

Review / Revisão

## Myeloproliferative neoplasms: a review of diagnostic criteria and clinical aspects

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*Chronic myeloproliferative disorders, currently called myeloproliferative neoplasms (MPN), according to the 4th edition of the World Health Organization (WHO) classification are clonal diseases of hematopoietic stem cells, in which there is increased proliferation of the myeloid series (granulocytic, erythrocytic, megakaryocytic series or mast cells) with effective maturation. The progression of all is characterized by marrow fibrosis or leukemic transformation. According to the WHO classification, the MPNs include: chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (IM), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia not otherwise categorized (CEL-NC), mastocytosis (M) and myeloproliferative neoplasm unclassifiable (MPNU). It is worth noting that the molecular basis of CML (BCR/ABL1), as well as PV, ET, IM (JAK2V617F and exon 12, MPL W515L/K) and M (KITD816V) have been identified and have, in common, constitutive activation of tyrosine kinase due to acquired hematopoietic stem cell defects. The JAK2V617F mutation is observed in around 90% of PV cases and in around 50-60% of IM and ET leading to the question why a single molecular lesion induces three different clinical manifestations. There is already evidence that additional genetic and epigenetic events contribute to the pathogenesis, including MPL W515L/K mutation. Some clinical aspects, the pathophysiology and diagnostic criteria of MPNs are presented in this paper. Rev. Bras. Hematol. Hemoter. 2010;32(4):308-316.*

**Key words:** Myeloproliferative disorders; chronic myeloid, leukemia, polycythemia vera; thrombocythemia, essential; primary myelofibrosis; mutation.

### Introduction

Myeloproliferative diseases, currently called myeloproliferative neoplasms (MPN) according to the fourth edition of the World Health Organization (WHO) classification, are clonal disorders of hematopoietic stem cells, in which there is increased proliferation of the myeloid series with effective maturation, which leads to peripheral blood leukocytosis, increased erythrocyte mass or thrombocytosis. Several types of MPN progress to fibrosis or leukemic transformation.<sup>(1)</sup>

The myeloproliferative group of disorders includes the following diseases: chronic myeloid leukemia (CML), polycythemia vera (PV), chronic idiopathic myelofibrosis (MF), essential thrombocythemia (ET), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia not otherwise specified (CEL-NS), mastocytosis (M) and myeloproliferative neoplasm, unclassifiable (MPNU).<sup>(1)</sup>

Some diseases, previously named CEL that present PDGFR $\alpha$ , PDGFR $\beta$  and FGFR1 rearrangements are now grouped as myeloid and lymphoid neoplasms with eosinophilia.<sup>(1)</sup>

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## Chronic myeloid leukemia

CML is a disease of mutated pluripotent hematopoietic cells which result in the Philadelphia (Ph) chromosome.

### Clinical aspects

CML has an incidence in the USA of 1.5 cases per 100,000 inhabitants per year; the median age of patients is 60 years old, but it can occur in any age group<sup>(2)</sup> with a slight predominance of males (1.3 male: 1 female).<sup>(3)</sup> Among 240 cases of CML treated at UNIFESP between 1990 and 2000, the median age was 43 years old.<sup>(3)</sup>

The most striking aspect of CML is leukocytosis with a 'left shift' and splenomegaly. The usual clinical manifestation is weakness, fatigue, increased abdominal size or bloating after meals (average size of the spleen was 5.8 cm in the UNIFESP cases<sup>(3)</sup>) but with subsequent weight loss. These manifestations are frequent in the chronic phase (CP) of the disease.

The natural history of CML is an evolution, on average over four years in the CP to blast crisis (BC). The disease is characterized clinically by two or three phases: the first is CP, which may be followed or not by an accelerated phase (AP) and the acute phase or BC. In these advanced stages, the cells do not differentiate and there is a predominance of blasts (myeloid, lymphoid or undifferentiated). In BC, patients are usually symptomatic with fever, bone pain, bleeding and sweating. Tables 1 and 2 show the diagnostic criteria of AP and BC.

Table 1. Diagnostic criteria for accelerated phase <sup>(1)</sup>

Peripheral blood or bone marrow	10-19% of blasts Basophils > 20% Platelets < 100,000 cells/ $\mu$ L or 1,000,000 cells/ $\mu$ L persistent and not therapy related, increase in white blood cell count despite of therapy
Physical exam	Increased spleen
Karyotype	Clonal evolution

Table 2. Criteria for blast crisis <sup>(1)</sup>

Peripheral blood or bone marrow	Blasts > 20%
extramedullary	Proliferation of blasts
Bone marrow biopsy	Grouping of blasts occupying a whole trabeculae

### Physiopathology

The pathophysiology of CML is based on the presence of the Ph chromosome, i.e. the translocation t(9;22)(q34;q11) or the molecular rearrangement of the BCR and ABL1 genes, an acquired genetic abnormality. The

BCR/ABL1 gene fusion transcribes mRNA that encodes a protein with tyrosine kinase activity. Depending on the breakpoint in BCR, the fusion product may be: M-BCR (major), m-BCR (minor) or  $\mu$ BCR (micro). M-BCR is the most common product in CML and results in a protein of 210 kD (p210BCR-ABL1). The m-BCR, which encodes P190BCR-ABL1, occurs in two thirds of acute lymphoid leukemia cases and less frequently in CML and  $\mu$ BCR (p230BCR/ABL) is rarely observed.

