HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Guidelines

Guideline on myeloproliferative neoplasms: Associacão Brasileira de Hematologia, Hemoterapia e Terapia Cellular Project guidelines: Associação Médica Brasileira – 2019

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Myeloproliferative neoplasms (MPNs) have been studied for a long time. However, many of the key advances for its proper understanding and approach have occurred in the last 20 years, a fairly recent period in medicine.

The concept of MPNs encompasses various diseases that, despite numerous features in common, show unique peculiarities – quite distinct from each other – with significantly distinct treatments and prognoses. The accurate understanding of those aspects is essential for the proper medical approach.

The earliest description of these diseases occurred at the beginning of the nineteenth century.[1] The concept of Myeloproliferative Diseases was determined by William Dameshek in 1951, including chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and Erythroleukemia.[2]

In 1960, Peter Nowell and David Hungerford discovered the Philadelphia Chromosome, the first chromosomal abnormality specific to a malignant neoplasm.[3] In 1973, Torbjön Caspersson characterized the chromosome Philadelphia from the cytogenetic point of view.[4]

Consequently, it was possible to classify MPNs as *Ph-positive* (in the case of CML) and *Ph-negative*. However, the therapeutic arsenal for those diseases remained considerably similar. In 1996, Brian Druker discovered *Imatinib*.[5] Soon, the use of tyrosine kinase inhibitors in CML has been considered a paradigm break in the treatment of CML. However, Ph-negative MPNs displayed no significant advances in its understanding and management.

In 2005, the JAK2V617F mutation was simultaneously described by four different laboratories.[6–9] The mutation appeared in the majority of patients with Ph-negative MPN. Next, there was a description of the MPLW515L mutation in 2006,[10] then the JAK2 exon 12 in 2007[11] and, more recently, changes in the Calreticulin gene (CALR) were described in 2013.[12,13] Those findings resulted into a more accurate understanding of the pathophysiology regarding those diseases, as well as the development of novel treatments.

The Classification of Hematopoietic and Lymphoid Tissue Tumors of the World Health Organization demonstrated significant importance. The introductory edition was published in 2001 and was the first document to incorporate genetic information into the diagnostic algorithms of the most varied diseases.[14] The classification had its second publication in 2008[15] and a third one recently, in 2017.[16] In this last edition, both genetic and molecular aspects and histopathological features have been reviewed and emphasized. Such approaches comprise a crucial step forward for the diagnostic standardization of MPNs worldwide.

Therefore, substantial scientific advances have been achieved regarding MPNs along the last twenty years, which included higher accuracy in diagnosis, new risk classifications, and therapeutic approach updates. Thus, frequent updating by hematologists is crucial.

However, the resources available for the diagnosis and treatment of these diseases are extremely variable among the various regions of the world, even within a continental country like Brazil. Thus, these Brazilian Guidelines on Myeloproliferative Neoplasms of the Brazilian Association of Hematology, Hemotherapy and Cell Therapy comprise an essential publication to update and guide the Brazilian hematologists in the best driving of patients with NMP Ph classic negative (PV, ET, and MFP) within our reality.

PICO 1: Myeloproliferative neoplasms – diagnostic criteria

Myeloproliferative Neoplasms (MPNs) constitute a group of clonal diseases of hematological progenitor cells that affect one or more myeloid lineages. In this setting, the Guidelines of the Brazilian Medical Association (AMB) project have been supported and participated by the Brazilian Association of Hematology, Hemotherapy and Cell Therapy (ABHH) since 2012, and is currently in its 12th edition. The present endeavor comprises the work of visiting hematologists with experience in the diagnosis and treatment of Philadelphia Chromosome-Negative Myeloproliferative Neoplasms to assist hematologists in their clinical practice. This instruction originates from the systematic review of the literature, through the acronym P.I.C.O., where “P” refers to patients, “I” refers to intervention or indicator, “C” refers to comparative and “O” refers to the outcome. Each issue was conducted without period restriction in the Medline database and generated a result of critically evaluated and selected studies to answer the clinical doubts. The details of the methodology and the resolutions of the guidelines show in Annex I.

Introduction

Myeloproliferative neoplasms (MPNs) constitute a group of hematologic clonal diseases that affect one or more myeloid lineages with an abnormal and abundant proliferation.[16,17]

The World Health Organization (WHO) currently groups the MPNs into seven categories: *Chronic myeloid leukemia* (CML), *chronic neutrophilic leukemia* (CNL), *polycythemia vera* (PV), *essential thrombocythemia* (ET) *primary myelofibrosis* (PMF), *chronic eosinophilic leukemia not otherwise specified* (NOS) and, *unclassifiable chronic myeloproliferative neoplasm* (MPN-U). Mastocytosis is no longer included in that group.[16]

BCR-ABL1 fusion gene causes CML, identified by the translocation (9; 22) known as the *Philadelphia chromosome* (Ph), subject of prior guideline. Among the so-called Ph-negative MPNs, the most common ones include PV, ET, and PMF, herein the subject of this publication.

PV, ET, and PMF share an anomaly in the JAK-STAT pathway, usually caused by genetic mutations on *Janus kinase 2-JAK2 V617F*, *thrombopoietin receptor gene-myeloproliferative leukemia-MPL* or *calreticulin gene-CALR*, which are mutually exclusive, and necessarily BCR-ABL negative (Table 1). PV, ET, and PMF share an anomaly in the JAK-STAT pathway, usually caused by mutations of the Janus kinase 2-JAK2 V617F genes, thrombopoietin receptor gene-myeloproliferative leukemia-MPL or calreticulin gene-CALR, that are mutually exclusive, and necessarily are BCR-ABL1 negative (Table 1). PV, ET, and PMF may arise alongside other mutations, as well as some cytogenetic abnormalities.[16,17] Despite the significant advances

Table 1 – Diagnostic criteria PV, ET, prefibrotic PMF and overt fibrotic PMF[16](D).

