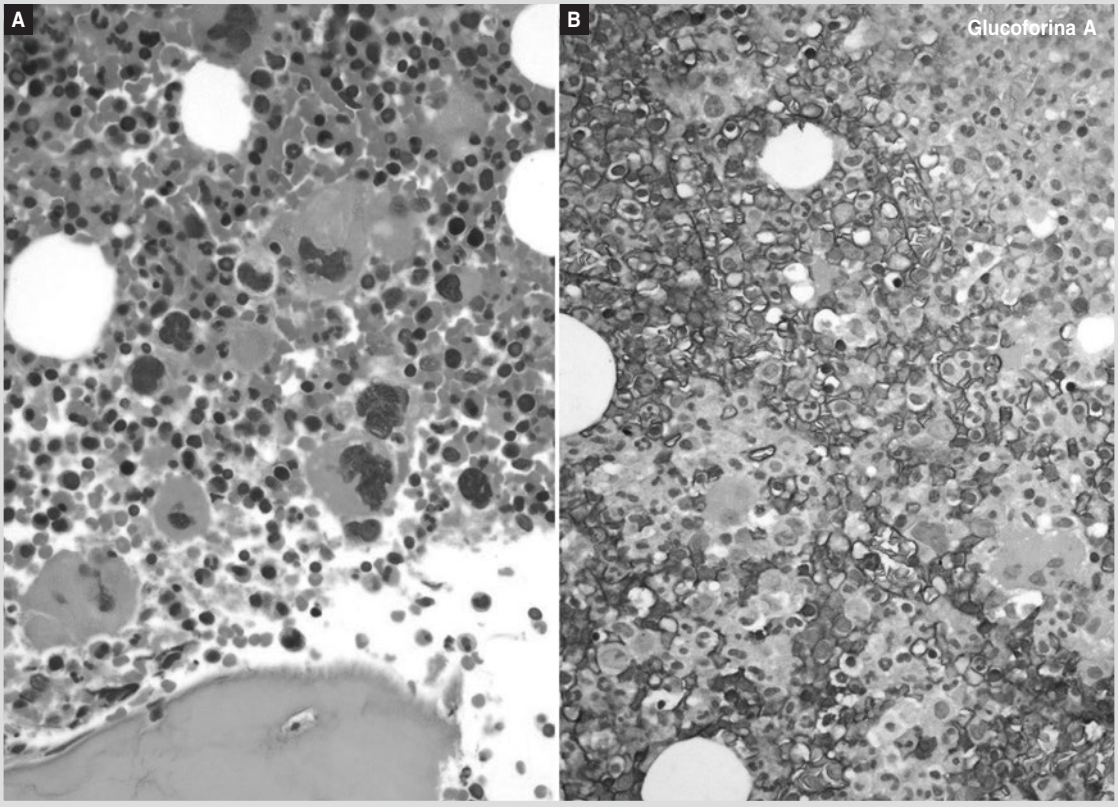


Figure 2. A: *bone marrow biopsy in a case of polycythemia vera. There is an increase in the number of megakaryocytes with small, medium and large shapes, with irregular nuclear lobulations, and forming groups with paratrabecular disposition.* **B:** *immunostaining with glycophorin A highlights erythroid series increase.*

have hyperlobulated nuclei. There is a mixture of pleomorphic megakaryocytes with different cell size (small, medium and large), which are grouped in intertrabecular or paratrabecular clusters and or/disperse (it is important to remember that megakaryocytes normally don not group with each other, but they are disperse in the BM). There is an increase in reticular fibers in some cases. Occasionally, trabeculae show irregular thickening, even reaching osteosclerosis, and benign lymphoid aggregates, reactive histiocytosis and granulomas can be observed. Treatment can reduce cellularity, but not necessarily reverts fibrosis. In occasions, there is an increase in the number of plasma cells (which are polyclonal), and there may be numerous mature eosinophils[5,6]. Iron, which can be evidenced by means of Perls staining, is generally decreased or absent due to excessive erythropoiesis, which constitutes a point in differential diagnosis with other MPNs[16].

Immunohistochemistry has no particular pattern, but it is useful to identify the increased erythroid series, which becomes evident with glycophorin A, spectrin and/or CD71. In case accelerated phase or leukemic transformation is suspected, myeloid blasts can be stained with CD34 and CD117[19].