The consequence of the BCR/ABL1 fusion protein is cell growth and transformation independent of cytokines, loss of apoptosis, alteration in adhesion of hematopoietic cells to the extracellular matrix due to increased integrin activity and genomic instability. The constitutive activity of tyrosine kinase in the cytoplasm causes the phosphorylation of substrates of various signal transduction cascades that affect cell growth and differentiation.<sup>(4,5)</sup>

About 95% of CML cases have the Ph chromosome, and the others, Ph and BCR/ABL1 negative, are classified as a separate entity, atypical CML.<sup>(1)</sup>

### Diagnostic exams

*Whole blood count:* CP patients usually have anemia, leukocytosis and a normal or increased platelet count. (3) The differential of leukocytes shows an increase in circulating granulocytes with 'left shift' and an increased number of basophils. In the AP, the number of blasts is between 10% and 19%, basophilia is  $\geq$  20%, and platelet count <100,000 or > 1,000,000 cells/ $\mu$ L. In BC, the percentage of blasts is > 20%.

*Myelogram:* the CP presents with hypercellularity due to the intense proliferation of granulocytes, resulting in a granulocyte-erythroblast ratio of from 10 to 20:1 with preserved maturation. The number of blasts in the CP is < 5%. The megakaryocytic sector appears hyperplastic. There may be eosinophilia. In the AP, the percentage of blasts is between 10% and 19% and there may be dysplasia. In BC there are > 20% blasts.

*Karyotype:* this is the best test to identify the Ph chromosome present in 90% -95% of patients with criteria consistent with CML. In < 5% of cases variant changes involving two or more chromosomes in addition to the 9 and 22 chromosomes may be observed. Such situations have been previously detailed.<sup>(6)</sup> In another < 5% of cases, additional abnormalities may be observed such as double Ph, i(17q), trisomy 8 and trisomy 21, among others.

*Fluorescence in situ hybridization (FISH):* can be used to detect the BCR/ABL rearrangement at diagnosis and has been recommended for situations in which there are no metaphases for analysis or when the Ph chromosome is absent in karyotyping. Another advantage is that FISH can be performed using peripheral blood samples. Thanks to the use of dual-fusion probes, several abnormal situations

with the loss of another signal or double fusion can be detected simultaneously.<sup>(6,7)</sup> Between 9% to 33% of CML cases have the der(9q) deletion; patients with this characteristic have shorter expected survival than those without such deletions. A study conducted in 120 patients with CML Ph+/ BCR/ABL1+ treated at UNIFESP revealed der(9q) deletion or del 5' ABL in 15% of cases. These patients showed lower overall survival and shorter duration of chronic phase than patients without this deletion.<sup>(7)</sup>

**Bone marrow biopsy (BMB):** presents hypercellular with increased neutrophils and their precursors. The megakaryocytes are typically smaller than normal nuclei and hypoglobular. About 40% of patients showed increased reticulin fibers. In the AP, prominent proliferation of small and dysplastic megakaryocytes can be observed with an increase in reticulin fibers. In BC, there are extensive foci of blasts.<sup>(1)</sup>

An investigation of the BCR/ABL1 transcript by RT-PCR is indicated at diagnosis for < 5% of cases in which the Ph chromosome is not detected by karyotyping and, for half of them, the BCR/ABL1 rearrangement is present.<sup>(8)</sup> BCR/ABL1-negative cases should be investigated for other myeloproliferative disorders. FISH and RT-PCR have also been used in cases of fibrosis in which it is impossible to carry out karyotyping.

The quantification of the transcript by real time PCR is now recommended to monitor anti-tyrosinase treatment.<sup>(9-12)</sup>

**Polycythemia vera**

PV is a clonal neoplastic disease characterized by increased total erythrocyte mass regardless of the action of usual mechanisms of erythropoiesis regulation.