	Polycythemia Vera[a] requires the 3 major criteria or the 2 first major criteria plus the minor criterion	Essential Thrombocythemia requires the 4 major criteria or the 3 first major criteria plus at least one of the minor criterion	Prefibrotic/initial stage Primary Myelofibrosis requires the 3 major criteria plus at least 1 minor criterion	Fibrotic Primary Myelofibrosis requires the 3 major criteria plus at least 1 minor criterion
Major Criteria	1. Hemoglobin >16.5 g/dL in men Hemoglobin >16.0 g/dL in women or, Hematocrit >49% in men. Hematocrit >48% in women or, increased red cell mass more than 25% above mean normal predicted value.	1. Platelet count $\geq 450 \times 10^9\ L^{-1}$.	1. Megakacariocytic proliferation and atypia, with no reticulin fibrosis >1, with medullary hypercellularity for age, granulocytic proliferation and generally decreased erythropoiesis.	1. Megakacariocytic proliferation and atypia, accompanied by reticulin fibrosis and/or collagen fibrosis grades 2 or 3.
		2. BM biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers.	2. Not meeting criteria for PV, ET, CML, MDS or other myeloid neoplasms.	2. Not meeting criteria for PV, ET, CML, MDS or other myeloid neoplasms.
	2. BM biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size).	3. Not meeting criteria for PV, ET, CML, MDS or other myeloid neoplasms.	3. Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker,[b] or absence of minor reactive BM reticulin fibrosis.[c]	3. Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker,[b] or absence of reactive myelofibrosis.[c]
	3. Presence *of JAK2V617F or JAK2 exon 12* mutation.	4. Presence of JAK2, CALR, or MPL mutation.		
	Polycythemia Vera[a] requires meeting either all 3 major criteria, or the first 2 major criteria plus the minor criterion	Essential Thrombocythemia requires the 4 major criteria or the 3 first major criteria plus the minor criterion	Prefibrotic/initial stage Primary Myelofibrosis requires the 3 major criteria and at least 1 minor criterion	Fibrotic Primary Myelofibrosis requires the 3 major criteria and at least 1 minor criterion
Minor criteria	- Subnormal serum erythropoietin level	- Presence of clonal marker or absence of reactional plaquetosis.	- Anemia not attributed to a comorbid condition	- Anemia not attributed to a comorbid condition
			- Leukocytosis $\geq 11 \times 10^9\ L^{-1}$ - Palpable splenomegaly - LDH level above the upper limit of the institutional reference range	- Leukocytosis $\geq 11 \times 10^9\ L^{-1}$ - Palpable splenomegaly - LDH level above the upper limit of the institutional reference range - Leukoerythroblastosis

[a] Major criterion 2 (bone marrow biopsy) may not be required in cases with sustained erythrocytosis (Hb >18.5 g/dL or hematocrit >55% in men and Hb >16.5 g/dL or Ht >49.5% in women). However, the presence of fibrosis at diagnosis can only be detected through BM biopsy and has prognostic value.

[b] In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (e.g., ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, SF3B1) are of help in determining the clonal nature of the disease.

[c] BM fibrosis secondary to infection, autoimmune disorder, or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

Table 2 – Semiquantitative grading systems of medullary fibrosis[a,16] (D).

Grade	Description
MF – 0	Scattered linear reticulin with no intersections corresponding to normal bone marrow
MF – 1	Loose network of reticulin with many intersections, especially in perivascular areas
MF – 2	Diffuse and dense increase in reticulin with extensive intersections,occasionally with only focal bundles of collagen and/or focal osteosclerosis[b]
MF – 3	Diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis[b]

[a] The density of the fibers should be measured in areas with hematopoiesis only. If the pattern is heterogeneous, the final gradation is determined by the highest degree present in more than 30% of the marrow area.
[b] In MF – 2 and MF – 3 grades, additional trichrome staining is recommended.

over recent years toward the characterization of genetic alterations, bone marrow morphological evaluation remains an important diagnosis tool.[17] JAK2, MPL, and CALR mutations are not unique to these three conditions and may be present in other myeloid neoplasms, or even absent in some cases of PMF and ET.[16]

WHO diagnostic criteria[16] updated 2016 review, include the differentiation between prefibrotic/early stage PMF from the PMF in the overt fibrotic stage; the decrease in hemoglobin and hematocrit indices for the diagnosis of PV; the emphasis on the histological criteria of PV and ET in bone marrow biopsies, and the standardization of criteria for fibrosis grading degrees, among others.[18]

Results

Table 1 shows the WHO diagnostic criteria (2016) for MPNs. Table 2 shows the criteria for grading medullary fibrosis.

The post-PV or post-ET MF are thus classified when the patient was previously diagnosed with PV or ET and progressed to full MF (Table 3).

Recommendation

- Although allogeneic bone marrow transplantation remains the only curative treatment, several medications have brought significant advances in symptom control and increased survival, such as inhibitors of the JAK-STAT pathway, Interferons, and others.
- Therefore, it is essential that hematologists be familiar with the advances and challenges in approaching those diseases, as well as their adequate prognostic characterization.

PICO 2: Myeloproliferative neoplasms – what is the role of bone marrow biopsy in the diagnosis of myelofibrosis, polycythemia vera, and essential thrombocythemia?

Structured question

The clinical question was framed through the PICO framework components with: (P (Patient), I (Intervention), C (Comparison), O (Outcome).

P: Myelofibrosis, polycythemia vera, essential thrombocythemia

I: Bone marrow biopsy

C

O

Scientific database researched

The scientific database researched was PubMed. A manual search was performed for reviews references (narrative or systematic).

Table 3 – Criteria for myelofibrosis secondary to PV or ET[19] (D).

MF secondary to PV	MF secondary ET
Required criteria	
1. Documentation of a previous PV diagnosis as defined by WHO criteria	1. Documentation of a previous ET diagnosis as defined by WHO criteria
2. Medullary fibrosis grade 2–3 on a scale of 0–3 or grade 3–4 on a scale of 0–4	
Additional criteria (2 required)	
1. anemia (below standard reference value concerning age, sex, and height) or sustained loss of need for phlebotomy and/or cytoreductive therapy	1. Anemia (value below normal reference in relation to age, sex and altitude), with a decrease of >2 g/dL in the patient's plateau
2. Leukoerythroblastosis	
3. Increased splenomegaly, defined as >5 cm from the patient's threshold or appearance of previously absent splenomegaly	
4. Increase in Lactate Dehydrogenase (above reference value)	
5. Development of any of the following 2 constitutional symptoms (loss of >10% of body weight in the last 6 months, night sweats, unexplained fever (>37.5 °C)	

Evidence search strategy

PubMed-Medline
Strategy: (essential thrombocythemia OR essential thrombocythemia OR thrombocytosis OR polycythemia OR polycythemia OR erythrocytosis OR myelofibrosis OR aleukemic megakaryocytic myelosis OR myeloid metaplasia OR osteomyelofibrosis OR myelosclerosis) AND (bone marrow) AND (diagnosis/broad [filter] OR comparative study OR evaluation study). *n* = 2179.

Results of selected evidence

Of the total articles retrieved, 2179 were selected to support the synthesis of evidence regarding the role of bone marrow biopsy in the diagnosis of patients with myelofibrosis (MF), polycythemia vera (PV) and essential thrombocythemia (ET) in longitudinal observational studies alone. Narrative reviews and case reports were excluded from the evaluation. The evidence was assessed according to the Oxford classification, which establishes strength evidence from the study design of choice.

Thiele et al.[24] (B)

Design: Retrospective study.

Population: Evaluation of bone marrow biopsy samples conducted independently and blindly, comprising 334 patients (mean age 60 years) who presented a significant increase or borderline in hemoglobin levels.

Outcome: Elucidating the discriminative impact of bone marrow biopsy independently of laboratory parameters in the diagnosis of polycythemia vera (according to morphological findings based on a semiquantitative evaluation of standardized characteristics such as cellularity and particularities of megakaryocyte morphology expressed by the quantity, size, appearance, and nuclear lobulation).

