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NATIONAL CONSENSUS ON PHILADELPHIA (PH) NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS AT ISSSTE

AUTHORED BY:
MARTHA ALVARADO IBARRA

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Alvarado Ibarra Martha*

National Medical Center "November 20", Mexico

***Corresponding Author**

Martha Alvarado Ibarra, National Medical Center "November 20". ISSSTE, Mexico

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Authors names and Affiliations

Herrera Olivares Wilfrido: *Hospital Regional ISSSTE Puebla, Puebla, Mexico*

Mena Zepeda Veronica: National Medical Center “November 20”, Mexico

Alvarado Ibarra Martha: National Medical Center “November 20”, Mexico

Pichardo Cepín Yayra Mercedes: National Medical Center “November 20”, Mexico

Martínez Ramírez Mario Alberto: Hospital General ISSSTE, Veracruz, Mexico

Flavio Rojas Castillejos: Centro Medico Nacional 20 Noviembre

Miranda Madrazo María Raquel: National Medical Center “November 20”, Mexico

Álvarez Vera José Luis: National Medical Center “November 20”, Mexico

Guerra Alarcón Lidia Virginia: National Medical Center “November 20”, Mexico

Espitia Ríos María Eugenia: National Medical Center “November 20”, Mexico

Mujica Martínez Aldo: National Medical Center “November 20”, Mexico

Montoya Jiménez Leire: National Medical Center “November 20”, Mexico

Pacheco Arias María Augusta: National Medical Center “November 20”, Mexico

Reynoso Pérez Ana Carolina: National Medical Center “November 20”, Mexico

Morales Hernández Alba Edna: National Medical Center “November 20”, Mexico

Bates Martín Ramón Alberto: National Medical Center “November 20”, Mexico

Palma Moreno Orlando Gabriel: National Medical Center “November 20”, Mexico

Aguilar Andrade Carolina: National Medical Center “November 20”, Mexico

Vilchis González Shendel Paulina: National Medical Center “November 20”, Mexico

Arano Luna Luara Luz: National Medical Center “November 20”, Mexico

Merino Pasaye Laura Elizabeth: National Medical Center “November 20”, Mexico

Loera Fragoso Sergio J: Hospital General Dr. Santiago Ramón y Cajal, Durango, New Mexico

Guajardo Leal Martha Lilia: Hospital General ISSSTE, Veracruz, Mexico

Reyes Brena Gustavo N: Hospital Regional de Alta Especialidad del Bajío, Guanajuato

Hernández Ruiz Eleazar: Hospital Regional Presidente Juárez ISSSTE, Oaxaca

Ramírez Romero Eva Fabiola: Hospital Regional Presidente Juárez ISSSTE, Oaxaca

Morales Adrián Javier de Jesús: Hospital Regional ISSSTE, Mérida

Medina Guzmán Liliana: Hospital Star Médica, Querétaro

López Arroyo José Luis: Hospital General ISSSTE, Ciudad Juárez

Añorve Hernández Erika: Hospital Regional Lic. Adolfo López Mateos, Ciudad de México

Pérez Zúñiga Juan Manuel: Hospital Regional Ignacio Zaragoza, Ciudad de México

Banda García Luisa I: Hospital General de Zona IMSS 11 Jalapa, Veracruz

Salazar Ramírez Oscar: Hospital General ISSSTE Dr. Darío Fernández Fierro, Ciudad de México;

Silva Vera Karina: Hospital General del ISSSTE, Tampico

Saavedra González Azucena: Hospital ISSSTE de Alta Especialidad Bicentenario de la Independencia, Estado de México

Grimaldo Gómez Flavio Adrián: Ciudad Instituto Nacional de Cardiología de México

Introduction

Chronic Myeloproliferative Neoplasms (cMPN) are an heterogeneus disease group of hematopoietic clone disorders (hematopoietic progenitor cells) characterized by the proliferation of one or more myeloid lines [1] in the bone marrow. In the third and fourth version of the WHO classification, 2008 and 2016 respectively, there are no significant changes, except for some molecular aspects. They are divided into classic chronic myeloid leukemia and Philadelphia chromosome-negative chronic myeloproliferative neoplasms: Polycythemia Vera (PV), Essential thrombocytemia (ET), and myelofibrosis (PMF). This classification is based on clinical, laboratory, anatomopathological in bone marrow, molecular, and cytogenetic criteria, being essential the JAK2 (Janus Kinase 2) mutational analysis [2,3] (Table 1).

Table 1: Schematic classification of Myeloproliferative Neoplasias.

Chronic Myeloproliferative Neoplasms (cMPN)	
Classic Ph Negative MPN	Polycythemia Vera (PV) Essential Thrombocythemia (ET) Primary Myelofibrosis (PMF)
Rare cMPNs	Chronic Neutrophilic Leukemia Chronic Eosinophilic Leukemia Unclassifiable Myeloproliferative Neoplasm
Chronic Myeloid Leukemia (CML)	

Discovering that Philadelphia negative classic myeloproliferative neoplasms present several molecular anomalies (including the JAK2 V617F mutation) opened several horizons in the diagnosis, prognosis and monitoring of such disorders. However, the knowledge development in molecular genetics requires parallel advances for a better approach in the methods of detection and reporting of the mutations associated with the disease, in order to make the diagnosis, prognosis and monitoring of cMPN more efficient and rational [4].

Genetic and enzymatic studies based on inactivation patterns of the X chromosome have revealed an origin of multipotent progenitor cells for the neoplastic clone, both in myelodysplastic syndromes (MDS) and in cMPN [5]. 90% to 95% of the PV cases present a somatic mutation in the JAK2 gene (JAK2 V617F); 5% exon 12 mutation [6], and about 60% of the patients with JAK2 V617F mutation for ET or PMF. Other recurrent specific mutations by MPN subtypes (such as MPL and CALR gene mutations), and several other associated to myeloid disorders, opened a new era in the understanding of the MPN biology (Table 2).

Table 2: Summary of acquired mutations in Ph negative MPN [7].

Gene	Chromosomic location	Protein	Type of mutation	Frequency of mutations in MPN (%)		
				PV	ET	MF
JAK2	9p24	JAK2	V617F (exon 14)	95-97	60	60
			Exon 12: K539L, deletions and ins	1-3	0	0
CALR	19p13.3-p13.2	Calreticulin	Exon 9 insertions and deletions	Unfrequent	15-30	23-35
MPL	1p34	TPO Receptor	Exon 10: W515K/L/A; S505N	0	3-5	5-10
LNK/SH2B3	12q24	LNK	Exon 2	1	3-6	3-6
CBL	11q23	CBL	Exons 8-9	Unfrequent	Unfrequent	5-10
TET2	4q24	IDH1/2	Inactivating mutations	10-20	5	10-20
IDH1/IDH2	2q34/15q26	DNMT3A	Frequently IDH1-R132 or IDH2-R140	Unfrequent	Unfrequent	3-6
DNMT3A	2p23	Ikaros	Inactivating mutations	5-10	1-5	5-10
IKZF1	7p12	ASXL1	Deletions	Unfrequent	Unfrequent	Unfrequent
ASXL1	20q11	EZH2	Inactivating mutations	2-5	2-5	13-25
EZH2	7q36	SF3B1	Inactivating mutations	1-3	1	5-10
SF3B1	2q33.1	SRSF2	Inactivating mutations	Unfrequent	3	4-7
SRSF2	17q25.1		Inactivating mutations	Unfrequent	Unfrequent	9-17

Overview

In 1879, the German surgeon Gustav Heuck (1854-1940) described PMF and the distinctive morphological characteristics between PMF and Chronic Myeloid Leukemia (CML), the first presenting bone marrow fibrosis, osteosclerosis and extramedullary hematopoiesis (EMH).

Louis Henri Vaquez (1860-1936), a French physician, was the first to describe PV in 1892. In 1903, William Osler (1849-1919) studied PV further as a new clinical entity and distinguished it from relative polycythemia and secondary polycythemia. ET was described in 1934 by Emil Epstein (1875-1951) and Alfred Goedel, both Austrian pathologists. In 1951 William Dameshek described the concept of "myeloproliferative disorders", grouping Chronic Myeloid Leukemia (CML), Polycythemia Vera (PV), Essential Thrombocytemia (TE), Primary Myelofibrosis (PMF), and erythroleukemia [6,7].

Between 1967 and 1981, Philip Fialkow, an American scientific physician, established that the four classic MPNs are disorders of the stem cells. In 1972, Janet Rowley an American genetist, characterized the Ph chromosome as a reciprocal translocation between chromosomes 9 and 22; T (9; 22) (q34; q11) [8]. In 1996, Nicholas Lydon, a British scientist, and Brian Druker, an American scientist, leaded the discovery and the therapeutical use of imatinib (a tyrosin kinase inhibitor) in CML. These important discoveries distinguished CML from other MPN, and the term "MPN" is only used now to refer to PV, ET and PMF; nevertheless, in the MPN category for the World Health Organization (WHO), CML is still included as well as other entities such as chronic Neutrophilic (CNL) and Eosinophilic (CEL) leukemias [9-11].

Thanks to the CML model as a general neoplastic disease, and to the development of "targeted therapies", such as imatinib, advancements have changed the natural history of oncologic diseases. In 2005, the gain-of-function mutation of JAK2 (JAK2 V617F) was described in BCR-ABL negative MPNs, elevating the perspective of a treatment strategy for PV, ET and PMF similar to that of CML. JAK2 mutation is one of the promoters of proliferation and maturation of the hematopoietic progenitors, and it has become a potential therapeutic target for treatment.

Background

As done for other pathologies (CML, MM, TIP, acute leukemias, and lymphomas), the MPN consensus, carried out by ISSSTE Hematologists, has the objective to become a reference frame for the clinical decision making, based on major evidence and recommendations currently available. The different diffusion and implementation phases of the clinical recommendations should be planned and duly overviewed by the corresponding regulatory bodies of such Institution, as well as government agencies.

Consensus Limitations

The consensus does not cover pediatric populations, nor other myeloproliferative neoplasms, such as: chronic neutrophilic leukemia, chronic eosinophilic leukemia and other non-classifiable. Scope: this consensus is mainly aimed for specialists in hematology.