**Clinical aspects**

PV predominantly occurs in the sixth and seventh decades of life (0.7 to 2.5:100,000 of the population per year) with a median survival after diagnosis of approximately 15 years.<sup>(1)</sup> It is more common in men than in women. Thrombosis is usually the most common cause of death and, in late stage disease, there is risk of medullary fibrosis or transformation into acute leukemia.<sup>(1)</sup>

Symptoms include headache, plethora, tiredness, dizziness and sweating. Itching is present in approximately 40% of patients and is attributed to increases in histamine and the number of mast cells in the skin. Thrombotic episodes (strokes, Budd-Chiari syndrome, myocardial infarction, pulmonary embolism or deep vein thrombosis) are among the most common complications, occurring in approximately 33% of patients. Bleeding has also been reported (25% of cases). There is an increase in the incidence of peptic ulcers.

The disease progresses through three phases: the prodromal or pre-polycythemia phase, in which there is only mild or borderline erythrocytosis; plethoric phase with the symptoms described above and the late exhaustion or consumption stage in which there is fibrosis and complaints that include weakness, anemia and abdominal discomfort due to evident splenomegaly.

**Physiopathology**

The discovery of the acquired JAK2 V617F mutation, which is the exchange of guanine for thymidine resulting in a substitution of valine by phenylalanine at codon 617 of the JAK2 gene, gave us an understanding of the pathogenesis of this group of diseases.<sup>(13)</sup> This mutation leads to constitutive activation of tyrosine kinase and, although this mechanism is not completely understood, results in myeloid cell proliferation and differentiation.<sup>(13)</sup> Other activating mutations of JAK2 (e.g., exon 12) have also been described, but are rarer.<sup>(13)</sup>

The JAK2 V617F mutation is observed in about 90% of cases of PV, but also in about 50% of cases of MF and ET.<sup>(13,14)</sup>

Table 3. Criteria for the diagnosis of PV

Major criteria:
Hb > 18.5 g/dL for men or 16.5 g/dL for women or other evidence of increased red cell mass
Presence of the JAK2 V617F mutation or other functionally similar aspect (e.g., exon 12)
Minor criteria:
Hypercellular BMB for age with panmyelosis (prominent proliferation of the erythroid, granulocytic and megakaryocytic series)
Serum erythropoietin below the normal range
<i>in vitro</i> formation of endogenous erythroid colonies

**Diagnostic exams**

According to the WHO, two major criteria and one minor criterion or the simultaneous presence of one major and two minor criteria are needed for the diagnosis of PV (see Table 3):<sup>(1)</sup>

In practice, the presence of two major criteria is sufficient in 97% of cases of PV. However, to minimize the consequences of false-positive molecular testing, the additional presence of at least one of three minor criteria is required. Alternatively, the combination of the first major criterion and two minor diagnostic criteria enables the inclusion of rare cases of PV that can truly be negative for known JAK2 mutations.

In addition, a high Hb/Ht ratio is required for the diagnosis of PV, i.e. without erythrocytosis, the diagnosis of PV is not possible.<sup>(13)</sup> Thus, the interpretation of probable cases of PV with iron deficiency may be confusing. Iron

replacement can not possibly be prudent, as a substantial increase in hemoglobin may be dangerous for the patient.<sup>(1,13)</sup> There was some controversy related to the criteria established by the WHO, such as accepting a high Hb/Ht ratio as a substitute for the measurement of the red cell mass per se.<sup>(14)</sup> On applying these criteria to 77 patients with PV and 66 with apparent erythrocytosis, Johanson et al. detected absolute erythrocytosis in only 35% of men and 63% of women with PV, although 14% of men and 35% of women apparently without erythrocytosis presented it in the analysis of the erythrocyte mass and plasma volume.<sup>(15)</sup> So, Hb and Ht are also accepted as criteria when they reach the 99th percentile of the normal reference value of the specific method for age, gender and altitude of residence, or Hb > 17 g/dL in men and > 15 g/dL in women associated with an documented and sustained increased Hb > 2 g/dL over the basal level for the individual that can not be attributed to a correction of iron deficiency or a high red cell mass > 25% than the expected mean.

The following are necessary for the diagnosis of the post-polycythemia fibrotic phase: 1) a prior documented diagnosis of PV according to the WHO criteria and 2) grade 2-3 (on a scale of 0-3) or 3-4 (on a scale of 0-4) fibrosis of the bone marrow together with two of the following: 1. anemia or sustained loss by phlebotomy or cytoreductive medication for erythrocytosis, 2. peripheral leukoerythroblastosis 3. defined progressive splenomegaly both by palpable spleen more than 5 cm from the left costal margin or the appearance of a palpable spleen; 4. the development of more than one of the three constitutional symptoms: loss of > 10% weight within the previous six months, and night sweats and fever > 37.5°C without apparent cause.

*Whole blood count:* shows an increased number of blood cells, hemoglobin and hematocrit; leukocytosis may be present with a shift to myelocytes; basophilia, eosinophilia, and monocytosis may be seen. Platelets may be increased in number, usually between 500,000 and 1,000,000 cells/ $\mu$ L.