Results: Clinical data and follow-up showed that in only 13 patients (4%), BM biopsy failed to clearly differentiate PV cases (208 patients) from secondary polycythemias (113 patients).

Florena et al.[25] (B)

Design: Retrospective study.

Population: Bone marrow biopsies from 142 patients (mean age of 62 years) with ET diagnosis by polycythemia vera Study Group (PVSG) criteria were assessed using the WHO classification. The study covered megakaryocyte morphology, amount of fibrosis and clustering index with the determination of microvessel density, number of CD34 positive cells and percentage of cells and megakaryocytes.MIB-1 positive.

Outcome: Analyzing the value of bone marrow biopsy in the diagnosis of essential thrombocythemia.

Results: Based on the WHO classification, 142 biopsies were classified as follows: ET (21%); idiopathic myelofibrosis (IMF) grade 0 (30%); IMF grade 1 (34%); IMF grade 2 (10%) and ET/MF (5%). A peculiar proliferative feature of megakaryocytes was detected in all ET cases. Microvessel density showed more pronounced, and the amount of CD34 positive cells were higher in the MFI cases in contrast to the observed in the ET cases.

Thiele et al.[26] (B)

Design: Retrospective study.

Population: Biopsies obtained from bone marrow of 272 patients with PV (in strict accordance with the criteria established by the PVSG – *Polycythemia Vera Study Group*) and 35 patients with reactive thrombocytosis were analyzed. Sixteen morphological parameters based on immunohistochemistry along with a semiquantitative analysis were scrutinized.

Outcome: Analyzing the value of bone marrow biopsy in the diagnosis of essential thrombocythemia.

Results: In contrast to the presumptive clinical diagnosis of essential thrombocythemia, histological features of the bone marrow revealed great heterogeneity. Significant impact variables, unidentified in the control group (patients with reactive thrombocytosis), included fibrotic content, number and cytological abnormalities of megakaryocytepoiesis, a degree of nuclear lobulation and presence of giant forms. In contrast to idiopathic myelofibrosis, essential thrombocythemia was characterized by marked proliferation of the megakaryocyte lineage without maturation defects and no relevant increase in reticulin fibers.

Thiele et al.[27] (B)

Design: Retrospective study.

Population: A retrospective morphological analysis of BM biopsies from 168 patients diagnosed with ET based on the criteria proposed by the PVSG – Polycythemia Vera Study Group.

Outcome: Analyzing the value of bone marrow biopsy in the diagnosis of essential thrombocythemia.

Results: Histopathological examination suggested three distinct groups for patient classification: *True Essential Thrombocythemia*, *Questionable Essential Thrombocythemia*, and *False Essential Thrombocythemia*. Those groups obtained characterization through clinical data obtained at admission. Real cases of essential thrombocythemia were found in 53 patients with or without discrete splenomegaly, the absence of relevant anemia or leukoerythroblastic figures in peripheral blood. Remaining patients had clinical signs and symptoms compatible with the initial pre-fibrotic phase (52 patients) or early idiopathic myelofibrosis with severe essential thrombocythemia (68 patients).

Rager et al. (B)

Design: Retrospective study.

Population: 44 patients with isolated diagnosis of thrombocythemia were evaluated retrospectively. All patients had an initial diagnosis of ET based on the diagnostic criteria established by the PVSG – Polycythemia Vera Study Group.

Outcome: Analyzing the value of bone marrow biopsy in the diagnosis of essential thrombocythemia.

Results: Given the clinical and biological follow-up, the diagnoses of 4 patients out of 6 patients were altered, from PMF to IMF. One patient alone out of the 14 patients identified as having primary myelofibrosis had their diagnosis altered. Nevertheless, the diagnosis of true essential thrombocythemia in 21 cases remained unaltered.

Brousseau et al.[28] (B)

Design: Retrospective study.

Population: BM Biopsies of 127 patients with ET diagnosis were revised.

Outcome: Evaluating the feasibility of the histopathological diagnosis of prefibrotic primary myelofibrosis (pre-PMF) cases as described by the WHO, as well as evaluating the clinical implications of pre-PMF in a series of patients previously diagnosed as having essential thrombocythemia according to the criteria established by the PVSG – Polycythemia Vera Study Group.

Results: Two distinct pathologists applied the WHO criteria to BM biopsies and further reclassified 127 cases as 102 patients with ET, 18 patients with pre-PMF, and 7 patients with fibrotic overt (overt PMF). In 35% of the cases, consensus was reached on the diagnoses.

Gisslinger et al.,[29] (B)

Design: Retrospective study.

Population: biopsies extracted from the database of patients with platelets $>450 \times 10^9\,L^{-1}$, with no evidence of reactive cause, normal iron stores and BCR-ABL1 negative.

Outcome: BM reclassification occurred according to WHO criteria and items A1 to A3 of the *British Committee for Standards in Haematology* (BCSH). The two cohorts were compared regarding their clinical aspects, prognosis and adverse events during follow-up.

Result: Regarding ET diagnosis, 238 patients met the BCHS criteria, and 232 patients met the WHO criteria. The BCSH group showed significantly higher values of lactic dehydrogenase and palpable spleen, as well as lower fibrosis-free survival and worse prognosis. Patients previously diagnosed with ET by BCHS criteria were submitted to a WHO-oriented reclassification that identified a heterogeneous population comprising 141 patients with ET (59.2%), 77 with pre-PMF (32.4%), 16 with PV (6.7%), and 4 with MF (1.7%).

Madelung et al.,[30]

Design: Retrospective study.

Population: 272 biopsies, of which 229 samples of patients with MPN and 43 samples from controls, from 3 Danish services and 1 Swedish service.

Outcome: Assessing inter-and intraobserver reproducibility, and the concordance between morphological and clinical findings.

Results: Concordance on morphological data was 53%, increasing to 83% after clinical data inclusion. Pathologists recognized 41 of the 43 control cases.

Discussion

The WHO classification for myeloproliferative neoplasms (MPNs) defines polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) as distinct entities based on clinical data, genotype, phenotype and histologic findings of bone marrow biopsy.[16] However, it is a fact that such diseases can transform phenotypically to each other, still presenting a tendency to evolution. Thus, the use of clinical criteria alone proved to be inadequate for diagnosis, which reiterates the importance of histological evaluation, given their inclusion as a criterion in WHO classification (D).[31,32]

However, precise definitions between MPNs that present thrombocythemia, as well as identification of the initial stages of the MFP, are still controversial, but of great importance given the difference in life expectancy associated with those diseases (B).[33] Furthermore, it is crucial to recognize that some histological characteristics of the bone marrow can be determined with reasonable reproducibility, specifically those associated with cellularity and degree of fibrosis. However, the evaluation of distinct cytological features, particularly related to megakaryocyte morphology, is less reliable, which may hinder the validity of the distinction between diseases based on subjective morphological criteria (B).[24,26,27,34,35]