Rationale

To homologate the behavior and medical practice for the adequate diagnosis, treatment and follow-up of the Ph negative MPN, with the aim of improving the safety and quality of attention for the patients; and to obtain epidemiological data from our institution that may be extrapolated to the Mexican population.

Epidemiology

According to a recent study based on records from the United States, prevalence rates are higher for PV (44-57 / 100,000) and ET (38-57 / 100,000) compared to MF (4-6 / 100,000). This may be due to a longer median survival for PV and ET (8-10 years), compared to MF (2-5 years) [12]. PV and ET may progress to myelofibrosis (MF), and the three entities may transform into Acute Myeloid Leukemia (AML). In systematic registration studies, incidence rates of classic Ph-negative MPN fall into a range of 0.1-2.8 cases / 100,000 / year in the United States and the European Union. Estimated incidence rates are higher for PV (0.4-2.88 / 100,000 / year) compared to ET (0.38-1.7 / 100,000 / year), and MF (0.1-1 / 100,000 / year), according to data bases of several registries from the European Union [13].

The lack of registries in Mexico impedes knowing the incidence. In the group work leaded by Ruiz-Argüelles in Mexico, there were 24 chronic myeloproliferative neoplasms in a population of 8,069 patients who visited their sites during 1989 to 2001. It was concluded that chronic myeloproliferative neoplasms are rare diseases in the Mexican Mestizo population, in opposition to a higher incidence in the Caucasian population [8,14-16]. In a recent study in the Hospital General de México, from January 2001 to July 2014, there were 38 patients diagnosed with chronic myeloproliferative neoplasms, 58% of them were female (n=22), with a median age of 58.61 years (range: 24-82). The diagnoses were: 55% (n=21) essential thrombocytemia; 32% (n=12) primary myelofibrosis, and 13% (n=5) polycythemia vera [11].

Pathophysiology

The described mutations responsible for cMPN are: JAK2 (located in the 9p24 chromosome), CALR (Calreticulin, located in the 19p13.2 chromosome), and MPL (myeloproliferative leukemia virus oncogene, located in chromosome 1p34). The present data support the hypothesis that the MPN phenotype is mainly related to the activated receptor types. The expression of the mutated gene in diverse cell lines generates cytokine-independent growth, or hypersensitivity to growth factors [17]. The JAK2 mutation activates the three main myeloid cytokine receptors: Erythropoietin Receptor (EPO), Granulocyte Colony Stimulating Factor Receptor (G-CSF), Granulocyte and Monocyte Colony Stimulating Factor (GM-CSF) and Thrombopoietin Receptor (TPO); while mutations CALR and MPL are restricted to the activation of the thrombopoietin receptor gene. This explains why the JAK2 mutation associates to PV, ET and PMF, while CALR and MPL mutations associate to ET and PMF [13].

Molecular Biology

JAK2 Gene Mutations: V617F Mutation and exon 12 Mutation

JAK2 protein is a kinase part of the signaling transduction pathway JAK-STAT that uses the type I cytokines receptors (EPO, G-CSF, GM-CSF or TPO). In 2005 the presence of the JAK2 V617F mutation was described in cMPNs, which consists on the change of a guanine by a thymidine in nucleotide 1849 located in exon 14 of the JAK2 gene, causing a change in codon 617 which replaces Valine (V) with Phenylalanine (F) [18]. This amino acid is located in the JH2 pseudokinase domain of the JAK2 protein, which has inhibitory activity on the kinase domain. As a consequence of the JAK2 V617F mutation, a constitutive activation of the JAK2 protein occurs in the absence of binding of the ligand to the hematopoietic receptor that causes a gain of function, that is, a permanent activation of this signal transduction pathway. There are different techniques to study the presence of the mutation, where the real time PCR allele-specific is the technique with higher sensitivity [19].

CALR Mutations

At least 60 different types of mutations have been described in the gene encoding the protein calreticulin (CALR). This protein is located in the endoplasmic reticulum and regulates different cellular processes of proliferation, phagocytosis and apoptosis. The mutations detected consist of deletions and insertions that affect the last exon of the gene (exon 9) and cause a premature breakdown of the protein, replacing its terminal carbon and modifying its negative charge and its function. CALR mutations have been described in 50 to 70% of patients with ET and PMF who do not have a JAK2 or MPL mutations, so it could play an important role as a diagnostic marker for these entities [17]. The A52-bp16 deletion and the 5-bp insertion are two specific mutations representing approximately 80% of these mutations [14].

MPL Mutations

There are several mutations in myeloproliferative neoplasms that affect the gene that codes for the thrombopoietin receptor (MPL), causing a gain of function by activating the signal transduction pathway dependent on this receptor. These mutations are grouped in exon 10 of the gene, five of them are recurrent in ET and PMF (W515L, W515K, W515A, W515R and S505N). The most frequent are W515L and W515A, which cause conformational changes that result in a constitutive signaling activation [14,20]. Alterations described in this region are present in 5% in PMF, and 1% in ET. No mutations have been described in the MPL gene in patients with PV.

Recently, mutations have been described in a small percentage of patients with Ph-negative NMP in different genes, which we can classify by their function in:

a. Genes involved in intracellular signaling: LNK, CBL [21].

b. Genes involved in epigenetic regulation: TET2, ASXL1, IDH1/IDH2, IKZF1, EZH2, DNMT3A [13,19].

c. Genes involved in the messenger RNA processing (or splicing): SF3B1, SRSF2, U2AF1 [22].

Mutations in these genes are detected in a limited percentage of patients, specially, in those with PMF. Its diagnostic role and prognostic potential value are not clear yet [23]. In the triple negative case (JAK2, CALR and MPL, which in the WHO classification criteria include the presence of a clonal marker as a minor diagnostic criterion in TE or a major alternative criterion in MFP, in the absence of the three main alterations), the search for other associated mutations such as ASXL, EZH2, TET2, DH1 / ITH2, SRSF2 or ESF3B1 is useful to determine the clonal nature of the disease [14,20].

Clinical Presentation

Constitutive symptoms (fever, night sweats and weight loss [24]) are more frequent in PMF patients than in those with PV or ET [2]. Table 3 shows the burden of symptoms experienced, related to the disease, in patients with cMPN presented one year before diagnosis. There are several validated tools in English for the evaluation of symptoms related to the disease [25,26]. The Myelofibrosis Symptoms Evaluation Form (MF-SAF) is a 20-item tool used for symptoms associated with MF, including: fatigue, symptoms associated with splenomegaly (early satiety, abdominal pain or discomfort, inactivity and cough), sweating, itching, bone pain, fever and weight loss [27]. It is advisable to evaluate basal symptoms and monitor the state of symptoms during treatment for all patients.

Table 3: Symptoms reported by patients with CMPN.

Symptoms			
Fatigue	**84% PMF**	**85% PV**	**72% ET**
Pruritus	52%		
Nocturnal Diaphoresis	49%		
Bone pain	44%		
Fever	14%		
Weight loss	13%		

Polycythemia Vera

i. Arterial and venous thrombosis: these are the most frequent complications and the main cause of death. One third of the cases presents before the diagnosis. Two thirds of the cases are arterial and, among the most frequent venous thrombosis we found DVT-DET [28].

ii. Bleeding: occurs in 15 to 30% (cause of mortality in 3%) Table 4.

Table 4: Thrombo-Hemorrhagic complications of PV and ET.

Thrombotic and Hemorrhagic Manifestations	Site of Manifestation
Microvascular Manifestations	Peripheral microcirculation
	Erythromelalgia - Acroparesthesia - Acrocyanosis
	- Gangrene
Micro and Macrovascular Thrombotic Manifestations	Brain and ocular circulation
	Headache
	Blindness, paresis, instability, dysarthria, scintillating scotomas
	Transient ischemic accidents (TIA)
	Stroke
	Coronary circulation
	IAM - Unstable angina
	Peripheral arterial circulation
	Intermittent claudication
	Arterial thromboembolism
	Abdominal vessel thrombosis
	Suprahepatic portal vein thrombosis
	(Budd-Chiari) – splenic
	Mesenteric venous thrombosis
	Venous thromboembolism (VTE)
	Deep venous thrombosis (DVT)
	Pulmonary thromboembolism (PTE)
Hemorrhagic Manifestations	Mucocutaneous (hematomas, epistaxis, gingivorrhagia, etc.)
	Chronic or acute digestive hemorrhage
	Urogenital (hematuria, metrorrhagia)
	Hemorrhages secondary to surgery, trauma, delivery or cesarean

Other symptoms: Aquagenic pruritus (40%), heat intolerance.

iii. Signs: plethoric facies (erythrosis), conjunctival chemosis, gout, palpable splenomegaly (70%), renal lithiasis, pulmonary hypertension.

The typical or classical evolution can be expressed in 2 phases:

a. Polycythemic Phase: increase of phenotypically normal circulating red blood cells, with hemoglobin (Hb) and hematocrit (Hct) persistently elevated and, less frequently, leukocytosis, thrombocytosis, splenomegaly, hepatomegaly and other foci of extramedullary hematopoiesis (HEM).

b. Post-PV MF Phase: presence of immature myeloid precursors and/or dacryocytes in PB, decrease in Hb not related to treatment, increase of DHL, decrease in platelets, and increase in the number of leukocytes and progressive splenomegaly.

c. There is a preclinical phase of PV or "early PV" characterized by a normal erythrocyte mass, Hb and Hct values in normal or slightly increased upper limit and with some PV alterations.

Essential Thrombocythemia

Up to 50% of ET patients are asymptomatic at the time of the diagnosis and may remain like that for years. In most cases thrombocytosis appears as a finding in a routine blood count, and in the rest, it presents with vasomotor symptoms due to microcirculation obstruction (23-43%) or thrombosis and/or hemorrhage of variable magnitude from 11 to 25% at the beginning [29,30]. Incidence of thrombosis is directly proportional to age. 1.7% in patients younger than 40 years vs. 15% in people >60 years per patient/year. Severe thrombocytosis is more

commonly associated with hemorrhages than thrombosis; this paradox has been related to an alteration of the von Willebrand factor (vWF) associated with thrombocythemia, characterized by the loss of large vWF multimers [31].