*Myelogram and BMB:* usually hypercellular with hyperplasia of all elements (even though a lack of hypercellularity does not discard diagnosis). Atypical megakaryocytes varying in size but with predominantly large elements and hyperlobular nuclei are observed. There may be a slight increase of reticular fibers but fibrosis only occurs in cases that progress to the exhaustion stage. Perls staining does not identify a hemosiderin deposit in the biopsy.<sup>(16)</sup>

*Karyotyping:* chromosome abnormalities are seen in from 10% to 30% of cases at diagnosis with the most common being: +8, +9, del(20q), gain of material on 1q, del(1q) and del(13q). With progress of the disease, the rate of cytogenetic abnormalities are seen in somewhere around 80% of cases, especially those in the post-polycythemia

fibrotic phase and nearly 100% of cases that evolve to acute leukemia.

*Research of the JAK2 V617F mutation and others:* investigations of the V617F and JAK2 exon 12 mutations by allele-specific PCR and sequencing are necessary to detect other possible mutations.

Patients with idiopathic abdominal venous thrombosis should be submitted to an investigation of the JAK2 gene as they may progress to PV. However, the presence of the mutation by itself in these cases does not define the case as PV, but as an unclassified myeloproliferative disorder.<sup>(17,18)</sup>

### Differential diagnosis

In secondary polycythemia (SP) there is an increase in the number of circulating red blood cells and red cell mass in response to stimulation of the bone marrow by erythropoietin (Epo) or abnormal functioning of mutant Epo receptors. Unlike PV, these patients do not show increased leukocyte and platelet counts or splenomegaly. SP is associated to cardiopulmonary disorders, chronic obstructive pulmonary disease, sleep apnea syndrome, smoker's polycythemia, polycythemia of renal tumors that produce Epo, polycystic kidney disease, polycythemia of high altitudes and large myomas, among other causes. Additional studies such as blood gases, abdominal ultrasound, polysomnography and gynecological evaluations may be helpful in the differential diagnosis.<sup>(19,20)</sup>

### Primary myelofibrosis

Primary myelofibrosis (MF) originates from a clonal neoplastic transformation of pluripotent hematopoietic cells (stem cells) accompanied by intense reactive changes of the bone marrow stroma with collagen fibrosis, osteosclerosis and angiogenesis.

### Clinical aspects

The estimated incidence is 0.5 to 1.5 cases per 100,000 inhabitants per year. A quarter of patients are asymptomatic and the diagnosis is made by splenomegaly or by chance. The other cases present with symptoms secondary to anemia (weakness, fatigue, palpitations and dyspnea), splenomegaly (satiety, discomfort or pain in the upper left quadrant of the abdomen), hypermetabolic state (weight loss, night sweats or fever), extramedullary erythropoiesis, bleeding (petechiae, gastrointestinal tract bleeding), bone changes (bone or joint pain due to osteosclerosis), portal hypertension (ascites, esophageal or gastric varices, hepatic encephalopathy, portal or hepatic vein thrombosis) and immunological abnormalities (circulating immune complexes or autoantibodies).

The disease has two phases: the pre-fibrotic phase starting with hypercellular bone marrow which progresses to replace nearly all the hematopoietic tissue by reticular fibers (the fibrotic phase). Survival varies from 3 to 10 years. The causes of death are: leukemic transformation (5% to 10% of cases), infection, bleeding, thrombosis, heart failure, liver failure, occurrence of another cancer, respiratory failure and portal hypertension.<sup>(21)</sup>

**Physiopathology**

In MF, the fibrosis is due to clonal proliferation of hematopoietic cells leading to hyperplasia of megakaryocytes and monocytes that release fibrogenic growth factor. The JAK2 V617F mutation has been detected in approximately 50% of patients, who have high white blood cell counts. Neutrophilia is seen in JAK2 V617F negative individuals who have less need for transfusion (presumably the mutation protects from severe anemia), but clinically more aggressive disease represented by worst survival. The JAK2 V617F is present in the homozygous form in 13% of cases, a situation that is associated with chromosomal abnormalities that may have an adverse function in the biogenesis of the disease. A mutation in the transmembrane domain of the thrombopoietin receptor (cMPL) was observed in 9% of JAK2 V617F negative (or MPLW515L/MPLW515K) patients but also in JAK2 V617F positive patients. So the current idea is that MPL mutations favor the development of thrombocytosis while JAK2 V617F predisposes to erythrocytosis. However, it is hard to blame each mutation as the sole cause of MF; perhaps the disease is the accumulation of multiple genetic lesions and epigenetic events.<sup>(22)</sup>