The grasp of retrospective baseline studies on patients initially diagnosed for ET has demonstrated that a significant amount of those samples failed to present histomorphologic criteria for such diagnosis and are therefore had their diagnoses reclassified to pre-fibrotic MFP. This fact was analyzed retrospectively with a revision of 142 BM biopsies in patients previously diagnosed with ET according to the PVSG criteria. After a careful evaluation, and following the WHO criteria, the diagnoses of ET were restricted to only 21% of the cases; the others were re-designated as MFP (B).[25]

Another retrospective study on the histomorphology of BM biopsies from patients previously diagnosed with ET via PVSG criteria confirmed the reclassification of particular cases to pre-fibrotic MFP (B).[26]

In another study, with 1104 BM biopsy samples from patients diagnosed with ET submitted to central revision, 180 cases (16%) were reclassified as PMF. The investigation found a significant worsening for survival ($p = 0.03$), leukemic transformation ($p = 0.007$) and fibrosis progression ($p = 0.019$).[36]

An updated retrospective study compared the clinical impact of BM morphology in the diagnosis of ET using different criteria from different entities. Clinicopathological characteristics, prognosis and adverse events during the follow-up of patients with ET were compared. From an Austrian database of 626 patients, BM biopsy of patients with platelets $>450 \times 10^9\,L^{-1}$, with no evidence of reactive cause, normal iron stores, and BCR-ABL1 negative would be revised and reclassified to ET in case WHO or BSCH criteria A1 to A3 were met. 238 patients were found and 232, respectively. The BCSH group displayed significantly higher values of lactic

dehydrogenase and a more palpable spleen in the presentation, as well as the less progression-free survival of fibrosis and worse prognosis. After reclassification by WHO criteria of patients previously identified with ET by BSCH, a heterogeneous population was identified, including 141 individuals with ET (59.2%), 77 with pre-fibrotic MF (32.4%), 16 with PV (6.7%), and 4 with MF (1.7%). BSCH factors A1 to A3 allow the ET diagnosis even without the BM histomorphological analysis but fail to differentiate ET from pre-fibrotic MF. The demonstration of greater clarity in the differentiation of the several MPNs applying the WHO criteria provides an enhanced prognostic evaluation and therapeutic indication (B).[29]

Intra- and interobserver reproducibility using the 2008 WHO MPN definitions, in addition to clinical collaboration for diagnostic concordance, was assessed by 3 Danish center, and 1 Swedish center. Seven pathologists reviewed 272 biopsies of 229 patients with MPN and 43 controls. The concordance degree was analyzed based on histological data alone and after the association with the clinical data. 20 biopsies were distributed 3 times to assess intraobserver concordance. The consensus reached regarding cellularity, fibrosis graduation, quantification of granulopoiesis and megakaryocytes was higher than the accord reached regarding the type of grouping, size, and shape of megakaryocytes. The morphological evaluation of exclusion was concordant in 53% of the cases, and with the inclusion of clinical data, it increased to 83%. There was a high degree of concordance between the pathologists participating in the differentiation between MPN and the histological reactional alterations.[30]

A WHO-based retrospective study was conducted on 397 patients positive for JAK2 *V617F*/Exon12 with PV bone marrow characteristics and their thrombosis outcomes, progression to MF, acute leukemia and overall survival. The goal was to assess the diagnosis of PV in patients with lower hemoglobin levels than required by the 2008 WHO criteria. Inclusion criteria comprised the presence of the JAK2 *V617F* (or Exon12) mutation, the serum erythropoietin measurement, no evidence of iron deficiency, and the availability of representative BM biopsies without prior treatment effect. Of these, 257 (65%) fulfilled the full WHO-2008 criteria for the diagnosis of PV, and the remaining 140 patients (35%) were classified as masked PV (mPV) with hemoglobin levels at diagnosis varying from 16.0 to 18.4 g/dL in men and 15.0 to 16.4 g/dL in women. In all cases of mPV, the characteristics of the bone marrow biopsy were consistent with the diagnosis of PV. After a mean follow-up of 3.8 years (0–29.8) and 4.5 years (0–21.1) for mPV and PV, respectively, the time for the first thrombotic event was superimposed on both groups. Overall survival was worse in patients with mPV compared to PV ($p = 0.011$). Annual mortality in mPV was almost twice as high as PV, mainly due to an excess of hematological transformation for MF and Acute Leukemia.[37]

Following the current WHO diagnostic guidelines, one study evaluated 690 patients with ET, of which 422 (61%) were JAK2 positive and 397 cases with PV positive for the JAK2 *V617F* or JAK2 exon12 mutation, of which 140 (35%) samples were consistent with mPV. Conversion from ET to PV in this series was rarely documented, occurring in only 1% and 5% of cases of JAK2 negative and JAK2 positive, respectively.[38]

A study conducted by the LeukemiaNet consortium evaluated the consensus and interobserver concordance among an international panel of six hematopathologists on the characterization and reproducibility of the BM histological characteristics used to diagnose early-stage myeloproliferative neoplasms, in particular, the differentiation of the so-called masked polycythemia vera (mPV) of JAK2 ET. Eighty-eight BM specimens were evaluated independently and blindly, without knowledge of clinical data. The specimens included 48 cases of mPV, 31 cases with clear PV and 19 cases of control. The latter group included samples of positive JAK2 ET, PMF, myelodysplastic syndrome, and several reactive conditions. The inter-rater concordance was high (overall agreement of 92.6%, kappa 0.812), particularly concerning the separation of mPV from ET. Virtually all mPV cases were correctly classified as PV according to BM morphology.[35]

Recommendation

The histomorphological analysis of the bone marrow by WHO criteria is extremely important for the proper classification, differential diagnosis, prognostic and therapeutic definition of MPNs. Such analysis is feasible and accurate with experienced hemopathologists, adequate clinical reports to pathologists, and good quality BM samples. Special attention is required for accurately distinguishing ET from pre-fibrotic MFP, as well as distinguishing ET JAK2 positive from mPV, where the prognosis and appropriate therapy are notably dissimilar.

PICO 3: Myeloproliferative neoplasms – the role of mutations in diagnosis

Myeloproliferative neoplasms are clonal neoplasms of hematopoietic stem cells where an exacerbated proliferation of one or more myeloid series occurs, leading to peripheral blood leukocytosis, increased erythrocyte mass, or thrombocytosis. The goal of this guideline is to evaluate the role of mutations in the diagnosis of primary myelofibrosis, polycythemia vera, and essential thrombocythemia. We used the structured form to formulate the question synthesized by the acronym P.I.C.O., with “P” for patients with myeloproliferative neoplasms, “I” for mutation indicator and “O” for the diagnostic outcome. We selected 31 studiesfrom the medline database with no restriction period, to answer the clinical question. Details of the methodology and results of this guideline are displayed in Annex II.