The pathophysiology of hemorrhage is also caused by:

i. Pre-existing anatomical defects (gastroduodenal ulcer, polips, tumors, etc.)

ii. Portal hypertension secondary to hepatic and splenic venous thrombosis.

iii. Thrombosis of the duodenal arcade or myeloid metaplasia, and

iv. NSAIDs chronic use.

During the physical examination a moderate splenomegaly may be found in up to 10% of patients, and hepatomegaly in 10 to 15%. All clinical entities presenting thrombocytosis should be excluded [32].

Primary Myelofibrosis

The most frequent symptom of PMF is fatigue, present in 50 to 70% of the patients [23,33]. Splenomegaly related symptoms have been described in 25 to 50% of the patients, while a small number present weight loss. From 5 to 20% present other signs such as: fever, bone pain and night sweating. Approximately, 15 to 30% of the patients are asymptomatic, with splenomegaly in 90%, hepatomegaly in 40 to 70%, or abnormal blood findings.

Splenomegaly and hepatomegaly are due to the marked extramedullary hematopoiesis associated to PMF. In a series of 566 PMF patients, 16% showed pruritus related to cytokines release. Stages of evolution. It begins with a proliferative stage, reaching the characteristic features of progressive anemia with dacryocytes (tear-shaped erythrocytes), leukoerythroblastosis in peripheral blood (immature myeloid and erythroid elements), splenomegaly, fatigue, bone pain, night sweats, and weight loss, with a bad quality of life and a reduced survival. Some evolve to leukemic transformation.

Diagnosis

Diagnostic criteria

The latest WHO classification, updated in 2016, perfected and expanded the use of objective diagnostic criteria; however, it is important to be familiar with the previous classification systems [33].

Polycythemia Vera (Tables 5 and 6).

Table 5: WHO 2008 Diagnostic criteria for PV.

Major criteria	Hb> 18.5 g/dL in men, > 16.5 g/dL in women or other evidence of increased erythrocyte volume. ***
	Presence of JAK2V617F or another mutation, such as JAK2 exon 12.
Minor criteria	BM pathology with hypercellularity, panmyelosis, with important erythroid, granulocytic and megakaryocytic proliferation. Proliferation and clustering of small and large (pleomorphic) MK. Lack of iron. Limited or no inflammatory reaction (plasmacytosis, cell debris).
	Levels of erythropoietin (EPO) below the normal reference range.
	Formation of spontaneous erythroid colonies *in vitro* (SEC)

*** Hb or Hct >99th percentile of the reference range for the method used according to age, sex, residence altitude or Hb > 17 g/dL in men and 15 g/dL in women. If this value represents a documented and consistent increase of at least 2 g/dL above the baseline level of the individual, not attributable to a correction of Fe deficit or increase of red cell mass> 25% above the normal predictive value. PV diagnosis: 2 major criteria + 1 minor criterion or 1 mayor criterion + 2 minor.

Table 6: WHO 2016 Polycythemia Vera.

Major Criteria
1. HB >16.5 g/DL in men, and >16 g/DL in women, or Hct >49% in men and 48% in women, or elevated erythrocyte mass (>25% of the normal value).
2. Bone biopsy: hypercellularity for age with panmyelosis, including erythroid, granulocytic hyperplasia and proliferation of megakaryocytes with pleomorphism and mature megakaryocytes.
3. Presence of JAK2 V617F or JAK2 in exon 12.
Minor Criteria
1. Decreased normal EPO serum levels according to the reference value. For a PV diagnosis 3 major criteria are required, or the first 2 major plus the minor criterion.
2. The bone biopsy can be omitted in case of persistent erythrocytosis and Hb > 18.5 g/dL in men (55.5%) and >16.5 g/dL in women (49.5%) for the third major criterion and the minor criterion. Initial myelofibrosis may be present in 20% of the patients and predicts the progression to post-PV myelofibrosis.

Diagnostic Algorithm

We must suspect of any patient with an increase of the red blood cells, or an increase of hemoglobin/hematocrit, and arterial oxygen saturation >92%. PV should be suspected in patients with Budd-Chiari syndrome and portal vein thrombosis, splenic or mesenteric, particularly in women younger than 45 years. In this context, the portal hypertension or hypersplenism may cover the increase in the blood cells count. Additional evidence suggesting a PV diagnosis includes:

i. Splenomegaly

ii. Thrombocytosis and/or leukocytosis

iii. Thrombotic complications

iv. Erythromelalgia or aquagenic pruritus

v. Microvascular symptoms (e.g. headache, paresthesia)

Peripheral Blood Smear (PBS):

In the initial stages, the peripheral blood usually has an excess of normocytic red blood cells. Thrombocytosis is common (median platelet count 466,000/ μ L) and approximately 15% of cases can mimic essential thrombocythemia. Leukocytosis is observed in the absence of fever or infection (median white blood cell counts 10,400/ μ L, range 3000 to 172,000/ μ L). On the contrary, in the myelofibrosis stage a leukoerythroblastic pattern develops with dacryocytes, poikilocytosis and erythroblasts.

Bone Marrow Aspiration and Bone Biopsy:

The Polycythemia Vera Study Group (PVSG) did not include specific bone marrow findings as major or minor criteria for diagnosis. These data were obtained by analyzing 281 bone marrow pretreatment biopsies in the first PVSG study and the following was observed:

a. The most common anomaly was the absence of stainable iron bodies in 94%;

b. Cellularity was variable: from 36 to 100% (median 82%, normal 35 to 50%);

c. The number of megakaryocytes and the amount of reticulin were variable, although both were generally increased.

The bone marrow biopsy usually shows hypercellularity and trilinear growth with erythroid, granulocytic and megakaryocytic proliferation. Findings in the bone marrow examination change as the disease progresses from a prodromal phase with mild erythrocytosis, to a phase with increased red blood cell mass to myelofibrosis with cytopenias, ineffective erythropoiesis, fibrosis, extramedullary hematopoiesis, and hypersplenism. Bone marrow biopsy findings are important in the WHO diagnostic criteria. In a study of 526 subjects who met the WHO criteria for the diagnosis of PV: 74 (14%) had reticulinic fibrosis of a lower degree (grade 1) in their initial examination and only 2 showed higher grade fibrosis.

Serum Erythropoietin

Patients with PV usually have low levels of EPO. Although low EPO levels are very specific for PV, the above-normal levels are unusual and suggest secondary erythrocytosis with a specificity of 98%. In two studies, for example, serum EPO levels in 42 patients with PV were compared with control subjects with other causes for polycythemia (e.g. hypoxia). The sensitivity and specificity of serum EPO levels below the normal reference range for the diagnosis of PV (using the criteria of the polycythemia vera study group as gold standard) were 64 and 92% to 99%, respectively. Sensitivity increased to 72% in patients who were analyzed twice. EPO serum concentration remained low even when the red blood cells mass normalized after a phlebotomy. Therefore, most of the new diagnostic criteria for PV include a low serum EPO level [34].

JAK2 V617F

JAK2 V617F mutations constitute the most frequent molecular alteration in MPNs, being detected in the majority of the PV patients (>95%), and in approximately, half of those (50 to 60%) with ET and PMF. The frequency of JAK2 V617F in PV and TE in children is lower (around 40%) than in adults, being important to rule out hereditary conditions in these cases.

Its diagnostic value consists of the usefulness as a clonal marker, making possible a differential diagnosis between neoplastic conditions with respect to reactive ones. It constitutes one of the current diagnostic criterion for PV, ET and PMF established by the World Health Organization. However, the absence of JAK2 V617F does not exclude the diagnosis of PV, ET or PMF (although this is unlikely for PV). This mutation does not allow to discriminate between the different MPN (PV vs. ET vs. PMF), also requiring clinical, laboratory and histological diagnostic criteria for its classification. Detection of the JAK2 V617F mutation can be performed in PB or BM using PCR techniques, such as allele-specific PCR or RFLP (restriction fragment length polymorphism), or by DNA sequencing. The determination of the allelic burden is still not very standardized, being carried out in the majority of the cases by real-time PCR [35,36].

Other Molecular Alterations

There are other molecular alterations in CMPN, although they are less frequent than JAK2 V617F. Mutations in exon 12 of the JAK2 gene are detected in 4% of PV cases and represent 60 to 80% of the JAK2 V617F-negative PV. This indicates that the possibility of PV in the absence of a JAK2 mutation (either V617F or exon 12) is exceptional. Patients with an exon 12 mutation tend to have isolated erythrocytosis, although their evolution does not differ from that in JAK2 V617F-positive patients. The study of JAK2 exon 12 would be indicated in patients with a strong suspicion of PV, negative for JAK2 V617F [36].

Mutations of the thrombopoietin receptor MPL are found in patients with ET (1 to 4%) and PMF (5 to 11%) without clinical or prognostic relevance. Its study as a diagnostic marker would be justified in patients with ET or PMF JAK2 V617F negative. More recently, mutations have been identified in genes involved in epigenetic regulation, such as mutations in the TET2 gene, which are detected in about 7% to 17% of patients with PV, ET and PMF. Other low frequency mutations in PMF, post-PV or post-ET MF, or in the leukemic phase include: CBL, LNK, IDH1/2, ASXL1, EZH2, DNMT3, among others.

Currently, the detection of these mutations in the routine study of MPN patients is not included. When an MPN is suspected, the initial study includes the evaluation of the JAK2 V617F mutation and BCR-ABL rearrangement. In the absence of JAK2 V617F, the study of JAK2 exon 12 mutations is suggested in case of strong suspicion of PV, or MPL exon 10 mutations in patients with presumed ET or PMF. The study of the BCR-ABL rearrangement is useful to exclude CML, especially in patients negative for JAK2 V617F or in those with characteristics suggestive of this entity, such as basophilia, deviation to the left in the leukocyte formula or atypical BM histology.