Table 4. Criteria for the diagnosis of MF

Major criteria
1. Presence of megakaryocytic proliferation and atypia, usually accompanied by reticular or collagen fibrosis or in the absence of significant fibrosis, the megakaryocytic changes must be accompanied by increased marrow cellularity at the expense of granulocytic proliferation with decreased erythropoiesis (pre-fibrotic stage);
2. Absence of WHO criteria for PV, CML BCR/ABL1+, myelodysplastic syndrome or other neoplasias;
3. Presence of the JAK2 V617F mutation or other clonal marker (MPLW515K/L) or, in the absence of a clonal marker, no evidence that the marrow fibrosis or other alterations are secondary to infection, inflammation, hairy cell leukemia, lymphoid neoplasia, metastasis and toxic myelopathy.
Minor criteria
1. Leukoerythroblastosis
2. Increased serum lactate dehydrogenase
3. Anemia
4. Splenomegaly

**Diagnostic exams**

At least three major and two minor criteria are necessary for the diagnosis of MF.

The tests needed for diagnosis are:

*Whole blood count:* usually shows normochromic and normocytic anemia (Hb < 10 g/dL in 60% of cases). Microcytic and hypochromic anemia is also identified in 5% of cases associated to iron-deficiency. The morphology of red blood cells shows poikilocytosis, dacryocytes and erythroblasts in circulation. Leukopenia is present in one quarter of the cases and leukocytosis in one third. The leukocyte count may have a shift to younger forms, even blasts and pseudo-Pelger-Huet anomaly. Both thrombocytosis and thrombocytopenia may be observed with the presence of macrothrombocytes.<sup>(23)</sup>

*Myelogram and BMB:* in the pre-fibrotic phase hypercellularity can be seen with myeloid hyperplasia of myeloid sectors. In the fibrotic phase, the aspiration is usually dry.<sup>(1,23)</sup> Megakaryocytes are abnormal and atypical (pleomorphic, large, but can also be small) forming clusters adjacent to the sinuses and bone trabeculae. Reticular fibrosis is minimal at first. In the fibrotic phase there is reticular or collagen fibrosis. Osteosclerosis may be present.<sup>(1,23)</sup>

*Karyotyping:* It may be very difficult to obtain samples for analysis due to marrow fibrosis. Changes are evidenced in 60% of cases with del(13q), del(20q), partial trisomy 1q, as well as +8 and +9. This is an important examination to differentiate between MF and CML (with the Ph chromosome) and myelodysplastic syndrome [3q21q26 or del(5q) abnormalities]. Cases with alterations involving chromosome 5 or 7 are related to prior use of chemotherapeutic agents in the treatment of a myeloproliferative disorder.<sup>(24)</sup>

Investigations of the JAK2 V617F mutation and others: can be performed on peripheral blood samples by PCR followed by sequencing or not.

**Differential diagnosis**

Fibrosis is a phenomenon that can occur in other myeloproliferative diseases such as CML, ET and PV, or even hairy cell leukemia, myelodysplastic syndrome with fibrosis, myelodysplastic/myeloproliferative syndrome, unclassifiable chronic myeloproliferative neoplasm, acute megakaryocytic leukemia, acute leukemia with fibrosis and other hematologic malignancies with no metastasis to the bone. Clinical conditions that may present bone marrow fibrosis as secondary events are the chronic granulomatous diseases (tuberculosis and histoplasmosis), inflammatory diseases, systemic lupus erythematosus, pulmonary hypertension and diseases related to metabolism of the parathyroid hormone (hyperparathyroidism and

hypoparathyroidism). The clinical and laboratory aspects are distinct from MF and should be taken into account in the diagnosis.

### Essential thrombocythemia

ET is characterized by a high platelet count with megakaryocytic hyperplasia, although other bone marrow sectors are affected qualitatively or quantitatively.

#### Clinical aspects

The incidence is 1 to 2 cases per 100,000 inhabitants/year. The median age of patients at diagnosis is 60 years. One-third to one-quarter of patients are symptomatic at diagnosis and 25% to 48% have splenomegaly. Vasomotor symptoms, characterized by headache, syncope, atypical chest pain, sight disorders, livedo reticularis and erythromelalgia (burning of hands or feet associated with redness and warmth) are observed in approximately 40% of cases.

Bleeding, thrombotic events and vascular complications are the leading causes of morbidity and mortality in ET. Extreme thrombocytosis is associated with increased risk of gastrointestinal bleeding.

Hemorrhagic phenomena are observed in 26% of cases. The majority of thrombotic events are deep vein thrombosis and pulmonary embolism. Hepatic or portal vein thrombosis (Budd-Chiari syndrome) occurs particularly in younger patients.