Introduction

Myeloproliferative neoplasms – [MPNs] are clonal neoplasms of hematopoietic stem cells where an exacerbated proliferation of one or more myeloid series occurs, leading to peripheral blood leukocytosis, increased erythrocyte mass, or thrombocytosis. Those diseases present heterogeneous clinical manifestations, with the progression characterized by medullary fibrosis or leukemic transformation. The mutation in the Janus kinase 2 tyrosine kinase gene (JAK2) has been proven to be a significant molecular marker for the diagnosis of MPNs and was incorporated in 2008 by the World Health Organization (WHO) as a primary diagnostic criterion of these

diseases[8,9] (B) (D).[39] The somatic mutation JAK2 V617F is a point mutation characterized by the exchange of guanine by thymine at nucleotide 1849 of the cDNA at the exon 14 of the gene, which results in a constitutively active cytoplasmic protein. This protein promotes the activation of numerous signaling molecules and pathways, such as JAK/STAT, PI3K/AKT and RAS/MAPK, all involved in the transformation and proliferation of hematopoietic progenitors.[8,9]

Results

The molecular analysis for the presence of the mutation in the JAK2 gene from peripheral blood leukocytes or bone marrow biopsies was carried out from 140 clinical samples of 130 patients diagnosed with hematological diseases, being 80 with myeloproliferative neoplasms (MPNs), 50 with hematological diseases not related to MPNs, and 10 from health subjects (control group). Out of that, 74 tested positive for a mutation in exons 14 ($n = 36$ with MPNs, $n = 31$ with hematological diseases not related to MPNs, and seven did not have disease). The frequency of mutations identified in all samples was 41.5%, all of which were heterozygous. The JAK2V617F mutation occurred in 82.1% of patients with polycythemia vera (23/28), 53.1% in patients with essential thrombocythemia (17/32), and in 40% of individuals with primary myelofibrosis (4/10). The mutation rate in the JAK2 gene in patients with myeloproliferative neoplasm was 62.5% (50/80). Four V617F mutations in the JAK2 gene arose for hematological diseases not related to MPNs, and no JAK2 V617F mutation was verified in patients of control group (B).[40]

In another study, 168 patients with myeloproliferative neoplasms were enrolled ($n = 114$ for essential thrombocythemia, $n = 36$ for polycythemia vera, and $n = 18$ for primary myelofibrosis). The mutation in the calreticulin gene (CALR) arose in 16.7% of all patients with MPNs. Approximately 22% of patients with essential thrombocythemia and 16.7% of patients with primary myelofibrosis presented this mutation. The mutation in the JAK2 gene occurred in 86.1% of patients with polycythemia vera, 59.6% with diagnosis of essential thrombocythemia, and 50% of patients with primary myelofibrosis. The trial did not verify mutations in exon 12 in the JAK2 gene. Mutations in the MPL gene occurred only in patients with essential thrombocythemia (5/114). The incidence of triple negative (JAK2, MPL, and calreticulin) was 13.9%, 14%, and 33.3% for polycythemia vera, essential thrombocythemia and primary myelofibrosis respectively (B).[41]

Forty-eight patients diagnosed with essential thrombocythemia and 14 with primary myelofibrosis were assessed for the incidence, clinical characteristics, and impact of the mutation of the calreticulin gene. The mutation of the CALR gene arose in 24.2% of the patients. Individuals diagnosed with essential thrombocythemia who suffered mutations in the calreticulin gene were younger, had a lower white blood cell count, and experienced fewer thromboembolic events compared to patients who tested positive for the mutation in the JAK2 gene alone. In JAK2-negative patients, the presence of the CALR mutation did not modify the clinical manifestation, prognosis, and DIPSS score (MFP) (B).[42]

Patients diagnosed with polycythemia vera ($n = 20$), essential thrombocythemia ($n = 17$), and primary myelofibrosis ($n = 21$) were analyzed for the mutation in the JAK2 and MPL gene. The V617F mutation in the JAK2 gene showed in 90% of patients with polycythemia vera, 42.8% of patients with primary myelofibrosis and 47% of those with a diagnosis of essential thrombocythemia. Patients with polycythemia vera but negative for the JAK2 V617F mutation had a lower number of platelets and leukocytes compared to those positive for this mutation. Primary myelofibrosis patients who were positive for the JAK2 V617F and MPL W515L mutations had a higher degree of bone marrow fibrosis when compared to those negative for the JAK2 V617F mutation. The MPL mutation W515L was positive in one patient with primary myelofibrosis and another individual with essential thrombocythemia (B).[43]

Another study analyzed the clinical and laboratory characteristics in MPN patients with and without the JAK2 V617F mutation. The JAK2V617F mutation appeared in 95%, 68% and 77% of patients with polycythemia vera, essential thrombocythemia, and primary myelofibrosis respectively. Among all patients with MPNs, 19% had thrombotic events. All patients diagnosed with polycythemia vera with thrombosis were positive for the JAK2 mutation, and 76.1% of patients with essential thrombocythemia who were positive for this mutation presented thromboembolic events (B).[44]

We examined the information collected from a cytogenetic analysis of 184 patients with Philadelphia-negative myeloproliferative neoplasms (essential thrombocythemia $n = 107$ and primary myelofibrosis $n = 77$). The prevalence of the JAK2 V617F mutation was significantly higher among patients with primary myelofibrosis (75.3%) contrasting patients with essential thrombocythemia (59.8%). Fourteen of 77 patients with primary myelofibrosis (18.2%) simultaneously presented JAK2 V617F and ASXL1 mutations. Only 8.4%the patients diagnosed with essential thrombocythemia suffered the ASXL1 mutation, out of whom approximately 66.6% had the JAK2 V617F mutation. In patients with primary myelofibrosis, the JAK2 V617F mutation resulted in no difference in overall survival. Multivariate analysis confirmed the independent prognostic relevance of the ASXL1 mutation on overall survival in patients with primary myelofibrosis (OR = 2.75 with 95% CI: 1.3–5.5) (B).[45]

Were analyzed data on 101 patients diagnosed with essential thrombocythemia to assess the impact of mutations in the JAK2, MPL and CALR genes on the occurrence of thromboembolic events. The incidence of major thromboembolic events reported was 13.8%. Sixty percent of patients suffered the JAK2V617F mutation and 3.9% of the MPL mutation W515L. The mutation of type 2 of the calreticulin gene appeared in only three cases, without the JAK2 V617F mutation. There was no association between the mutation in the JAK2 gene and thromboembolic events (RR = 1.3 with 95% CI: 0.4 to 4.2 and $p = 0.668$) (B).[46]

Recommendation

All patients with suspected MPNs should be tested for mutations of the jak-stat pathway activating genes: JAK2, CALR, and MPL. Mutations in these three genes present high

sensitivity and moderate specificity for the diagnosis of PV, ET, and MF and should be analyzed together with other clinical and laboratory diagnostic criteria for the correct diagnosis of the patient. Due to the low prevalence of JAK2 exon 12 mutations, testing should particularly apply to patients with clinically suspected pv of mutation of jak2 v617f negative and have a low serum erythropoietin level. Individuals diagnosed with et or mf who tested negative for the jak2 v617f mutation require screening for mutations of the calr gene. mutations in other genes altered recurrently in patients with myeloid neoplasms may help in establishing a clonality process of a case with suspected mpn. however, routine diagnostic evaluation is unnecessary because of its low sensitivity and specificity, particularly indicated for cases that are negative for the mutations of jak2 v617f, calr, and mpl w515 and exhibiting clinical and laboratory findings suggestive of myeloproliferative syndromes.