ET (Epstein-Goedel disease)

The first description of ET is attributed to Emil Epstein (1875-1951) and Alfred Goedel, pathologists of the *Kaiserin Elisabeth-Spital* in Vienna. In 1934, they published the case of a 54-year old patient with moderate erythrocytosis and leukocytosis and sustained platelet increase who had suffered constant headaches and hemorrhages for 4 years and died 2 months after amputation of a right toe[4]. They called this condition "hemorrhagic thrombocytemia".

ET annual incidence ranges from 0.59 to 2.53/100,000 inhabitants, and its prevalence is about 30/100,000, which is similar to that of PV. Mean age at diagnosis is 65 to 70 years, but, occasionally, it can occur in children, and it is relatively common in women at the third or fourth decades of life. Clinical signs and symptoms are related to coagulopathy and include thromboembolism, hemorrhage and mild hepatosplenomegaly and sometimes erythromelalgias; however, many patients can be asymptomatic and only be detected in routine exams. Clinical course is usually indolent, with possible transformation to fibrosis and osteosclerosis, other MPN, MDS or acute leukemia in approximately 1 to 55% of patients. The risk for transformation is related to the type and duration of treatment[19].

ET is a MPN that mainly affects the megakaryocytic lineage and is considered an exclusion diagnosis, since thrombocytosis is a common phenomenon among the other MPNs[19]. It is characterized by sustained thrombocytosis higher than (>) 450 x 10^9/l in peripheral blood, and neutrophilia with normal or slightly decreased hemocrit is often found. Platelets display anisocytosis (coexistence of small and large platelets), and occasionally they are irregular (motley), with pseudopod formation and cytoplasmic granule decrease. BM smear shows variable myeloid:erythroid ratio. In hematopoietic cells maturation there is no major alteration in untreated patients and, characteristically, there are numerous megakaryocytes with large shapes and nuclear hyperglobulation. Iron deposits are decreased or absent, which is a finding that not necessarily reflects iron deficiency. The detection of mutations in the *JAK2* (more commonly), *CALR* (calreticulin), *MPL* (thrombopoietin receptor) or *TET2* (tet methylcytosine dioxygenase 2) genes is helpful to differentiate ET from reactive thrombocytosis, but BM histological examination is also helpful to differentiate it from other MPNs[20].

In histological slices, BM is hypercellular, predominantly affecting the megakaryocytic lineage and without marked left-shift in granulopoiesis and with adipose tissue preservation. Megakaryocytes are large or gigantic, with abundant cytoplasm, which sometimes displays emperipolesis[21]. It is quite characteristic of ET that megakaryocytes exhibit multiglobulated nuclei that can resemble "deer antlers" (Fig. 3). Conversely to the megakaryocyte dense clusters observed in PV and PMF, megakaryocytes in ET are arranged in small paratrabecular clusters or clusters close to the sinusoids or appear as isolated megakaryocytes. This features differ from pre-fibrotic phase early PMF, where megakaryocytes are dysplastic with marked atypia, show dense grouping and exhibit hyperchromatic and hyperlobulated nuclei (cloud-shaped or bulbous), with marked alteration of the nucleus:cytoplasm ratio. In addition, frequently there is hypercellularity with increased neutrophil granulopoiesis with left-shift (megakaryocytic-granulocytic myelosis) (vide infra)[22]. In ET, there is grade 0 or 1 fibrosis and, therefore, if ET diagnosis is being considered and BM displays marked fibrosis (grade 3), the diagnosis most probably does not correspond to ET[19].

PMF (Heuck-Askanazy-Assmann disease)

PMF, agnogenic myeloid metaplasia, is characterized by gradual evolution of a pre-fibrotic initial phase