The rates of transformation to PV, MF and AML are 2.7%, 4% and 1.4% respectively. There may also be transformation to myelodysplastic syndrome. The transformation to leukemia may take 1.7 to 16 years. Most transformed patients received prior cytoreductive therapy however transformation may also occur without treatment, suggesting that the event is a natural sequel of the disease, a result probably of its biology and the time from diagnosis and not to prior therapy. In relation to post-thrombocythemia fibrotic stage, the clinical features are identical to primary myelofibrosis.

#### Physiopathology

Clonality studies show that about 55% of ET are polyclonal (6 of 10 monoclonal and 2 of 13 polyclonal had thrombosis,  $p < 0.05$ ). The relationship between thrombocytosis and thrombopoietin (TP) is not well defined but serum TP is normal or slightly increased. It is believed that megakaryocytic progenitors may be hypersensitive to TP, although there are reports of autonomous growth of cultured megakaryocytes from patients with ET, calling into question the involvement of pathways of intracellular signal transduction. The acquired somatic point mutation, JAK2

V617F is present in primary cases of ET, but never observed in cases of secondary disease.

### Diagnostic tests

The four diagnostic criteria of the WHO classification must be met for diagnosis (Table 5): <sup>(1,23,25)</sup>

Table 5. Criteria for the diagnosis of essential thrombocythemia

1. A sustained platelet count > 450,000 cells/ $\mu$ L;
2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with an increased number and size of mature megakaryocytes. Absence of any significant increase or 'left shift' of neutrophil granulopoiesis or erythropoiesis;
3. Absence of the WHO criteria for PV, MF, CML BCR/ABL1+ or myelodysplastic syndrome [absence of del(5q), t(3;3)(q21;q26), inv(3)(q21;q26)] or other myeloid tumors;
4. The presence of the JAK V617F mutation or others

The criteria for the diagnosis of post-thrombocythemia myelofibrosis are: 1. documented prior diagnosis of ET according to the WHO criteria; 2. grade 2-3 (on a scale of 0-3) or 3-4 (scale of 0-4) fibrosis associated with two other criteria of the following: 1. anemia or a decrease of > 2 g/dL of the baseline Hb; 2. peripheral leukoerythroblastosis; 3. increased splenomegaly defined both by an enlarged spleen palpable 5 cm beyond the left costal margin or the appearance of a palpable spleen; 4. increased lactate dehydrogenase; and 5. the appearance of more than one of the following constitutional symptoms: loss of in weight > 10% in the preceding six months or night sweats and fever without apparent cause.

Of the available diagnostic tests, karyotyping should always be performed to investigate the presence of the Ph chromosome, which defines the disease as CML, thus useful in the differential diagnosis. There are no cytogenetic abnormalities typical of ET and the rate of alterations is about 5%. The most frequent abnormalities identified are trisomy 8 and 9, in addition to 13q and 20q deletions. Abnormalities such as del(5q), t(3;3)(q21;q26,2) and inv(3)(q21q26.2), which are associated with thrombocytosis, are characteristics of myelodysplastic syndrome and acute myeloid leukemia.

### Differential diagnosis

Transient processes that can lead to reactive thrombocytosis include: acute hemorrhage, recovery from thrombocytopenia, acute inflammation, infection, response to exercise and drug reactions. Chronic processes that also induce thrombocytosis are: iron deficiency, hemolytic anemia, asplenic state, chronic inflammatory disease, chronic infectious diseases and cancer.

## Chronic neutrophilic leukemia

CNL is a rare myeloproliferative disorder characterized by persistent peripheral neutrophilia with hypercellular marrow and hepatosplenomegaly.<sup>(1)</sup>

### Clinical aspects

The incidence and etiology are unknown. CNL affects the elderly. Splenomegaly is an important characteristic and the complaints usually derive from this abnormality but hepatomegaly is also common. Mucosal or gastrointestinal tract bleeding may be observed. The disease has a slow progressive clinical course.<sup>(1)</sup>

### Diagnostic criteria

1. leukocytosis in peripheral blood > 25,000 cells/ $\mu$ L, with > 80% of the differential count composed of segmented and band neutrophils, < 10% of immature granulocytes (promyelocytes, myelocytes and metamyelocytes) and < 1% myeloblasts;

2. BMB shows increased percentage and absolute number of granulocytes, myeloblasts < 5% of nucleated bone marrow cells, a normal pattern of maturation of neutrophils and normal megakaryocytes or with a 'left shift';

3. hepatosplenomegaly;

4. no physiological cause for the neutrophilia (infection, inflammation, tumors) is identified or, if present, myeloid clonality is demonstrated by cytogenetic or molecular studies;

5. absence of the Ph or BCR/ABL1 rearrangement;

6. absence of PDGFR $\alpha$ , PDGFR $\beta$  and FGFR1 rearrangements;

7. no evidence of PV, MF or ET;

8. no evidence of myelodysplastic syndrome or myelodysplastic/myeloproliferative diseases (no granulocytic dysplasia, no myelodysplastic changes or the other lineages and monocytes < 1000 cells/ $\mu$ L).