Discussion

The JAK2 V617F mutation is often identified among patients with myeloproliferative neoplasm – especially patients with PV (90%), followed by patients with MF and ET (50–60%). That mutation is not specific for any MPN and rarely occurs in other hematological diseases unrelated to MPNs such as refractory anemia with ring sideroblasts, acute myeloid leukemia, and systemic mastocytosis. Therefore, neither the absence nor the presence of such mutation interferes in the diagnosis (B).[47] In addition to the V617F mutation in the JAK2 gene, mutations in other genes that lead to the activation of the JAK-STAT pathway (e.g., CALR, MPL) also play an essential role in the pathogenesis, diagnosis, and prognosis of those diseases.

Polycythemia vera implicitly (approximately 90% of cases) refers to mutations in the JAK2 gene (V617F mutation and scantily mutations in exon 12), whereas only 50–60% of patients with essential thrombocythemia and primary myelofibrosis confirm the mutation V617F[9] (B) (D).[7] Notably, the JAK2 V617F mutation often exhibits low allelic charge[48] (B) (PMID) which requires techniques with higher sensitivity for mutation detection. Hence, a lower prevalence of the mutation in earlier studies is justified. For instance, in two studies published in 2005, which described the JAK2 V617F mutation in patients with polycythemia vera, essential thrombocythemia, and myelofibrosis, the prevalence of the mutation was around 70–80% in patients with PV, and 30–40% of patients with thrombocythemia and myelofibrosis (D).[7,49] Recent data show that the frequency of this mutation in these diseases is much higher. In a prospective cohort study of 338 PV patients published in 2010 using a quantitative allele-specific polymerase chain reaction (PCR) technique, the JAK2 V617F mutation occurred in 320 patients (94.6%) with PV (B).[50] Likewise, in a study conducted in 1548 patients with myeloproliferative neoplasms published in 2014, the JAK2 V617F mutation arose in 53.8% of the patients with essential thrombocythemia, and in 55.8% of the patients with myelofibrosis (B).[51] A Brazilian study yielded equivalent results in three distinct centers consisting of 373 patients with ET or MF (B).[52] The study shows the JAK2 V617F mutation in 50.9% of patients with ET and 58.9% of patients with MF.

The JAK2 V617F mutation can also occur in other myeloproliferative neoplasms and other hematological malignancies, but with a much lower prevalence. Varied studies show a prevalence of around 3–15% for this mutation and patients with chronic myelomonocytic leukemia (CMML), 5–10% for patients with atypical chronic myeloid leukemia (aCML), up to 20% for patients with unclassifiable myeloproliferative neoplasm, and 3–5% for patients with AML and/or MDS (B).[53–56] According to the results obtained in this study, the positivity rate of the JAK2 V617F mutation in patients with MPNs is significantly different from that obtained among patients with other hematological diseases unrelated to MPNs and in rarer subtypes of MPNs. Hence, the incidence of the V617F mutation in the JAK2 gene may be a reliable diagnostic criterion in patients with MPNs.

Even rarer mutations of the JAK2 gene, located in exon 12, may occur in patients with PV without the V617F mutation. According to a study published in 2007, ten patients with PV were tested for mutations throughout the coding region of the JAK2 gene, and four mutations arose in exon 12 of gene (B).[10] Patients with PV and mutation of the JAK2 gene exhibit a distinct clinical picture of PV patients and JAK2 V617F mutation, which is characterized by isolated erythrocytosis in bone marrow and peripheral blood, unlike patients with JAK2 V617F-positive PV, which often proliferation of the three medullary strains, thrombocytosis, and leukocytosis. According to Schnittger et al., in a comprehensive review comprising 15,542 patients with suspected MPN, mutations of exon 12 of the JAK2 gene occurred in 4.68% of patients with PV (corresponding to 40% of suspected cases of PV, negative for the JAK2V617F mutation (B).[57] Unlike point mutation V617F JAK2 – which is recurrent – exon 12 mutations can be of varied types, including insertions, deletions, and point mutations. Hence, the need to perform complete sequencing of the exon to detect that change. Another distinct feature of exon 12 mutations is their occurrence in patients with PV only, unlike V617F, which may occur in different diseases (B).[10]

Among 40% of patients with essential thrombocythemia and primary myelofibrosis without the JAK2 V617F mutation, 3–5% had a mutation at codon 515 of the gene encoding the thrombopoietin – MPL (D) receptor.[11] Beer et al., in a retrospective study including 200 patients with essential thrombocythemia and primary myelofibrosis, detected the MPL mutation in 8.5% of patients negative for the JAK2 V617F (B) mutation.[58] The authors found that individuals with essential thrombocythemia who suffered the MPL mutation, in contrast to those who tested positive for the mutation in the JAK2 gene, had lower levels of hemoglobin and higher platelets at diagnosis (B).[58]

In a cross-sectional study conducted in 2011, Dos Santos et al. investigated the effect of mutations in the JAK2 and MPL genes on leukocyte count, platelet count, hemoglobin level, and age. According to that study, patients diagnosed with polycythemia vera negative for the JAK2 V617F mutation had a lower number of platelets and leukocytes compared to those who tested positive for this mutation (B).[43] Patients with primary myelofibrosis positive for the JAK2 V617F and MPL W515L mutations had a higher degree of fibrosis in bone marrow compared to those negative for the V617F JAK2 (B) mutation.[43] The MPL mutation W515L occurred in one patient

with primary myelofibrosis and another with essential thrombocythemia.

The mutation of the CALR gene is the last mutation leading to the activation of the JAK-STAT pathway in MPNs to be described. That mutation arose through exome sequencing studies in patients with MPNs who were negative for the JAK2 V617F (B) mutation.[12,13] The mutations of the CALR gene are always nucleotide insertion and/or deletion type mutations, and always occur in exon 9 of the gene. The CALR mutation can be found in 25–35% of patients with ET and MF, corresponding to 65–88% of the cases of these diseases that do not present the V617F mutation of the JAK2 gene 27 (B).[12,13]

Thus, following the JAK2 V617F mutation, the second most frequent mutation in patients with ET and MF is the mutation of the CALR gene. Klampfl et al. analyzed the presence of this mutation in a combined cohort of 1107 hematologic-neoplastic patients. Accordingly, the CALR mutation occurred only in patients with ET, MF and rare cases of refractory anemia with ring sideroblasts associated with thrombocytosis (RARS-T an ET-related neoplasm), a rather specific mutation for the diagnosis of ET and MF (B).[13]

Based on the medical literature, mutations of the JAK2 gene (exon 12 and V617F), CALR (frameshift indels in exon 9) and MPL – mainly codon W515, as well as other rarer mutations – are genetic alterations with high sensitivity for the diagnosis of MPNs. In the evaluation process of a patient with suspected MPN, the presence of mutations in one of these three genes confirms the clonality of the process. Concerning specificity, we can state that the V617F mutation is not specific for any subtype of MPN, and can occur, although at different frequencies, in patients with PV, ET, and MF. The JAK2 V617F mutation is more specific for the diagnosis of MPNs, but it can be found, albeit rarely, in other myeloid malignancies. Comparatively, mutations of the MPL and CALR gene are more specific for the diagnosis of ET and MF, as well as mutations of exon 12 of the JAK2 gene occur only in PV. Although all these mutations are valuable tools in the diagnostic process of patients with suspected NMPs, their isolated presence is only indicative of a neoplastic process. Thus, the correct diagnosis and classification should take into account all clinical and laboratory data of the patient.