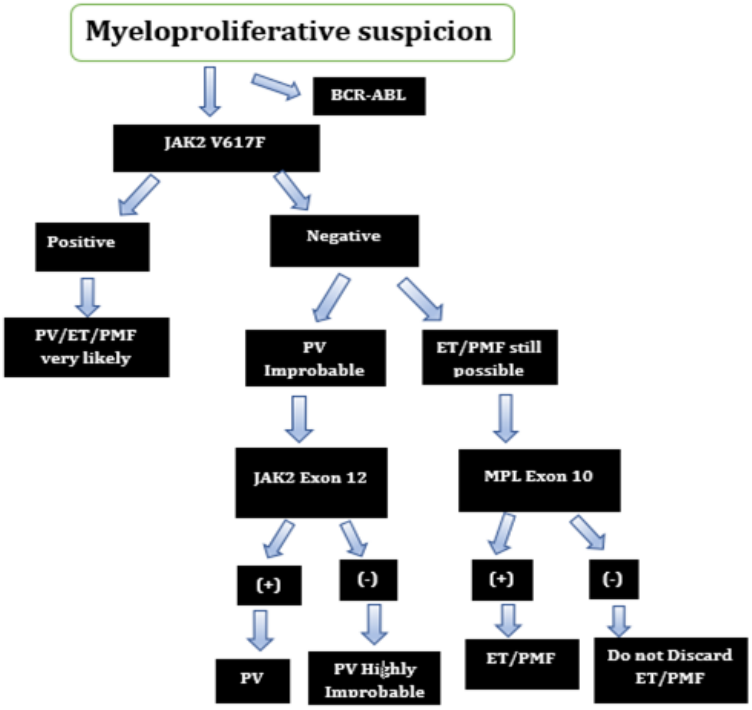

Figure 1: Molecular algorithm for CMPN.

In case of positivity for BCR-ABL, the cytogenetic study and FISH for BCR-ABL (the latter in case of absence of the Phi chromosome by cytogenetics) are fundamental to complement the diagnosis.

However, cytogenetic studies are relevant, since they allow to:

i. Confirm clonality and rule out reactive myeloproliferation,

ii. Exclude Phi chromosome to make the differential diagnosis,

iii. Evaluate if there is cytogenetic clonal evolution during the leukemic transformation, and

iv. Identify the worst prognosis population.

Incidence of cytogenetic alterations at the time of diagnosis is 15 to 25%. The most common alterations are: 20q-, +8, +9, +1q, 13q-. The acquisition of +1q, 5q-,7q- y 17p- it is associated to a poor prognosis (Figure 1) (Table 7).

Table 7: PV differential Diagnoses.

Classification of Absolute Erythrocytosis	
Primary Erythrocytosis PV	
Secondary Erythrocytosis Congenital	Hb with high affinity for O_2 Deficiency of mutase 2,3 biphosphoglycerate VHL mutation
Acquired	EPO mediated With hypoxia Central hypoxia process COPD Right-left Cardiopulmonary shunt CO poisoning Current smokers Hypoventilation such as: sleep apnea, altitude
	Local Renal hypoxia Renal artery stenosis End stage renal disease Hydronephrosis Kidney cysts (polycystic kidney disease) By EPO pathological production Tumors Hepatocellular carcinoma Kidney cancer Cerebral hemangioblastoma Parathyroid carcinoma / adenoma Uterine leiomyoma Pheochromocytoma Meningioma
	By exogenous EPO Associated with drugs Androgen preparations Post renal transplantation erythrocytosis
Idiopathic Erythrocytosis	

Essential Thrombocytemia (Table 8)

Table 8: WHO 2008 Diagnostic criteria for ET.

ET diagnostic Criteria (WHO)	1. Sustained platelet count > 450 x 109/L
	2. BM biopsy: proliferation of megakaryocytes with mature and large morphology, with normal or slightly increased proliferation of the granulocytic and erythroid series.
	3. WHO criteria for CML, PV, PM, MDS or any other myeloid neoplasm must not be met.
	4. Demonstration of JAK2V617F or MPL W515L/K mutation, or other clonal marker, or in the absence of JAK2V617F mutation, no evidence of iron deficiency or other causes of reactive thrombocytosis.
	The four diagnostic criteria must be met.

ET is defined as a clonal chronic myeloproliferative neoplasm that primarily involves the megakaryocyte (MK) line of the bone marrow (BM); It is characterized by persistent thrombocytosis (greater than 450,000/µl) and megakaryocytic hyperplasia, in the absence of erythrocytosis or leucoerythroblastosis (Table 9). It has a relatively benign clinical course, with a higher frequency of thrombotic complications between 15 and 25%, arterial being more frequent (60 to 70%) than venous thromboses; also, hemorrhagic complications and an increased risk of transformation to a more severe hematologic malignancy post-ET MF (4 to 8%) at 10 years, and much less frequent MDS and AML. Between 50 and 60% of patients with ET are positive for the JAK2 V617F mutation and between 1 and 4% are carriers of mutations in the thrombopoietin receptor gene (MPL gene).

Patients carrying JAK2 V617F have characteristics that resemble PV:

i. Higher Hb level, higher leukocyte and neutrophil count;

ii. Lower platelet count ($p < 0.001$ for each variable);

iii. With a greater population of erythroid and myeloid progenitors in BM (p = 0.005 and 0.02, respectively);

iv. Higher frequency of venous thrombosis;

v. Lower ferritin and EPO levels;

vi. It was in this group where all the transformations to PV occurred.

Table 9: WHO 2016 Essential Thrombocythemia.

Major criteria
1. Platelets > 450,000.
1. Bone marrow biopsy with megakaryocytic proliferation, increase in the number of mature megakaryocytes with hyperlobulated nuclei. No significant increase in neutrophils or erythropoiesis and, rarely, a reticulin increase (grade 1).
4. Not meeting criteria for BCR ABL, CML PV, PMF, myelodysplastic syndrome or other myeloid neoplasms.
5. Presence of Mutation: JAK2, CALR or MPL.
Minor criteria
1. Presence of a clonal marker or evidence of reactive thrombocytosis. For an ET diagnosis the 4 major criteria are required, or the first 3 major plus the minor criterion.

Diagnostic Tests

Peripheral blood smear characterized by:

a. Marked thrombocytosis in peripheral blood:

b. Platelet anisocytosis that goes from very small platelets to giant ones;

c. Although agranular platelets and those with abnormal shapes can be seen, they are not common;

d. Erythrocytes are normochromic and normocytic, but could be hypochromic and microcytic due to a concomitant iron deficiency;

e. The leukocyte count is usually normal or slightly elevated, with normal differential and mild basophilia in some cases;

f. A leukoerythroblastic picture with dacryocytes, poikilocytosis and circulating nucleated red cells suggests the transformation to a stage of post-ET myelofibrosis.

Bone Marrow Aspirate and Biopsy

Bone marrow biopsy classically shows normal or moderate hypercellularity and the growth of prominent megakaryocytes to giants with abundant mature cytoplasm, deeply lobulated and hyperlobulated nuclei.

The following are not characteristic of ET, and if present they suggest an alternative diagnosis:

a. Megakaryocytes with very atypical morphology,

b. Increase in Myeloblasts,

c. Myelodysplastic Characteristics, and

d. Significant reticulin Fibrosis (grade> 1) or collagen Fibrosis.

Histopathological Diagnosis

i. The cellularity is normal or moderately increased for the age of the patient;

ii. There is a preserved histological pattern;

iii. MK with medullary center location in groups or scattered large or giant size and abundant cytoplasm;

iv. Nuclei with deep hyperlobulation and irregular contours;

v. Frequent emperipolesis, not a specific finding;

vi. Normal or increased myeloid and erythroid series. The erythroid series is increased in cases of previous hemorrhages;

vii. Reticulin fibers have a normal pattern or are minimally increased in ET (the significant increase in reticulin or collagen fibers discards a diagnosis of ET), up to 3% may have minimal primary fibrosis and a previous therapy may induce fibrosis.

viii. The presence of hemosiderin is usually observed in 40 to 70% of cases;

ix. No blasts or dysplastic alterations of the granulocytic series are observed, and

x. Extramedullary hematopoiesis is rare.

Molecular and genetic alterations

See PV chapter.

Differential Diagnosis (Table 10)

Table 10: Causes of thrombocytosis, differential diagnosis.

Primary	Reactive
ET PV Overt MF MF prefibrotic phase CML MDS (5q-) Hereditary thrombocytosis	Acute and chronic infections (TBC-Pneumonia) Tissue injury (AMI, pancreatitis) Chronic inflammatory processes Bowel inflammatory disease Collagenopathy-Vasculitis Rebound thrombocytosis (post CT or PTI) Hemorrhage - Ferropenia and its correction Post-splenectomy Neoplasias (solid tumors, lymphomas) Drugs: vincristin, epinephrin, ATRA Cytokynes - Growth factors Renal failure - Nephrotic syndrome Extreme exercise Suppression of alcohol addiction

Primary Myelofibrosis (Table 11)

Table 11: WHO 2008 Diagnostic criteria for PMF WHO 2008 thrombocytopenia, differential diagnosis.

Major criteria	1. Presence of MK proliferation and atypia, accompanied by reticulin or collagen fibrosis or, in the absence of significant reticulin fibrosis, the change of MK must be accompanied by an increase in bone marrow cellularity, characterized by a granulocytic proliferation and, often, decreased erythropoiesis.
	2. Absence of WHO criteria for PV, CML, MDS or other myeloid disorders.
	3. Confirmation of JAK2 or other clonal marker (eg MPL, WK / L) or no secondary evidence in the absence of clonal markers.
Minor criteria	1. Leukoerythroblastosis
	2. DHL increase
	3. Anemia
	4. Splenomegaly
A diagnosis is established if three major and two minor criteria are met	

Primary myelofibrosis (PMF), formerly called idiopathic chronic myelofibrosis and agnogenic myeloid metaplasia, is a clone disease of the hematopoietic progenitor stem cell, characterized by progressive fibrosis of the bone marrow (BM) and the development of extramedullary hematopoiesis (EMH) (Table 12).

Table 12: WHO 2016 PMF criteria.