### Diagnostic tests

*Whole blood count:* shows leukocytosis (> 25,000 cells/ $\mu$ L), with a predominance of segmented and band neutrophils, immature granulocytes make up < 10% of the cells and myeloblasts < 1%.

*Myelogram and BMB:* hypercellular with increased granulocytes, blasts < 5% and normal pattern of maturation. Mild fibrosis is occasionally observed.<sup>(1)</sup>

*Karyotyping:* is normal in most cases. The Ph chromosome can not be present. Rare cases may show abnormalities common to other MPNs such as +8, +9, del(20q) and del(11q).<sup>(1)</sup>

## Chronic eosinophilic leukemia, not otherwise specified

In CEL, there is an autonomous and clonal proliferation of eosinophilic precursors, resulting in persistent myeloproliferation in bone marrow, peripheral blood and tissues. Organ damage occurs as a result of leukemic infiltration or the release of cytokines, enzymes and other proteins by eosinophils.<sup>(1)</sup>

Patients with CML BCR/ABL1+ are excluded from this category as are patients with PDGFR $\alpha$ , PDGFR $\beta$  and FGFR1 rearrangements.

CEL is more common in men at a ratio of 9:1 woman with the peak incidence being between 20 and 50 years old. Rare cases have been observed in infants and children.<sup>(26)</sup>

The diagnostic criteria for CEL are:<sup>(1)</sup>

1. eosinophils > 1500 cells/ $\mu$ L in peripheral blood;

2. absence of the Ph chromosome or other BCR/ABL1+ MPN (PV, ET and MF) and myelodysplastic/myeloproliferative disorders;

3. absence of t(5;12)(q31-35,p13) or other PDGFR $\beta$  rearrangements;

4. absence of FIP1L1/PDGFR $\alpha$  or other PDGFR $\alpha$  rearrangements;

5. absence of the FGFR1 rearrangement;

6. the blast count in peripheral blood and bone marrow is < 20% and there is no inv(16)(p13;q22) or t(16;16)(p13;q22) or other diagnostic characteristics of AML;

7. there are molecular or cytogenetic clonal abnormalities or blasts > 2% in the peripheral blood or > 5% in bone marrow.

### Clinical aspects

About 10% of patients are diagnosed by chance because they are asymptomatic. In other cases, symptoms such as fever, fatigue, cough, angioedema, muscle pains, rash and diarrhea are common.<sup>(1)</sup> Anemia, thrombocytopenia, mucosal ulceration, endomyocardial fibrosis and splenomegaly are also common.

The most important clinical finding is related to endomyocardial fibrosis with restrictive heart disease, which is irreversible. Heart disease triggered by the infiltration of eosinophils in the endocardium has a necrotic initial stage lasting an average of five weeks. In this stage, the disease is not clinically recognized and generally goes unnoticed in echocardiography and angiography as ventricular wall thickening has not occurred. Sometimes, just a right ventricular endomyocardial biopsy allows diagnosis at this stage. In the second 'thrombotic' stage, with an average duration of ten months, there is mural thrombus formation with the potential of embolization to the brain. Finally, in

the fibrotic third stage after two years, endomyocardial fibrosis occurs that results in mitral and/or tricuspid valve regurgitation when valve replacement may be necessary. The clinical presentation includes dyspnea, chest pain, congestive heart failure and cardiomegaly, as well as T-wave inversion on electrocardiograms.

Peripheral neuropathy, central nervous system dysfunction and pulmonary symptoms may also be present.<sup>(1)</sup>

### Diagnostic tests

The investigation of a patient with hypereosinophilia should follow a logical line of reasoning to discard causes of reactive eosinophilia and MPN with rearrangements as described above, as well as identifying clonality in eosinophils (a phenomenon that is difficult to prove) or an increase in blasts in the peripheral blood or bone marrow.

*Whole blood count:* eosinophilia persistently > 1500 cells/ $\mu$ L. Eosinophils in LEC are usually mature cells, with smaller numbers of interspersed myelocytes and promyelocytes. There is morphological heterogeneity, with sparse granulation and light areas in the cytoplasm, cytoplasmic vacuolization, nuclear hyper or hyposegmentation and an increased size, but these changes are indistinguishable between reactive and neoplastic cases. There are < 20% blasts.

*Myelogram and BMB:* The marrow is hypercellular due to eosinophil proliferation; erythropoiesis and megakaryocytopoiesis are usually normal but fibrosis can be observed in some cases.

*Karyotyping:* this must be performed systematically in bone marrow samples because it can detect clonal abnormalities observed in this disease or other abnormalities that lead to a correct diagnosis, for example, the presence of the Ph chromosome, which indicates LMC; t(5,12) which demonstrates chronic myelomonocytic leukemia with eosinophilia, etc..