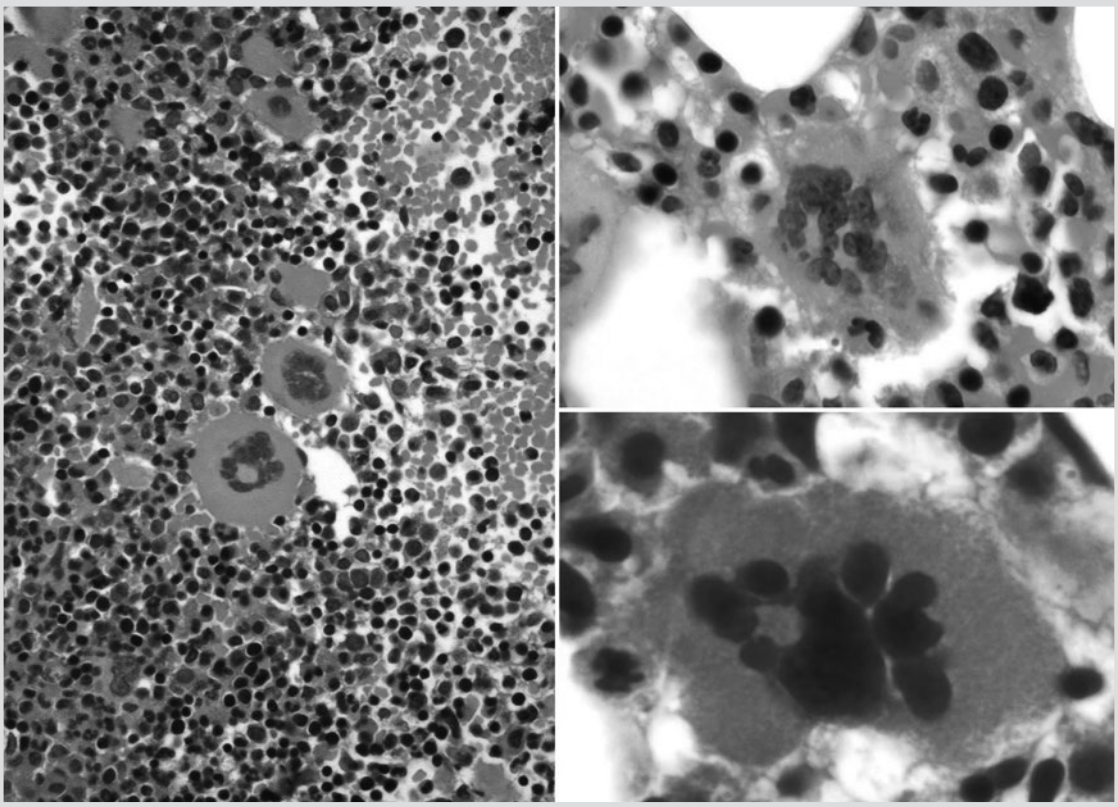


Figure 3. *Bone marrow in a patient with essential thrombocytopenia. Megakaryocytes are increased in number and are large with multilobulated nucleus.*

with hypercellular BM and absent or minimal reticulin fibrosis, which can evolve to clearly fibrotic phase (grade 3 reticulin fibrosis) that is positive to Masson staining (type I collagen), often accompanied by osteosclerosos[23]. The WHO classification (2008) emphasizes on exclusion of PV, ET, CML and MDS, and inclusion of molecular makers such as *JAK2* and *MPL*[24]. Characteristically, in PMF there is no Ph1 chromosome or *BCR-ABL* rearrangement, and *JAK2 V617F* mutation can be found in up to 50% of cases[25].

Clinically, patients with PMF may show splenomegaly with myeloid metaplasia, and in peripheral blood, tear-shaped erythrocytes (dacryocytes), presence of myeloid immature myeloid cells, erythroblasts and abnormal megakaryocytes (vide infra)[19]. Clinically, PMF is indistinguishable from PV and ET transformation to MF, and trying to distinguish it is probably unimportant, since its treatment is similar[19].

The reticulum (term proposed by M. Siegfried in 1892), or also called argyrophilic fibers, corresponds to type III collagen (which is comprised by 3 type α [III] collagen chains and contains 10% of carbohydrates), and becomes evident by means of Gordon-Sweet, Jones methenamine silver or Gömöri staining. With these stains, reticular fibers turn black. The advantage of the Gordon-Sweet technique lies in that reticular fibers (type III collagen), which appear in intense black color, can be distinguished from type I collagen, which stains grey-brown in color. It is important knowing that Masson staining only paints type I collagen (formed by two type α1 [I] collagen chains and one type α2 [II] chain and contain 1% of carbohydrates) in blue color and, thus, assessing reticular fibers is not useful[26]. Fibrosis is secondary to the production of platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), lysyl oxidase and tissue inhibitors of matrix metalloproteinases, released by megakaryocytes and abnormal platelets[26]. These factors stimulate collagen overproduction by fibroblasts and medullary endothelial cells[27].