Major criteria
1. Proliferation and megakaryocytic atypia, accompanied by reticulin or collagen fibrosis, grade 2 or 3.
2. WHO criteria not met for ET, PV, BCR-ABL positive CML, MDS or any other CMPN.
3. Presence of JAK2, CARL or MPL. In the absence of these mutations or clonal marker, ASXL1, EZH2, TET2, IDH1-IDH2, SRF2, SF381 or absence of reactive MF.
Minor criteria
Presence of at least 1 of the following (in 2 consequential determinations): a. Anemia not attributed to any other comorbidity, b. Leukocytosis > 11,000, c. Palpable splenomegaly, d. DHL increased above the upper limit, e. Leukoerythroblastosis.

The diagnosis requires at least 3 major and 1 minor criteria.
Fibrosis in reactive bone marrow can be due to: infections, autoimmune disorders or some chronic inflammatory condition, hairy cells leukemia or lymphoid neoplasm, metastatic or toxic, chronic exposure.

Diagnostic Tests

Peripheral blood smear is very characteristic, showing anisocytosis, poikilocytosis, dacryocytes, normoblasts and varying degrees of polychromatofilia.

Bone Marrow

Without marked fibrosis, which has classically been considered the hallmark of the disease. The marrow can be aspirated and/or biopsied directly or evaluated by magnetic resonance imaging (MRI). These techniques can be used for initial diagnosis, prognosis and follow-up.

Bone Marrow Aspirate

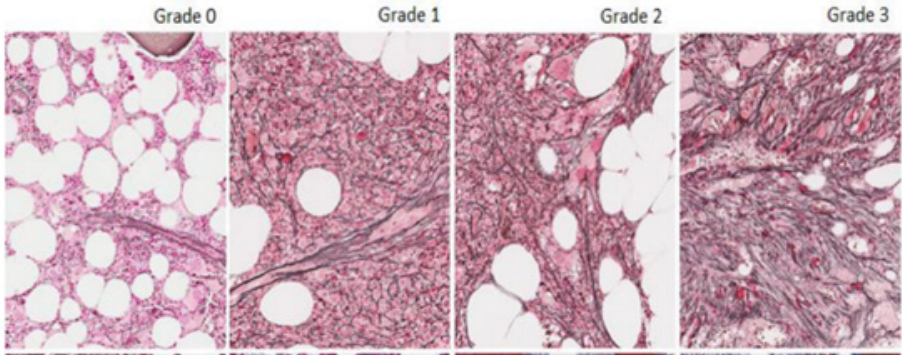

Figure 2: Degrees of myelofibrosis according to the WHO classification [37].

The bone marrow in PMF is often difficult to aspirate, due to a usual "dry" touch. In addition, the results of the aspiration alone, if successful, are not diagnostic. The most frequent findings are neutrophilic and megakaryocytic hyperplasia. Megakaryocytes are often morphologically abnormal. Granulocytes may show hyperlobulation and erythroid precursors may be normal or increased. These morphological changes in megakaryocytes help distinguish early / pre-fibrotic PMF from essential thrombocythemia (Figure 2) (Table 13).

Table 13: Degrees of myelofibrosis according to the WHO classification [37].

Grade EUMNET/WHO	Description
MF0	Dispersed linear reticulin, without intersections
MF1	Fine deposit of reticulin with several or frequent intersections
MF2	Dense, diffuse deposit with extensive intersections and occasional foci of collagen
MF3	Dense and diffuse deposit with abundant intersections and presence of collagen bundles

Histopathological Diagnosis

Prefibrotic stage PMF

a. 30 to 40% are diagnosed in this phase.

b. Hypercellularity.

c. Megakaryocytic proliferation: variable size, pleomorphic aspect, nuclear lobulation anomalies, "cloud" or "balloon" nuclei, increased nucleocytoplasmic ratio, hyperchromasia, naked nuclei, pleomorphic, bizarre appearance and topographic alteration with paratrabecular location and distribution in dense nests.

d. Neutrophilic proliferation.

e. Frequent decrease in erythropoiesis with deviation to the left.

f. Increase in angiogenesis.

g. Minimal or absent reticulin fibrosis (grade 0 and 1).

h. CD34: <10%.

i. Progression to MF in 50 to 70%, unpredictable. The increase in maturation defects (dysplasia) of the MK is associated with a faster progression to fibrosclerotic stages.

Fibrotic Stage PMF

i. 60 to 70% are diagnosed in this stage.

ii. Gradual cellularity decrease.

iii. The BMB may be hypercellular but more commonly normo or hypocellular, presenting cellularity in "patches" of hematopoietic tissue separated by areas of connective or adipose tissue.

iv. Predominant megakaryocytic proliferation, prominent atypia: dense and compact nests, anomalies of nuclear "cloud" or "balloon" lobulation, hyperchromasia, increased nucleocytoplasmic ratio, naked nuclei.

v. Hematopoietic islands separated by connective or adipose tissue.

vi. Bone neoformation. Osteosclerosis.

vii. Reticulin (grade 2 and 3) and collagen fibrosis.

viii. Sinusoidal dilatation with intraluminal hemopoiesis.

ix. In cases of previous diagnosis of PMF, the presence of 10 to 19% blasts or CD34 + cell nests indicate an accelerated phase, and the presence of 20% or more blasts means transformation to acute leukemia.

Differential Anatomopathological Diagnoses

Prefibrotic PMF from ET:

a. ET: MK more dispersed, lose nests, increased size, with abundant cytoplasm and deep nuclear lobulations.

b. PMF: More atypical MK with size variations, dense nests, frequent neutrophilic proliferation.

MDS with Fibrosis

i. 5 to 10% of MDS cases may present fibrosis, usually RAEM.

ii. In general, there is no presence of organomegaly, showing prominent dysplasia in multiple cell lines in BM and PB.

iii. MKs are small and dysplastic in the BMB, unlike the large and bizarre MK in PMF.

Extramedullary Hematopoiesis

a. Spleen is the most common site, followed by the liver.

b. The spleen shows expansion of the red pulp by erythroid cells, granulocytes and MK.

c. MK are the most conspicuous component.

d. Investigate the presence of CD34 + myeloid sarcoma using immunohistochemical techniques [30].

Risk Classification

Table 14: Prevalence and incidence of thrombosis in CMPN.

CMPN	At diagnosis (%)	Accumulated Rate / Year (%)	Post-thrombosis Recurrence Risk
PV	30	2.5-5	8-19
ET	10-29	1.9-3	8-31
PMF	13	1.7-2.2	9-10.7
"Thrombosis may be venous or arterial. Recurrence of thrombosis presents at the initially affected site.			

Thrombotic and hemorrhagic complications are the main cause of morbidity and mortality in PV. Patients who do not receive any type of treatment have a very short survival due to the appearance of recurrent thrombotic episodes, especially cerebro-vascular accidents [37-39] (Table 14). The initial management of ET patients depends on the risk of thrombotic complications, calculated by prognostic scores. Important factors to evaluate the risk of thrombotic complications include a history of venous or arterial thrombosis, age > 60 years, JAK2 V617F mutation and cardiovascular risk factors (e.g. hypertension, diabetes mellitus, obesity and smoking) (Table 15).

In the case of positivity for the JAK2 V617F mutation, there is an increased platelet activation (P-selectin, tissue factor, thrombin generation and aggregation), leukocyte activation (leukocytosis, monocyte tissue factor, overexpression of CD14, CD40L and leukocyte alkaline phosphatase), activation of endothelial cells (thrombomodulin and von Willebrand factor), activation of coagulation factors (tissue factor) and presence of fibrinolysis products (Dimer D) [40]. Although treatment is not curative, modern therapy for PV can relief symptoms and prolong survival. The treatment objectives are: to reduce the risk of first and/or recurrence of thrombosis, prevent hemorrhagic events, improve the burden of symptoms and minimize the risk of evolution to post-PV Myelofibrosis (MF) and Acute Myeloid Leukemia (AML)/Myelodysplastic Syndrome (MDS) [32].

Table 15: a. Percentage of thrombosis and b.Bleeding in the different arterial and venous territories in CMPN [31].

CMPN	At diagnosis (%)	Accumulated Rate / Year (%)	Post-thrombosis Recurrence Risk
PV	30	2.5-5	8-19
ET	10-29	1.9-3	8-31
PMF	13	1.7-2.2	9-10.7
"Thrombosis may be venous or arterial. Recurrence of thrombosis presents at the initially affected site.			

Hemorrhage	PV	ET	PMF	No. of patients
Upper digestive tube	7	3	6	20
By intervention	4	0	0	6
CNS	0	0	3	3
Total	13	5	10	36

Polycythemia Vera Risk Stratification

Table 16: Risk factors and stratification according to low or high risk of thrombosis.

Prognostic Model	Risk group
Risk of thrombosis	
At least one of the following	
Age ≥60 years	Low risk: age <60 years without history of thrombosis.
Prior thrombosis	High risk: age ≥60 years and/or history of thrombosis.

Risk stratification is part of the treatment decision based on low or high risk [41] (Table 16). Although no drug has shown to reduce the risk of hematological transformation to MF or AML/MDS, current treatment approaches generally avoid agents known to increase this risk.

Risk stratification in Essential Thrombocytemia

Table 17: Thrombosis predictive model (see text).

Predictive model of thrombosis in essential thrombocytosis * IPSET-thrombosis
a. Age ≥ 60 years (1 point)
b. History of thrombosis (2 points)
c. Cardiovascular risk (1 point)
d. Presence of JAK2 V617F (2 points)
Low risk <2 points 1.03%
Intermediate risk = 2 points 2.35%
High risk ≥ 3 points 3.56%
*IPSET = International Prognostic Score for Essential Thrombocythemia.