Determined patients with primary myelofibrosis or essential thrombocythemia are referred to as “triple-negative” for JAK2 V617F, MPL W515 or CALR mutations. Due to the non-detection of such mutations by conventional methods, such cases pose diagnostic challenges and have prognostic significance. In particular, patients with primary myelofibrosis require identification through clinical, pathological, and other molecular methods (B).[59]

State-of-the-art sequencing studies have found novel mutations of the JAK2 and MPL genes in some of these patients with ET and MF negative for mutations of JAK2 V617F, CALR, and MPL W515 (B).[60,61] These mutations have a much lower frequency in this patient population. Laboratory studies show that they lead to the constitutive activation of the JAK-STAT pathway, being responsible for the disease phenotype. In a study published in 2016, approximately 10% of ET-negative patients for mutations known in the JAK2, MPL and CALR genes had new mutations in the MPL gene. According to Cabagnols et al., 9.3% of ET patients had mutations not described in the MPL gene.[60]

Other studies have reported the occurrence of mutations related to DNA methylation (TET2, DNMT3A and IDH1/2) and genes related to histone modification (EZH2, ASXL1) and mRNA splicing (SRSF2, U2AF1, SF3B1) in patients with Myeloproliferative Neoplasms. However, they arise mainly in patients with primary myelofibrosis. All of these mutations can occur to a greater or lesser prevalence in patients with other myeloid neoplasms[62] (D) should not to be considered in isolation as specific tool for the diagnosis of NPMs.

PICO 4: Myeloproliferative neoplasms – what is the role of cytogenetics in primary myelofibrosis, polycythemia vera, and essential thrombocythemia?

Structured question

The clinical question was framed through the PICO framework components with: P (Patient), I (Intervention), C (Comparison), O (Outcome).

P: Primary myelofibrosis, polycythemia vera, essential thrombocythemia

I: Cytogenetics

C

O

Scientific database researched

The scientific database researched was PubMed-Medline. A manual search was performed for reviews references (narrative or systematic).

Evidence search strategy

PubMed-Medline

Strategy: (essential thrombocythemia OR essential thrombocythaemia OR thrombocytosis OR polycythemia OR polycythaemia OR erythrocytosis OR myelofibrosis OR aleukemic megakaryocytic myelosis OR myeloid metaplasia OR osteomyelofibrosis OR myelosclerosis) AND (karyotype OR karyotypes OR karyotyping OR chromosome OR chromosomes OR cytogenetics OR cytogenetic OR chromosome deletion OR gene deletion OR translocation, genetic OR chromosome inversion OR chromosome duplication OR gene duplication OR monosomy OR monosomies OR ploidy OR aneuploidy OR tetrasomy OR trisomy OR diploidy OR haploidy OR polyploidy OR tetraploidy OR triploidy) AND (diagnosis/broad[filter] OR comparative study OR evaluation study). **N = 984**.

Results of selected evidence

The database search retrieved 984 articles, of which 59 studies underwent a title-abstract selection, followed by a full-text analysis in consonance with the PICO framework with the

further addition of eight observational studies. The main exclusion criteria included thematic inadequacy (cytogenetic detection method analysis/distinct diseases analysis), narrative reviews; case reports, and publication languages other than English. The evidence assessment followed the Oxford classification, which establishes strength evidence from the study design of choice.

Sever et al. Leuk Lymphoma. 2013;54(12):2667–70.[63]

Design: Retrospective longitudinal observational study.

Population: 133 patients (median age of 57 years) with a diagnosis of polycythemia vera, and adequate cytogenetic evaluation.

Outcome: Survival.

Result: Survival at five, ten, and fifteen years was 93%, 79%, and 64%, respectively. No significant difference was observed in overall survival among the 19 patients with cytogenetic abnormalities at any time of the disease compared to individuals ($n = 144$) who did not present chromosomal abnormalities ($p = 0.12$). During follow-up, 12% of 133 patients had the following conditions: myelofibrosis ($n = 11$), acute myeloid leukemia ($n = 4$), and myelodysplastic syndrome ($n = 1$). Eight of those 16 individuals had some cytogenetic abnormalities, most commonly detected at chromosomes 9 ($n = 3$), 12 ($n = 2$) and 20 ($n = 2$).

Boiocchi et al. Mod Pathol. 2013;26(12):1577–85.[64]

Design: Case series.

Population: 23 cases of post-polycythemia myelofibrosis, described in detail. Cytogenetic results were available for 19 patients.

Outcome: Disease progression.

Results: Patients with post-polycythemia myelofibrosis presented a higher degree of karyotype alterations, as well as a higher percentage of cases with complex karyotype and/or two or more clones. Chromosome 1 defects were the most common anomalies in post-polycythemia vera myelofibrosis.

Leon et al. Leuk Res. 2011;35(9):1188–92.[65]

Design: Retrospective longitudinal observational study.

Population: Retrospective cohort of 338 patients with myeloproliferative neoplasm. The investigation was conducted using conventional cytogenetics and evaluated for the presence of the JAK2 V617F mutation. Among these patients, 95/338 (28%) had a JAK2 V617F mutation, and 26% (25/95) had concurrent chromosomal abnormalities (the most common defects included the deletion of the long arm of chromosome 20, trisomy of chromosomes 8, 1, and 9, monosomy of the chromosome, and der(6)t(1;6)). Of these 25 patients, nine had polycythemia vera, five with essential thrombocythemia, three with primary myelofibrosis, four with myelofibrosis, three with myeloproliferative neoplasm (unspecified), and one with polycythemia vera and essential thrombocythemia.

Outcome: Prognosis.

Results: In addition to the presence of two extra chromosomes (1,9) (q10; p10), the t (1;9) translocation appeared in two patients with essential thrombocythemia, which in combination with the mutation JAK2 V617F showed progression to acute myeloid leukemia and myelofibrosis.

Gangat et al. Eur J Haematol. 2008;80(3):197–200.[66]

Design: Retrospective longitudinal observational study.

Population: 137 patients (median age of 64 years) with myeloproliferative neoplasm submitted to cytogenetic study. Normal results appeared in 85% of the patients. An isolated Y chromosome abnormality occurred in five patients, and other chromosomal abnormalities appeared in 15 individuals. These abnormalities consisted of trisomy of chromosome 8 (four patients), trisomy of chromosome 9, del(20q) and abnormalities in chromosome 1 (two patients), and trisomy of chromosomes 8 and 9, (13q), (3) (p13p21), dup (13) (q12q14) and (11) (q21) (one patient).