One of PMF's characteristics is a predominant proliferation of megakaryocytes and granulocytes which,

in stages of full fibrotic development of the disease, are associated with hepatosplenomegaly, peripheral blood leukoerythroblastosis, cytopenias, extramedullary hematopoiesis and BM increased vascular density. As a matter of fact, MFP is the condition with most angiogenesis among all MPNs, which may be a tool to differentiate PMF from other MPNs[28-30]. PMF occurs at middle age and in the elderly, although some cases have also been described in children[5]. Clinical signs and symptoms are related to cytopenias, coagulopathy and excessive hematopoiesis. Massive hepatosplenomegaly is often observed at late phases of the disease, and the course of the condition is progressive, with increased cytopenia and possible transformation to MDS and/or acute leukemia. Survival is significantly worse than in PV and ET. Cases of clonal B and plasma cell neoplasms have been reported in patients with PMF[5,29].

Peripheral blood usually shows anemia, neutrophilia with immature cells, irregular and hypogranular platelets and naked megakaryocyte nuclei. Over time, neutropenia, thrombocytopenia and circulating blasts may appear. BM aspirate smears can be hypercellular in MFP early phases and hypocellular in patients with advanced fibrosis. There is marked increase in the number of megakaryocytes displaying marked dysplasia, with strong variation in size, maturation, nuclear shape and irregular lobulations. Some nuclei are highly hyperchromatic with low nucleus:cytoplasm ratio (in favor of the nucleus). Iron deposits are decreased or absent, which is a finding not necessarily reflecting iron deficiency[1,5,19].

Between 30 and 40% of PMF patients experience a pre-fibrotic or early fibrotic stage. In the PMF prodrome, BM shows marked hypercellularity with proliferation of the granulocytic and megakaryocytic lineages, and there is a decrease and/or arrest in erythroid precursors' maturation. Reticulin fibrosis is minimal or absent at pre-fibrotic stage (with grade 0 or grade 1 reticulin fibrosis). If there is fibrosis, it tends to be focal and located around bone vessels and trabeculae. Characteristically, megakaryocytes show notorious cytological anomaly (accentuated dysplasia) (Fig. 4A) and display extensive grouping; therefore, megakaryocyte topography and morphology are the key in the recognition of PMF pre-fibrotic stage[19]. Generally, this megakaryocyte proliferation with marked grouping and abnormal paratrabecular location is pretty obvious and notorious; however, owing to extensive fibrosis, megakaryocytes are sometimes difficult to assess and, therefore, immunostaining with CD61, CD42b or anti-FVIII can be useful to identify them. As in BM aspirate, megakaryocytes in the BM biopsy can be found in clusters or appear dispersedly and be predominantly large with high degree of nuclear pleomorphism, with variations in size, increased nuclear folding and aberration in the nucleus:cytoplasm ratio caused by a large, hyperchromatic, lobulated nucleus[19] (Fig. 4B). In addition to this disordered nuclear lobulation, numerous megakaryocyte "nude nuclei" can be identified, as well as aggregated platelet groups and/or disperse platelets in the intertrabecular space, with some giant platelets[19,23,24]. As indicated in previous paragraphs, it is common for vascular proliferation to be increased, which can be made more evident with CD34 and CD31 immunostaining (Fig. 4C), and there may be lymphoid nodules in up to 30% of cases[19,30].

It should be emphasized that a very important cytological piece of information to consider for PMF diagnosis is that megakaryocytes are characterized for having the highest degree of cytological atypia (dysplasia) among all MPNs[22]. This dysplasia is one of the most important features to differentiate PMF pre-/early fibrotic stage from ET. Most patients with pre-/early fibrotic stage MF can experience transformation to overt MF (grade 3 fibrosis), associated with extramedullary hematopoiesis (myeloid metaplasia), which produces hepatosplenomegaly[19].