Predictive model of thrombosis in essential thrombocytosis * WHO-ET
Very Low Risk of Thrombosis
• No history of thrombosis
• Age < 60 years
• Non-mutated JAK2 V617F
• No cardiovascular risk factors
Low Risk of thrombosis

• No history of thrombosis
• Age < 60 years
• JAK2 V617F mutated and/or cardiovascular risk factors
High cardiovascular risk
• History of thrombosis and/or age > 60 years
• JAK2 V617F mutated and/or cardiovascular risk factors

In ET, as in PV, try to identify patients at risk of thrombosis (to establish preventive measures) directly impacts on the long-term morbidity and mortality. The current risk stratification for thrombosis in ET has 2 levels and considers categories of low and high risk depending on the absence or presence of: age > 60 years, or a history of thrombosis. In an international study of 891 patients with etiology defined by WHO, we identified additional independent risk factors, including cardiovascular risk factors and JAK2 V617F [42]. Below, we present the WHO-ET and the IPSET-thrombosis Models (Table 17).

Risk of Progression in PV and ET

i. Hematological complications: one of the main causes of death in PV is the transformation of the disease to post-PV myelofibrosis and/or evolution to AML/MDS. A large prospective study reported that the rate of such haematological complications was 1.3 episodes per 100 patients/year (21 cases of AML, one case of myelodysplasia and 38 cases of myelofibrosis in 1,638 patients) [43-46]. The risk of PV transformation to AML has an accumulated incidence of 7.9% at 20 years; for TE 0.6 to 5% within 20 years from the diagnosis [47] and 11% for PMF. In all cases, if they receive prior chemotherapy the incidence increases to 20% [48] (Table 18).

Table 18: Risk factors for myelofibrotic progression in PV and ET: age > 60 years, leukocytosis > 15,000 /cel/uL, extreme thrombocytosis > 1000x10^3 platelets/uL, and myelofibrosis > grade 2.

	Clinical	Genetic
Post-PV	Age Leukocytosis Disease duration Reticulin fibrosis Splenomegaly	JAK2V617F allelic load
Post-ET	Age Leukocytosis Anemia Reticulin fibrosis	Absence of JAK2 ASXL1 mutation

There is little information on the specific prognostic models for post-PV or post-ET myelofibrosis, therefore, it is considered that some models can be extrapolated from PMF, such as the IPSS (International Prognostic Scoring System) and its time-dependent variants (dynamic IPSS or DIPSS and DIPSS plus). However, since they are not specific for secondary myelofibrosis, they are suboptimal to predict survival. The most specific model to date is the MYSEC (Myelofibrosis Secondary to PV and ET) that includes clinical and cytogenetic variables (presented below) [20,49]. The quantitative monitoring of JAK V617F has been identified as a risk factor for progression to myelofibrosis when it remains above 50% or does not persist below 50% in myeloproliferative neoplasms, with an incidence rate of 20.7 (IRR) [50] (Tables 19 & 20).

Table 19: Survival in post-PV or ET MF.

Risk factor	Scoring	
Hemoglobin < 11 g	2	
Circulating blasts ≥ 3%	2	
Non-mutated CALR genotype	2	
Platelets < 150 000	1	
Constitutive symptoms	1	
Age in years	0.15	
Risk group	**Scoring**	**OS years**
Low	Below 11	NR
Intermediate 1	11 to <14	9.3
Intermediate 2	14 to <16	4.4
High	≥ 16	2

Table 20: Clinical and genetic risk factors for leukemia transformation from myelofibrosis secondary to PV or ET.

Transformation	Clinical Risk factor	Genetic Risk Factor
Post-PV Leukemia	Age	Abnormal karyotype
	Leukocytosis	TP53
	Reticulin fibrosis	RUNX1
	Splenomegaly	
Post-PV Leukemia	Age	TP53
	Leukocytosis	RUNX1
	Anemia	
	Reticulin fibrosis	
	Thrombosis	
	Platelets > 1 million	

Stratification in Primary Myelofibrosis

The main risk factors for leukemic transformation in PMF are: age > 65 years, presence of constitutional symptoms, anemia (Hb <10 g/dL), leukocytosis (> 25x103/uL), thrombocytopenia (<100x103/uL), circulating blasts ≥1%, grade of fibrosis in bone marrow, unfavorable karyotype, associated mutation (triple negative vs. JAK2/MPL vs. CALR) [41]. In an epidemiological study of patients in Olmsted County, Minnesota, the three-year survival rate was 52%. Longer survival times have been reported in several non-population-based studies, including the series used to construct the International Prognostic Scoring System (IPSS) for PMF, in which the median survival was 69 months. Several prognostic models are available to evaluate PMF, but the most recent and internationally recognized is the DIPSS Plus (Table 21, comparing the different scales).

Table 21: Comparison of different prognostic models in PMF.

Variable	IPSS	DIPSS	DIPSS Plus
Age > 65 years	✓	✓	✓
Constitutive symptoms (night sweats, fever, significant weight loss)	✓	✓	✓
Hb < 10g/dL	✓	✓	✓
Leukocyte count > 25000 / ul.	✓	✓	✓
Blasts in peripheral blood > 1%	✓	✓	✓
Platelet count < 100x109/L			✓
Need of RBC transfusion			✓
Unfavorable karyotype [+8, -7/7q, i (17q), inv (3), -5/5q-, 12p-, 11q23 rearrangements or complex karyotype]			✓
Prognostic Score calculation	1 point per risk factor	1 point per risk factor + Hb < 10: 2 points	1 point per risk factor

	IPSS	DIPSS	DIPSS Plus			
Risk group	**Risk factor (n)**	**Median OS (years)**	**Risk factor (n)**	**Median SV (years)**	**Risk factor (n)**	**Median S (years)**
Low	0	11,3	0	Not reached	0	15,4
Intermediate 1	1	7,9	1 or 2	14,2	1	6,5
Intermediate-2	2	4,0	3 or 4	4	2 or 3	2,9
High	>3	2,3	5 or 6	1,5	≥ 4	1,3

Overall Survival Models

Table 22: Risk factors and scoring for the prognosis stratification and calculation of overall survival for ET [23].

Risk factor	Scoring	
Age ≥ 60 years	2	
Leukocytes ≥ 11 000	1	
History of thrombosis	1	
Risk group	**Scoring**	**OS years**
Low	0	NR
Intermediate	1 to 2	24.5
High	≥ 3	14.7

PV is associated with a reduced overall survival, higher incidence of thromboembolic events and several hematological complications already discussed. The median survival of PV untreated symptomatic patients has been estimated in 18 months, but survival is 13 years at least, in patients receiving treatment. However, the overall survival of PV treated patients is lower than that of the normal population matched by age and sex. A more exhaustive analysis of the factors that influence survival in PV was an international study of 1,545 patients with this disease who were treated with a variety of agents. Age, leukocytosis, history of venous thrombosis and abnormal karyotype were identified as independent risk factors for survival. This study led to the following prognostic model for survival in PV (Figure 3) [51]. In the same way, ET has a prognostic score that allows an adequate stratification to calculate the overall survival (Table 22).

- **Age ≥ 67 years (5 point)**
  57 to 66 years (2 points)
- **Leukocytes ≥ 15 x 10 $^{9/L}$ (1 point)**
- **Venous thrombosis (1 point)**

Low risk 0 points mean OS 27.8 years

Intermediate risk 1 to 2 points OS 18.9 years

High risk ≥ 3 points OS 10.7 years

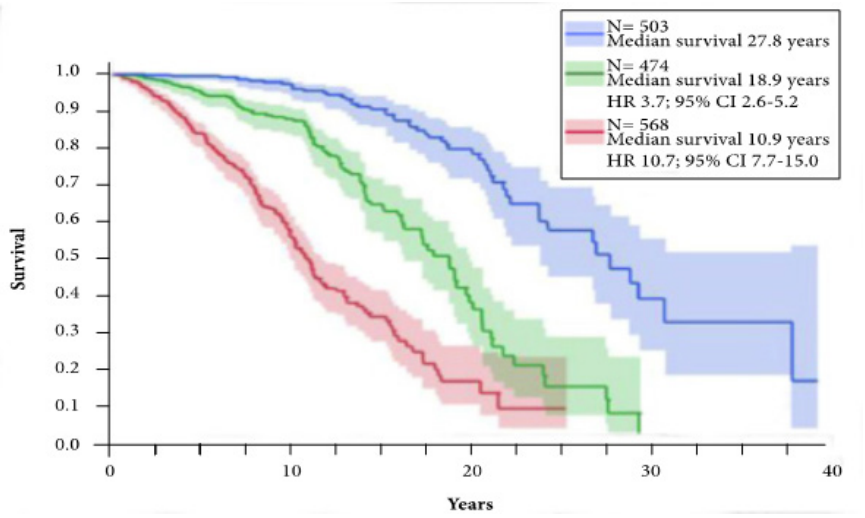

Figure 3:
a. Risk factors and score for prognostic stratification in polycythemia vera, and
b. Median survival according to risk classification.

Treatment

Polycythemia Vera (Figure 4)

The main objective of PV treatment is to prevent thrombo-hemorrhagic complications [28], avoiding, as far as possible, exposing the patient to drugs with leukemogenic potential [52]. On the other hand, no drug has shown to be able to reduce the risk of disease transformation to myelofibrosis or acute leukemia [53]. The hematocrit (Hct) control below 45% is associated with a lower cardiovascular mortality and a reduction in the rate of major thrombosis (level of evidence Ib). Platelet antiaggregation with low doses of acetylsalicylic acid (100 mg/d) is

associated with a lower cardiovascular risk, non-fatal cardiac infarction, non-fatal stroke and major venous thrombosis, without significantly increasing the rate of bleeding (level of evidence Ib) [54]. Cytoreductive treatment is associated with a lower rate of thrombosis compared to phlebotomy only (level of evidence Ib).