*FISH and RT-PCR:* These tests can detect FIP1L1/PDGFR $\alpha$ , PDGFR $\beta$  and FGFR1 rearrangements, which define these cases as other subtypes as will be discussed later.

### Differential diagnosis

A significant (> 5%) and sustained increase in circulating eosinophils is usually due to parasitic (eosinophilia severe), allergic (eosinophilic mild to moderate) or inflammatory diseases or rarer clonal or idiopathic situations, which present with severe tissue damage as a result of eosinophilic infiltration.<sup>(27,28)</sup>

CEL-NS can be distinguished from other clonal hematopoietic diseases in which eosinophilia is part of the neoplastic clone, such as CML, PV, ET, MF, myelodysplastic syndromes, AML [myelomonocytic with inv(16) or CBF $\beta$ /MYH11 rearrangement with maturation t(8;21) or the ETO/

AML1 rearrangement].<sup>(1)</sup> CEL-NS must also be differentiated from myeloid and lymphoid proliferations with eosinophilia with the FIP1L1/PDGFR $\alpha$ , PDGFR $\beta$  and FGFR1 rearrangements and should be distinguished from situations that occur with aberrant phenotypes of clonal T cell populations and abnormal cytokine or G-CSF production.<sup>(1,29)</sup>

Once clonal and reactive causes for hypereosinophilia have been discarded, there is still the poorly understood idiopathic hypereosinophilic syndrome (HES) to be considered.<sup>(1)</sup>

HES is a heterogeneous group of diseases characterized by eosinophilia in the peripheral blood or tissue, resulting in end organ damage. But the differentiation between HES and CEL is that there is no proven clonality and no increase in blasts. For the diagnosis of HES, the following criteria must be met: 1. eosinophils > 1500 cells/mL in peripheral blood for more than six months 2. reactive causes discarded, lack of clonality, 3. AML, MPN, SMD, SMD/MP discarded 4. absence of an immunophenotypically anomalous T cell population or production of cytokines.<sup>(1,30,31)</sup> 5. evidence of organ damage caused by eosinophilia.

Therefore, a disease is only defined as HES when criteria 1 to 4 are present, however, if there is no tissue injury it is still idiopathic hypereosinophilic syndrome.

The clinical presentation is highly variable, with patients totally asymptomatic and requiring no treatment and with long survival, while others have rapidly progressive and fatal disease, such as congestive heart failure or leukemic transformation.

The main target organs are the skin, heart and central nervous system with more than 50% of patients presenting clinical complications in each of these structures. Eosinophil infiltration of the lung, liver, spleen, digestive system, joints and kidneys occurs with variable frequency, but any organ or system can be involved.

### Resumo

*As síndromes mieloproliferativas crônicas, atualmente denominadas neoplasias mieloproliferativas (NMP), de acordo com a 4ª edição da classificação da Organização Mundial da Saúde (OMS), são doenças clonais de célula-tronco hematopoética, nas quais há a proliferação aumentada de uma ou mais das séries mieloides (granulocítica, eritrocítica, megacariocítica ou mastocítica) com maturação eficaz. A progressão de todas é caracterizada por fibrose medular ou transformação leucêmica. Pela classificação da OMS, as NMP incluem: leucemia mieloide crônica (LMC), policitemia vera (PV), mielofibrose idiopática crônica (MF), trombocitemia essencial (TE), leucemia neutrofílica crônica (LNC), leucemia eosinofílica crônica não especificada (LEC), mastocitose (M) e neoplasia mieloproliferativa inclassificável (NMI). É interessante notar que tanto a LMC (BCR/ABL1) como PV, MF e TE (JAK2 V617F e éxon 12, MPLW515L/K) e M (KITD816V) tiveram suas*



bases moleculares desvendadas e apresentam em comum a ativação constitutiva de tirosino-quinase graças às mutações adquiridas pela célula-tronco hematopoética. A mutação JAK2 V617F é observada em mais de 90% dos casos de PV, mas também em cerca de 50%-60% das MF e TE, levando ao questionamento de como uma única lesão molecular desencadeia três manifestações clínicas diversas. Já há evidências de que eventos genéticos e epigenéticos adicionais contribuem para a patogênese, tais como MPLW515L e MPLW515K. No presente manuscrito são apresentados os aspectos clínicos, a fisiopatologia e os critérios diagnósticos das diferentes NMP. Rev. Bras. Hematol. Hemoter. 2010;32(4):308-316.

**Palavras-chave:** *Transtornos mieloproliferativos; leucemia mieloide crônica; policitemia vera; trombocitemia essencial; mielofibrose primária; mutação.*

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