Advanced PMF classic image includes leukoerythroblastic reaction in peripheral blood smears with poikilocytosis with dacryocytes (tear-shaped erythrocytes), splenomegaly and anemia of variable degree, associated with marked BM reticulin and/or collagen fibrosis (grade 2 or 3 fibrosis). In addition to BM grade 3 fibrosis, osteosclerosis is an additional feature that indicates evolution to end stage. The medullary architectural aspect with hematoxylin and eosin of elongated cells resembling a "stream" (streaming effect) is a sign of underlying fibrosis (Fig. 5A). As in the pre-fibrotic stage, megakaryopoiesis with accentuated dysplasia is the most evident feature, in addition to the presence of megakaryocyte dense aggregates and numerous nude nuclei. In most cases of advanced PMF, as a result of fibrosis, small tortuous vessels and dilated sinusoids can be identified, with intraluminal, especially megakaryocytic, hematopoiesis[30]. Immunostaining with CD34 and CD117 for blast identification is important, since in PMF there are few blasts, and an accelerated phase is considered when there are between 10 and 20% of blasts, and transformation to acute myeloid leukemia if blast count exceeds 20%[19].

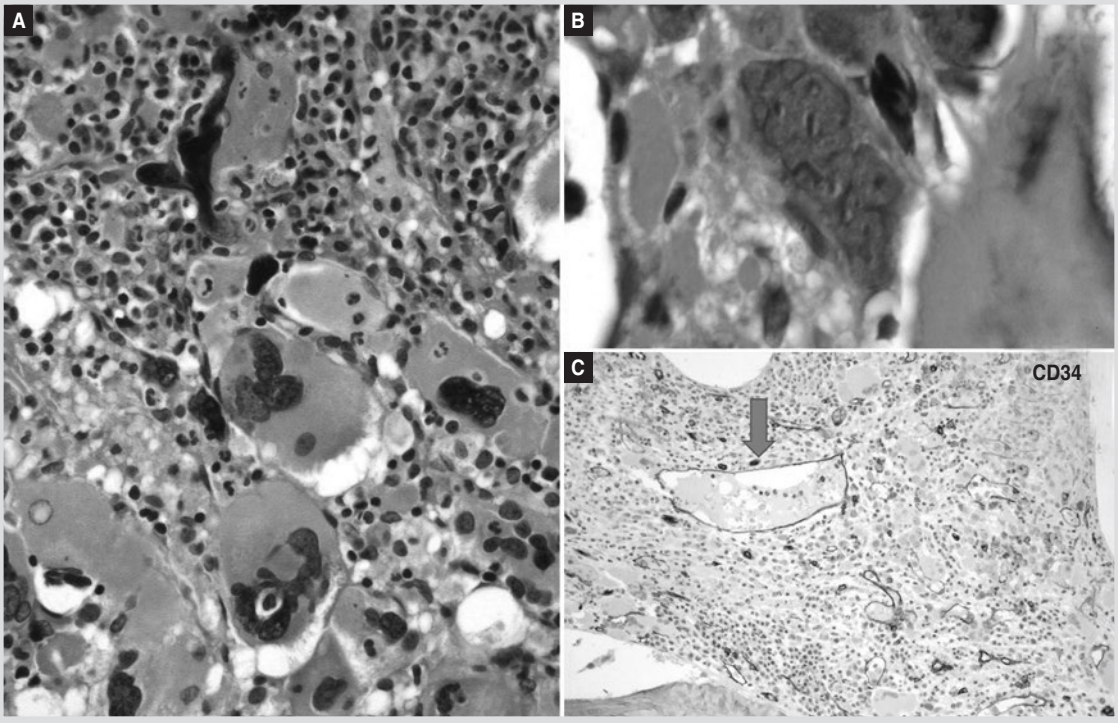


Figure 4. *Primary myelofibrosis. Cellularity is increased and megakaryocytes are prominent and abnormal, showing accentuated dysplasia* **(A)**. *Some megakaryocytes have lobulated nuclei with fine granular chromatin, which has been compared to "clouds"* **(B)**. *Among myeloproliferative neoplasms, primary myelofibrosis is the one displaying more angiogenesis.* **C:** *it is stained with CD34 and shows prominence of small neo-formed vessels and sinusoidal dilatation (arrow).*