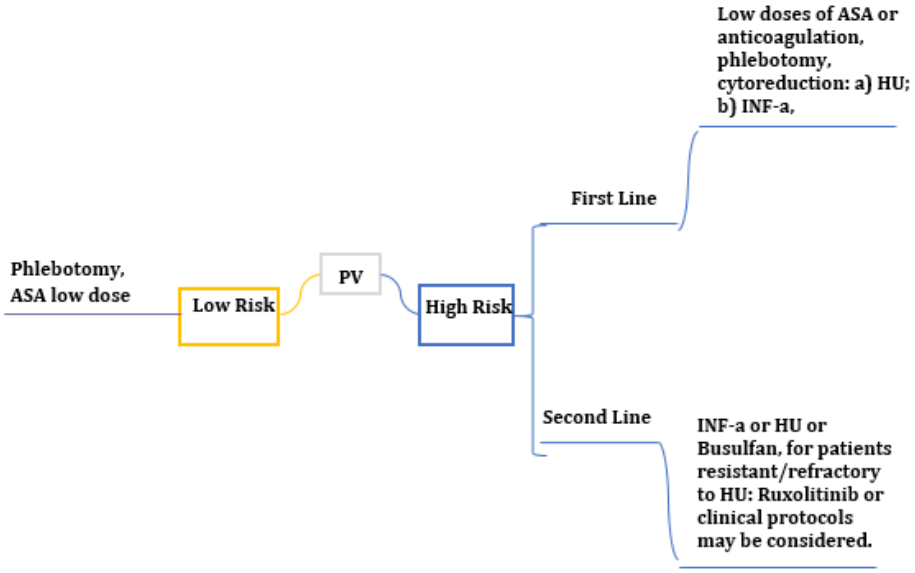

Figure 4: Schematic representation of treatment options according to risk in PV53.

Some cytoreductive agents (chlorambucil, busulfan, pipobroman) have demonstrated leukemogenic potential and should be avoided as far as possible (level of evidence Ib). Hydroxyurea (HU) is currently one of the first line treatments for patients with PV, since its chronic use does not favor a leukemogenic risk [55]. In general, once the cytoreductor treatment is started, it is recommended to try to normalize the Hct value, as well as leukocytes (<10x109/L) and platelets (<400x109/L). However, in some retrospective studies, maintaining normal blood values throughout the treatment is not associated with a lower incidence of thrombosis [33].

Therapeutic Options

Phlebotomy

Phlebotomy and erythrocytapheresis are treatment options for symptomatic patients who require immediate cytoreduction treatment. Administering 450 ml of venous blood (300 ml, in advanced age or heart disease) once or twice a week to obtain a Hct ≤42% (women) or ≤45% (men), since higher levels increase the risk of thrombosis. The disadvantages of using phlebotomies are: high rates of arterial and venous thrombosis in the first 2 years of treatment, no action on splenomegaly, pruritus or myeloproliferative activity.

Platelet Antiaggregant

As long as there is no contraindication for bleeding or intolerance, acetylsalicylic acid (ASA) is recommended at doses of 100 mg/day, associated to phlebotomies or cytoreductive treatment as prevention of thrombosis [56].

Hydroxyurea

The recommended dose is 15 to 30 mg/kg/day, individualizing each patient with their risk factors and dose adjustments. Around 10% will develop resistance to HU (median: 6 years). The frequent complications with its use are: malleolar or oral ulcers, gastric intolerance. Level of evidence Ia [57].

Interferon Alpha

the usual dose is 3 million units, 3 times per week (range, 1.5 to 9 million, 3 times per week), but its main limitations are the adverse effects that require stopping treatment in a third of the cases (flu-like symptoms, irritability, depression, hepatitis and gastrointestinal discomfort).

Pegylated interferon-alpha 2a, Pegasys

Better tolerated with dose adjustments to obtain an adequate hematological control, usual maintenance dose ~ 90 to 180 mcg/weekly). It is the only cytoreductor recommended during pregnancy, due to the lack of teratogenic effect. It induces a high proportion of molecular responses (reduction of the allelic load of mutated JAK2). It shows lower toxicity, better tolerability and a decreased frequency of administration compared with interferon alpha [58].

Busulfan

Initial dose of 2 mg/day PO. It is considered a second-line drug for patients who cannot receive HU or interferon. It should be avoided, since it can generate medullary aplasia and a leukemogenic effect.

Anagrelide

limited role in PV, restricted to the control of intense thrombocytosis in relatively young patients who do not require cytoreductive treatment for other reasons. Level of evidence Ia.

Essential Thrombocythemia (Figure 5)

Treatment of Low Risk Patients

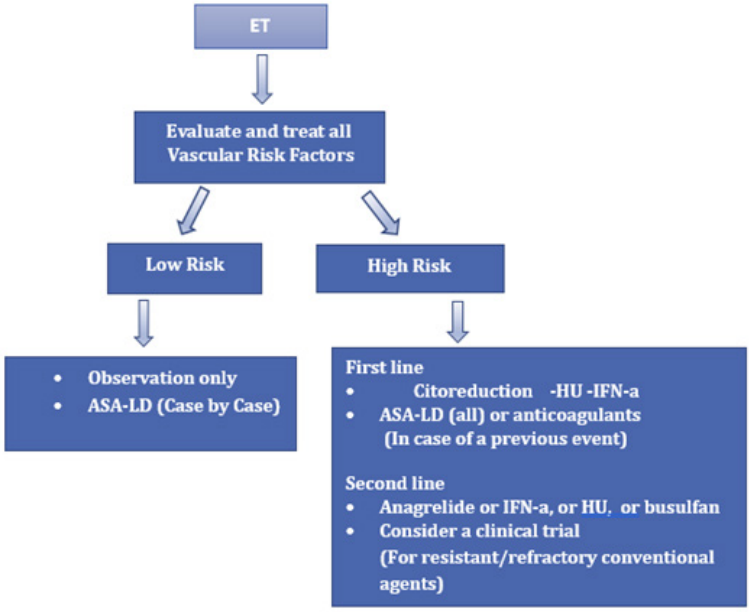

Figure 5: Schematic representation of treatment options according to risk in ET.

It is important to bear in mind that the incidence of thrombosis in patients with low-risk ET is similar to that of the general population and that the administration of antiplatelet agents to low-risk patients who do not receive cytoreductive treatment may increase the risk of bleeding. In low risk ET, we could recommend the administration of antiplatelet agents (acetylsalicylic acid 100 mg/day) to carriers of the JAK2 V617F mutation or with cardiovascular risk factors, as long as the platelet count is < 1000x103/ μ L. In addition to its role in the primary prevention of thrombosis, acetylsalicylic acid at low doses is the treatment of choice for patients who present microvascular symptoms (headache, paresthesia, erythromelalgia).

Treatment of High-Risk Patients

There is unanimous consensus in administering cytoreductive treatment to all patients with high-risk ET. Another indication of cytoreductive treatment is the persistence of microvascular symptoms despite antiplatelet therapy in low-risk patients. Among the different modalities of cytoreductive treatment, hydroxyurea constitutes the best option in the first line at doses of 15 mg/kg/day. Other treatment options are interferon alpha at a dose of 3 million U/3 to 7 days per week; radioactive phosphorus is to be considered in elderly patients, at doses of 1.7 mCi/m2 and a maximum dose of 5 mCi, evaluating the response at 3 months for dose adjustments; busulfan may be considered in elderly patients at a dose of 2 mg/day. Anagrelide, a second-line drug in the treatment of ET, without antiproliferative effect, non-leukemogenic, not producing myelodysplastic effects. The initial dose is 0.5 mg every 12h per day and increases of 0.5 mg per day per week until a response is observed. Avoid doses higher than 4 mg/day. The main adverse effects to be assessed are positive inotropism and vasodilation with palpitations, headache, edema due to increased vascular permeability, dyspnea and congestive heart failure. Contraindicated in patients with heart disease [59].

Myelofibrosis

Considering that there is no standard treatment for primary myelofibrosis, it was proposed to manage these patients based on their risk stratification (IPSS, DIPSS, DIPSS plus), in two large groups:

i. Low and intermediate-1 risk groups, which are asymptomatic, may select surveillance; conventional treatment or ruxolitinib are recommended.

ii. Intermediate-2 and high-risk groups should be considered or not for transplantation. In those not eligible for BMT, the treatment will be based on ruxolitinib, management of anemia or a clinical trial.

Conventional Treatment

It allows the temporarily control of the proliferative manifestations (constitutional syndrome, painful splenomegaly, leukocytosis, thrombocytosis).

Cytolytic Agents: The drug of choice is hydroxyurea. The initial dose is 500 mg/day PO and will increase according to the hematological tolerance.

Anabolic Agents

They improve anemia in 40% of cases and may increase platelets:

Danazol (600 mg/day) PO, this initial dose must be maintained for at least 6 months to assess a response. In case of response, the dose should be progressively lowered until a low dose (200 mg/day) for maintenance. It can cause hirsutism, liver function alterations and induce or stimulate the growth of prostate or liver tumors, therefore it is recommended to screen for prostate cancer (PSA) before starting treatment, and ultrasound controls.

Erythropoietin and Darbopoietin

They improve anemia in 40% of cases (they should only be administered in patients with periodical EPO baseline serum levels <125 U/L).

Corticoids

They can improve anemia in patients with associated immunological disorders, for example, prednisone (0.5 mg/kg/day) PO initially, with progressive decrease in case of response or, rapid withdrawal if no response is seen after a month of treatment.

Splenectomy

In selected patients, considering mortality (10%) and morbidity (40%), due to peritoneal hemorrhage, infection and thrombosis. Indicated in cases of cytopenia (especially anemia) or symtomatic splenomegaly, in those refractory to other treatments, and portal hypertension. Contraindicated in case of thrombocytosis.

Splenic Irradiation

Painful splenomegaly improves transiently (median: 4 to 6 months), however, a third part present severe and prolonged pancytopenia, associated with some mortality. Only indicated in patients who are candidates for splenectomy, whose general condition contraindicates this procedure.

Immunomodulatory Agents

Thalidomide (50 mg/day) in combination with corticosteroids at low doses (30 mg/day the first month, with progressive withdrawal in the following two months). They improve anemia in 25% of the cases and can raise the platelet count. Slightly effective in controlling splenomegaly. Common side effects: thalidomide (neuropathy, constipation) [60]. The use of lenalidomide (10 mg/day) may be considered in patients who have not responded to thalidomide or with a 5q- association. In a Spanish study it was found that in a group of patients in whom it was possible to analyze the response to treatment (n = 29), 16 (55%) showed a clinical-haematological response. The median time from the start of lenalidomide to the evidence of any response was 185 days (IC 95%: 49-121). Two patients (7%) showed complete response [61].