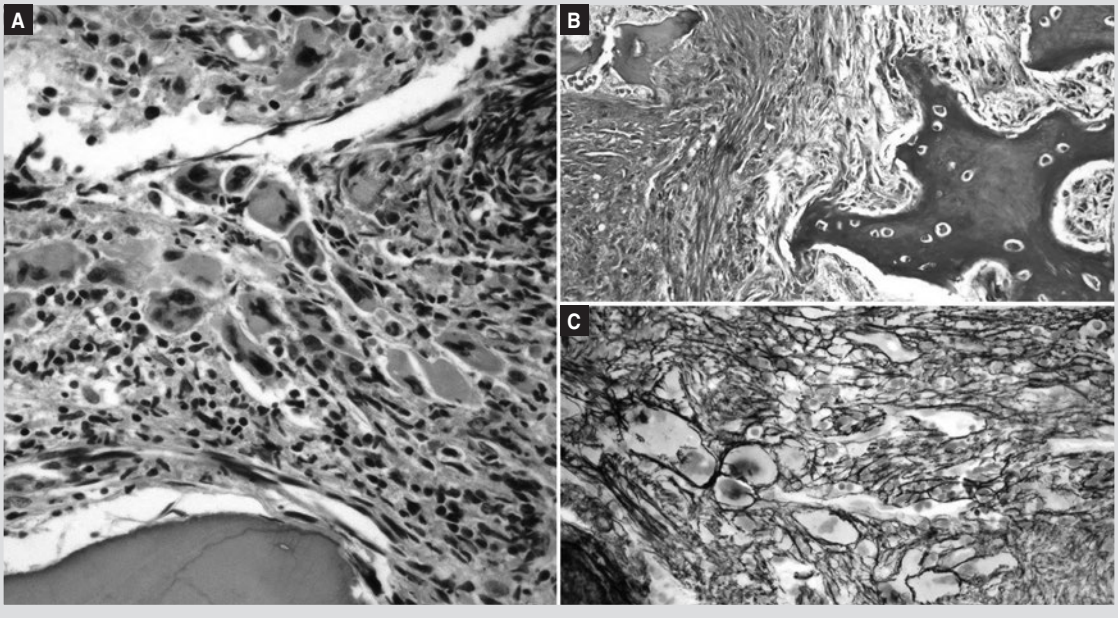


Figure 5. *Primary myelofibrosis.* **A:** *fibrosis compresses bone marrow cellularity and produces an effect resembling a "stream" (streaming effect). There are numerous atypical megakaryocytes.* **B:** *Masson staining showing mature collagen (type I).* **C:** *reticulum staining showing reticular fiber increase (type III collagen).*

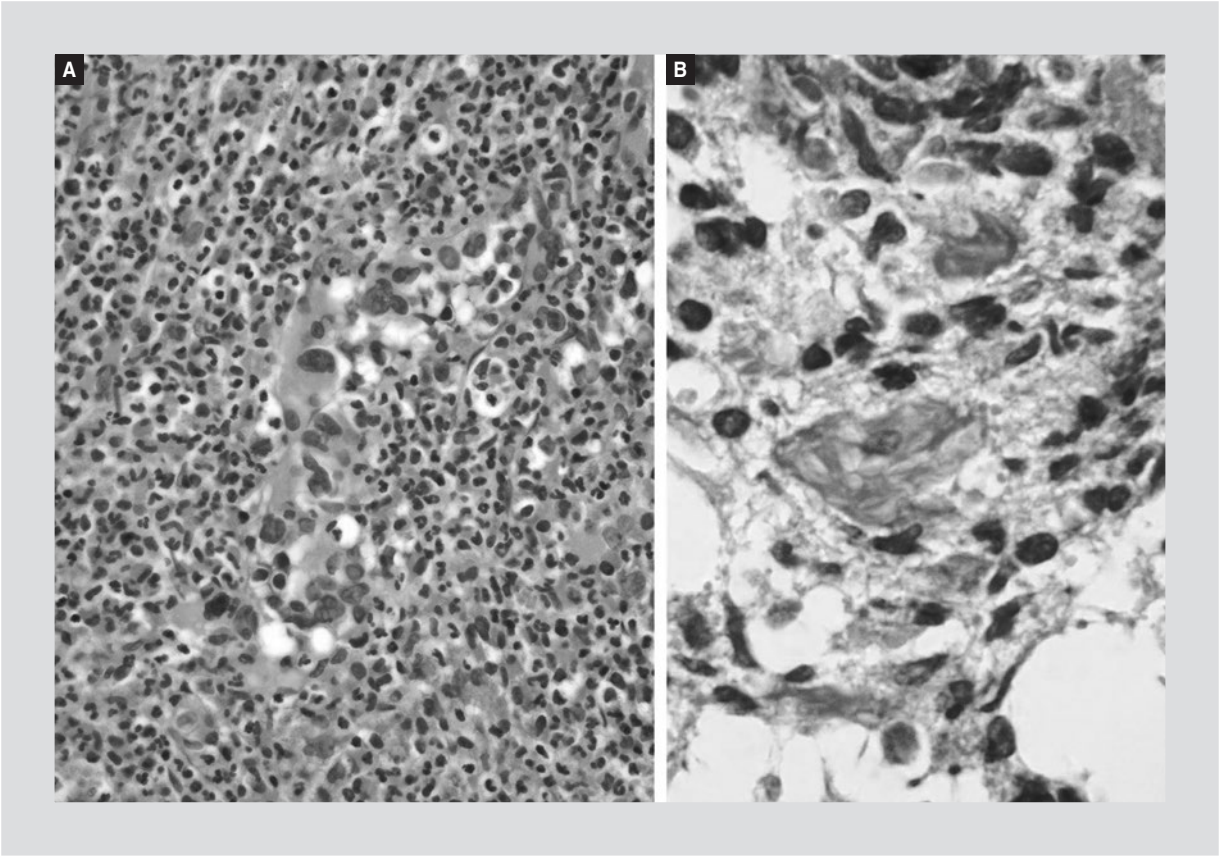


Figure 6. A: *chronic myeloid leukemia. Bone marrow shows 100% cellularity with a myeloid:erythroid ratio higher than 10:1, with numerous granulocytes and small megakaryocytes (dwarf megakaryocytes).* **B:** *PAS staining showing macrophages (pseudo-Gaucher) with PAS-positive filamentous material in their cytoplasm.*

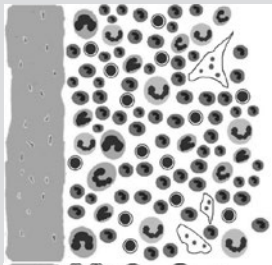
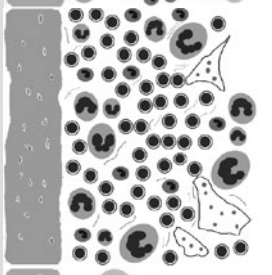
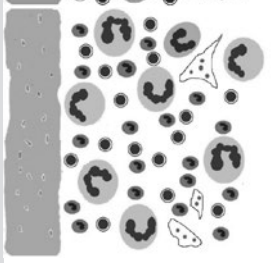
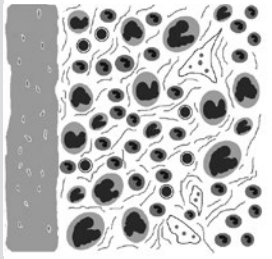
CML (chronic myeloid leukemia)

CML is one of the most common leukemias in adults and was the first hematological neoplasm where cytogenetic alterations and the development of leukemia could be associated[4,19].

It was Rudolf Virchow (1821-1902) in Germany and John Hughes Bennet (1812-1875) in the United Kingdom, in 1845, who, with a few months' difference, described patients with CML. Virchow named this condition "white blood" (*Weisses Blut*) and later "leukemia", and Bennet termed it "leukocytemia"[4].

BCR/ABL is the product of the t(9;22) (q34;q11) translocation that is present in up to 95% of patients with CML[31]. There are 3 main forms of this translocation (p190, p210 and p230 *BCR/ABL*) with different clinical and morphological manifestations. In CML cases, where the t(9;22) translocation occurs at the *BCR* breakpoint in the chromosome 22 5.8-kb region named M-bcr (major bcr) (which correspond to more than 90% of patients with CML), a 210-kb molecular weight protein is produced, known as p210 (p210 *BCR-ABL*). In these cases, the first morphological change observed on BM microscopic assessment is hypercellularity (higher than 90%) with marked proliferation of granulocytes at all maturation stages. The myeloid:erythroid ratio is 10-20:1 or more and mature neutrophils together with metamyelocytes predominate in cellularity. In the chronic phase, myeloblast count is usually lower than 5%, and the combination of blasts and promyelocytes is lower than 10%. Immature myelocytes are located around the bone trabecula forming an irregular cell thickness of more than 10 cell-layers, which has been named expanded myeloid cuff[5,19]. In most patients, eosinophil and basophil granulopoiesis is also increased. Although megakaryocytes can be decreased (in up to 30% of cases), there is generally a megakaryocyte increase with numerous small shapes (dwarf megakaryocytes) and hypolobulated, which sometimes are much more evident by means of immunostaining with CD61 or CD42b. There are cases where megakaryocytes are so numerous, that some