JAK2 tyrosine kinase Inhibitors

Very effective to control hyperproliferative manifestations of the disease (splenomegaly, constitutional symptoms, cachexia) and pruritus, both in JAK2-positive and negative patients. They may cause anemia and thrombocytopenia. Presently, ruxolitinib (Jakavi) is the only drug approved for adult patients with primary MF or secondary to ET or PV with symptomatic splenomegaly and/or constitutive symptoms. The initial dose should be adapted to the platelet count: 20 mg/12 h (if > 200x109/L), 15 mg/12 h (if 100 - 200x109/L), 5 mg/12 h if 50 - 100x109/L.

If treatment must be stopped, this should be done progressively, in order to avoid the sudden reappearance of the symptoms due to the increase of cytokines suppressed by the drug. Preliminary clinical data suggest an increase in the survival of patients with MF treated with ruxolitinib without significant reduction of medullary fibrosis or allelic load of mutated JAK2. Based on the COMFORT I clinical study, 41.9% of

the patients treated with ruxolitinib had a decrease of at least 35% of the spleen volume, and 97% had an improvement of the splenomegaly. In COMFORT II, 97% of the patients showed some improvement, however, the treatment does not seem to have the capacity to eradicate the neoplastic clone, myelofibrosis, since the allelic burden of the mutated form of JAK2 does not decrease substantially [37-39]. Other inhibitors in JAK studies are: Fedratinib, Momelotinib, Pacritinib

Transplantation

Currently the only therapeutic option with curative potential for PMF. Indicated in young patients with unfavorable prognostic factors (IPSS, DIPSS, DIPSS-Plus intermediate-2 or high risk). In patients aged 45 to 65 years who are candidates for transplantation, a reduced intensity conditioning should be used. The resolution of post-transplant fibrosis can be relatively late (6 and 12 months), so it is not advisable to perform a marrow biopsy before 6 months. However, in a literature review, the 5-year survival ranged between 50 and 67% in those with reduced intensity, and 31 to 61% in those with conventional myeloablative conditioning.

Special considerations: Pregnancy

IFNα represents the only therapeutic alternative for the treatment of CMPN, because in addition to its antiproliferative activity it can generate a cytogenetic, molecular and hematological remission. However, there are no therapeutic guidelines for its management in pregnancy, but the recommendations come from retrospective studies with lower level of evidence. Regarding PV, the use of IFNα should be done under special conditions, and a longer follow-up should be established, since published studies are scarce. It should be noted that IFNα has not presented teratogenic effects at the standard doses used [62,63].

Support Treatment

i. Supplementary measures Allopurinol 300 mg/day if hyperuricemia ≥8 mg/dL or lower with symptoms.

ii. Pruritus: avoid triggering situations (hot showers, intense skin friction), cytoreductive treatment (interferon is the most effective), antihistamines, serotonin reuptake inhibitors (paroxetine 20 mg/d), phototherapy, JAK2 inhibitors. Phlebotomies are not useful to control pruritus.

Follow-up

Table 23: Response criteria in Ph negative CMPN; a) Myelofibrosis, b) PV and ET.

Response criteria in Ph negative MPN		
Myelofibrosis response criteria		
Response categories		
CR	Bone marrow: Normal cellularity according to age, <5% blasts, grade 1 fibrosis or no evidence of fibrosis; plus Peripheral blood: Hb ≥10 g/dL, neutrophils ≥1x109/L, platelets ≥100x109/L and, <2% of immature myeloid cells.	Clinical: Resolution of symptoms related to the disease, not palpable liver and spleen, without evidence of EMH.s
PR	Peripheral blood: Hb ≥10 g/dL, neutrophils ≥1x109/L, <2% of immature myeloid cells; or Bone marrow: Normal cellularity according to age, <5% blasts, grade 1 fibrosis or no evidence of fibrosis; plus Peripheral blood: Hb ≥8.5 g/dL but <10g/dL, neutrophils ≥1x10 9/L, platelets ≥50x109/L but <100x109/L, <2% of immature myeloid cells.	Clinical: Resolution of symptoms related to the disease, liver and spleen not palpable, no evidence of EMH.
CI	Improvement of anemia, decreased splenomegaly and/or improvement of response symptoms, no progressive disease or increased severity of anemia, thrombocytopenia or neutropenia.	
Anemia Response	Transfusion-independent patients: an increase of Hb ≥2g/dL. Transfusion-dependent patients: become transfusion independent.	
Splenic Response	5-10 cm palpable basal splenomegaly below the LCM becomes non-palpable. Palpable basal splenomegaly > 10cm below the LCM with a 50% decrease. Palpable basal splenomegaly <5 cm below the LCM not eligible for splenic response. Splenic response requires demonstration by imaging (CT or MRI) showing a splenic volume reduction ≥35%	
Symptoms Response	Reduction ≥50% in the MPN-SAF TSS score.	

PS	Appearance of palpable splenomegaly at least 5 cm below the LCM, or an increase ≥100% in the palpable distance below the LCM of a basal splenomegaly of 5-10 cm. Increase ≥50% in the palpable distance below the LCM of a basal splenomegaly > 10cm; or Leukemic transformation confirmed by BMA with blast count ≥20%, or blast count in peripheral blood ≥20% associated with an absolute blast count > 1x109/L for at least 2 weeks.
EE	None of the categories listed above are met.
Relapse	No criteria for a CR response at least, after reaching CR, PR, or loss of anemia response that persists for at least 1 month. Recommendations to evaluate cytogenetic and molecular changes induced by treatment:
Cytogenetic Remission	At least 10 metaphases should be analyzed for the cytogenetic response evaluation, requiring confirmation by repeated testing within 6 months: CR: eradication of a pre-existing anomaly. PR: ≥50% reduction in abnormal metaphases (PR only applies to patients with at least ten abnormal metaphases at baseline)
Molecular Remission	The evaluation of the molecular response should be analyzed in peripheral blood granulocytes and confirmation should be made by repeated tests within 6 months: CR: eradication of a pre-existing anomaly. PR: ≥50% decrease of the allelic load (PR only applies to patients with a 20% load of mutant allele at baseline, at least).
Cytogenetic/Molecular Relapse	Reappearance of a previous cytogenetic and molecular abnormality that is confirmed by repeated testing.

Response criteria for Polycythemia Vera and Essential Thrombocythemia		
Grade of Response	**PV Response**	**ET Response**
CR	Hct <45% without phlebotomies	
	Platelets ≥400x10^9/L	Platelets ≥400x10^9/L
	Leukocytes ≥10x10^9/L	Leukocytes ≥10x10^9/L
	Normal spleen size through Imaging	Normal spleen size through imaging
	No symptoms related to the Disease	No symptoms related to the disease
PR	For patients not meeting the complete response criteria	For patients not meeting the complete response criteria
	Hct <45% without phlebotomies	Platelets ≥600x109/L or decrease > 50% from baseline
	Response in ≥3 of the other criteria	
NR	Any response not meeting the partial response criteria	Any response not meeting the partial response criteria

It is suggested to evaluate patients periodically (every 3 to 6 months) in relation to symptoms, response to treatment and timely detection of data for disease progression or clonal transformation, for PV and ET into 2nd MF, and AML/MDS in the case of PMF or 2nd MF (at this time, it is suggested to perform a bone marrow aspiration and biopsy if there is a higher than expected decrease in the cell count). The monitoring and response in Ph negative CMPN, as in other entities, should be based on the clinical, biological and molecular data that define each of the entities at the time of diagnosis. Some of these variables have been defined as part of the Myelofibrosis Response Criteria (Table 23).

The risk of progression to MF in PV at 15 years varies from 6 to 15%, although a duration >6 years is associated with a risk increase (2.8%), and the presence of JAK2 (V617F) is associated in more than 50% to the risk. In the case of leukemic transformation, the reported incidence of progression to AML is 5/1,000 patients and <5% at 10 years (mainly in cases with abnormal karyotype) [28]. However, there are data to guide this risk. Advanced age, anemia, high transfusion requirement, leukocytosis, leukopenia, thrombocytopenia, blasts in PB, systemic symptoms and cytogenetic abnormalities are associated with an unfavorable prognosis.

Although prognostic models for PMF are based on the assessment at the time of diagnosis, the acquisition of these new variables as risk factors during the course of the disease can substantially modify the outcome of patients. A dynamic model, which considers this type of modifications in the risk profile, such as DIPSS and aaDIPSS (age-adjusted in <65 years) has practical value. The presence of the JAK2 mutation (V617F) does not seem to be involved in a worse survival, although a low allelic burden seems to be related [64]. Currently, the survival time of each of the CMPN must be considered in relation to the type of therapy established:

i. No treatment,

ii. Support therapy,

iii. Curative treatment (HSCT for MF),

iv. Targeted therapy: other (pacritinib, fedratinib and momelotinib without showing effectiveness at the moment), therefore, ruxolitinib a JAK1/JAK2 inhibitor is the only drug to evaluate at the moment (ECC phase III by pathology: PV RESPONSE, RESPONSE2 and RELIEF; MF

COMFORT-I AND COMFORT-II).

In the context of targeted therapies, ruxolitinib reverses cachexia and controls systemic inflammation (potential reasons for its use) in PMF, and the long-term use stops the medullary fibrosis, and gradually reduces the allelic burden. MR and/or HP are rarely observed, but if adverse prognostic mutations are added, the probability of success with the treatment or its durability decrease (although an early initiation in low-risk or intermediate-1 patients could maximize the long-term benefits).

For PV: ruxolitinib reduces thrombotic events (6 vs.1) although the transformation to MF and AML occurs with or without treatment, and patients with cytopenias have an increased risk of mortality (HR: 3.5, IC 95%: 1.5-8.3, p = 0.003) because they represent an independent risk factor for leukemic transformation (HR: 20.3, IC 95%: 5.4-76.5, p <0.001). The problem to evaluate the results of the efficacy of ruxolitinib in PV is the selection of the outcomes to be evaluated (control of Hct, reduction of splenomegaly) in the absence of therapeutic alternatives in patients without response to treatment; however, ruxolitinb remains a treatment to be evaluated in PV resistant and/or intolerant to HU [65].

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