Table 2. Summary

	Chronic myeloid leukemia: Hypercellularity (90% or more) with marked granulocyte proliferation at all maturation stages. Myeloid:erythroid ratio 10-20:1. < 5% blasts (at accelerated phase, 5-10% blasts). Increase in megakaryocytes with numerous small forms (dwarf megakaryocytes) and hypolobulated. Pseudo-Gaucher-type macrophages. Variable fibrosis (grade 0 to 2).
	Polycytemia vera: Erythroid precursor predominant proliferation. Disperse megakaryocytes of different sizes (small, medium and large), grouped forming intertrabecular or paratrabecular clusters. Grade 0 to 1 reticulin fibrosis. Sinusoidal dilatation.
	Essential thrombocytemia: Eythropoiesis and granulopoiesis mild increase. Megakaryocytes with large shapes and "deer antler"-shaped nuclear hyperlobulation. Paratrabecular megakaryocyte clusters, close to sinusoids or disperse. Grade 0 or 1 reticulin fibrosis.
	Primary myelofibrosis Accentuated proliferation of the granulocytic (left-shift) and megakaryocytic lineages. Decrease and/or arrest in erythroid precursors' maturation. Dysplastic megakaryocytes, with variation in size, accentuated nuclear folding and aberration in the nucleus:cytoplasm ratio. Variable reticulin fibrosis from grade 0 to 1 (pre-fibrotic phase) to accentuated (grade 3).

authors have designed them as "Ph1 chromosome+ ET"[32]. Finding micromegakaryocytes such as those found in myelodysplasia is rather infrequent[33]. In most patients, erythropoiesis is decreased and, except for micromegakaryocytes, dysplastic characteristics in hematopoiesis are uncommon at chronic phase (Fig. 6A). In up to 70% of CML cases, there can be macrophages resembling Gaucher cells (pseudo-Gaucher cells), which some authors have proposed as a finding associated with good prognosis and potential indicators of increased survival[34] (Fig. 6B). These macrophages have in their cytoplasm traces of phagocyted cells and their cytoplasm acquires a birefingent fibrillar or "striated" appearance, which becomes more evident by means of PAS staining. Reticulin fibrosis is variable, ranging from moderate (grade 2) to accentuated (grade 3), particularly in advanced cases. Bone trabeculae are generally thick and irregular and, in advanced cases, there may be osteosclerosis. In CML accelerated phase, blasts in BM are between 10 and 19%, and there may be dysplastic changes in all 3 hematopoietic series, as well as prominent megakaryocytic proliferation, with micromegakaryocytes and hyperlobulated forms. In the blastic phase, there is more than 20% of blasts in the BM, and these form clusters of more than 20 blasts in the intertrabecular space[19].

When *BCR* cleavage occurs at the so-called m-bcr (minor-bcr) region, the chimeric RNA translation protein, produced by the *BCR/ABL* fusion, is a 190-kD molecular weight protein, known as p190 (p190 *BCR/ABL*). These cases are infrequent and are typically accompanied by marked monocytosis, and there can be also absence of basophilia and splenomegaly[5,19]. It is also important bearing in mind that these cases

can mimic chronic myelomonocytic leukemia and may be resistant to treatment with imatinib[19,35]. If *BCR* cleavage occurs at the so-called µ-bcr (micro-bcr) region, a 230-kD protein is produced, and it is characterized by prominent neutrophilic maturation[5,19].

Summary

MPNs are a heterogeneous group that share many histological features and, therefore, a specific diagnosis requires for clinical, molecular and histopathological characteristics to be integrated (Table 2). In all MPNs, BM is typically hypercellular, and megakaryocytic hyperplasia and dismegakaryopoiesis is a usual characteristic in this type of neoplasms. Hypervascularity, reticulin fibrosis and osteosclerosis are also frequently observed. BM findings tend to change over time, with dismyelopoiesis increase, increased blasts, fibrosis and osteosclerosis. Some cases can be transformed to MDS or acute, either myeloid or lymphoid, leukemia. The role of the pathologist is fundamental for the diagnosis of MPNs, but it is important to recognize that histopathological diagnosis must walk hand in hand with clinical assessment and laboratory results at the moment these neoplasms are classified.

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