Outcome: Disease progression.

Results: The leukemic transformation occurred in three patients, of whom only two exhibited cytogenetic information. Both presented normal results at the time of diagnosis of polycythemia vera. Complex cytogenetic showed only at the time of leukemic transformation.

Hidaka et al. Eur J Haematol. 2009;83(4):328–33.[67]

Design: Prospective longitudinal observational study.

Population: 202 patients (median age of 65 years) with primary myelofibrosis submitted for cytogenetic study.

Outcome: Disease progression.

Results: 80 individuals had cytogenetic abnormalities, which had a clear association with platelet counts. Although the presence of an abnormal karyotype unassociated with prognosis, patients with cytogenetic abnormalities other than 13q- or 20q- showed a worse prognosis than in patients with a normal karyotype or single abnormalities of 13q- or 20q-. Patients with an unfavorable cytogenetic profile (abnormal cytogenetics other than 13q- or 20q-) also showed a greater tendency for leukemic transformation compared to patients with a favorable cytogenetic profile (normal cytogenetics, single chromosome 13q- or 20q- abnormalities).

Hussein et al. Eur J Haematol. 2009;82(4):255–9.[68]

Design: Retrospective longitudinal observational study.

Population: 109 patients (median age of 63 years) with primary myelofibrosis submitted for cytogenetic study. Cytogenetic findings categories included “normal”, “abnormal”, “favorable” (either normal or with unique abnormalities of 13q- or 20q-) and “unfavorable” (all other abnormalities).

Outcome: Disease progression.

Results: Cytogenetic results were "abnormal" in 33% of patients and "unfavorable" in 21%. With a mean follow-up of 35 months, 41% of the patients died. Only cytogenetic findings referred to as "unfavorable" had an association with poorer survival.

Tam et al. Blood. 2009;113(18):4171–8.[69]

Design: Retrospective longitudinal observational study.

Population: 256 (median age of 61 years) with primary myelofibrosis submitted for cytogenetic study. Abnormal cytogenetic findings appeared in 36% of patients, and 165 (64%) had normal diploid karyotype. Of these, 131 (51%) went for cytogenetic study at the time of diagnosis, and 125 (49%) had an evaluation after the initial diagnosis.

Outcome: overall survival.

Results: Patients with favorable abnormalities presented survival similar to those observed for patients with normal diploid karyotypes (median of 63 and 46 months, respectively). Such abnormalities included single deletions in 13q or 20q, or chromosome 9 trisomy associated or not with another abnormality. Conversely, individuals with unfavorable abnormalities presented lower mean survival (15 months). These abnormalities included rearrangement of chromosome 5 or 7, or a number equal to or greater than three abnormalities. Patients with chromosome 17 abnormalities had an average survival of only five months.

Gangat et al. Eur J Haematol. 2009;83(1):17–21.[70]

Design: Prospective longitudinal observational study

Population: A cohort of 402 patients (median age of 56 years) with a diagnosis of essential thrombocythemia submitted for investigation using conventional cytogenetics.

Outcome: Prognosis (mean follow-up of 70 months).

Results: The prevalence of abnormality in a cytogenetic investigation was 7% (28/402). The most common cytogenetic abnormalities were trisomy of chromosome 9 ($n=4$), chromosome 1 abnormal ($n=3$) and trisomy of chromosome 8 ($n=2$). Parameters at the time of diagnosis significantly associated with abnormal cytogenetic study included splenomegaly, venous thrombosis, and anemia. During follow-up, patients with abnormal cytogenetics had neither lower survival rates nor greater transformation to acute myeloid leukemia or myelofibrosis.

Discussion

Primary myelofibrosis

Cytogenetic abnormalities appeared at the time of diagnosis in approximately one-third of patients with MFP (D).[71] According to a 10-year prospective study, with 200 PMF patients, the frequency of cytogenetic changes at the time of diagnosis was 40%. 49 patients had single abnormalities, including 22 patients exhibiting either the deletion of the long arm of chromosome 20 (del(20q)) or the deletion of the long arm of chromosome 13 (del(13q). Three patients had trisomy of chromosome 8 (B).[67] The findings showed that survival in patients with del(20q) or del(13q) was similar to that of patients with normal karyotype and significantly better than patients who had other cytogenetic abnormalities (B).[67] In corroboration to these findings, a retrospective study (mean follow-up of 35 months) with 109 PMF patients submitted for cytogenetic studies within one year after the initial diagnosis found an abnormal karyotype in 36 individuals (B).[68] Of these patients, 12% had unique abnormalities (del(20q) or del(13q)). Other unique cytogenetic abnormalities included chromosome 9 trisomy and chromosome 8 trisomy (three patients each). Only two patients had monosomy of chromosome 7 and (7q). In this study, patients who had cytogenetic abnormalities other than deletion of the long arm of chromosomes 20 or 13 showed significantly lower survival (B).[68] Another retrospective study verified the favorable prognostic effect of cytogenetic abnormalities characterized by the deletion of the long arm of chromosomes 20 or 13. Further, the investigation identified chromosome 9 trisomy, alone or associated with another abnormality, as another favorable prognostic cytogenetic category (B).[69]

Another retrospective study of 165 subjects included patients with: PMF ($n=112$) (68%), post-PV MF ($n=29$) (18%), and post-ET MF ($n=24$) (14%). In this investigation, 80% of the patients had not received chemotherapy at the time of karyotype analysis, which was abnormal in 94 patients (57%). These patients had PMF ($n=62$) (55%), post-PV MF ($n=18$) (62%), and post-ET MF ($n=10$) (42%). The incidence of clonal abnormalities increased with time, even without exposure to chemotherapy. The most frequent alterations were (20q), del(13q), abnormalities on chromosomes 1, 7, 8 and 9, and 12. Twenty patients (12%) developed acute leukemia during the study period. Patients with -7/del(7q), -5/del(5q), abnormalities in 12p, chromosome 1, and chromosomes 8 and 9 had a higher rate of leukemic transformation than those with 20q or 13q 22.[70]

Another series, with 109 PMF patients, showed that 33% of patients had clonal abnormalities at diagnosis or in the first year of treatment, before any chemotherapy. Of the total number of participants, 63 (58%) presented the JAK2V617F mutation. The presence of unfavorable cytogenetic abnormalities was significantly associated with worse survival, as well as the presence of JAK2V617F.[72]

Moreover, a further study analyzed the karyotype of 106 patients at the time of diagnosis, of which 35% had abnormalities. The most frequent abnormalities included changes in chromosome 13, del(20) (q11) and duplication 1q. The mean survival of patients with abnormal karyotype was 30 months, which is significantly lower when compared to 72 months in patients with normal karyotype.[73]

In another series with karyotype, 94 cases of PMF patients presented cytogenetic abnormalities in 32 individuals (34%), and included (20q), (13q), trisomy 8 and (12) (q22). The presence of abnormal karyotype was associated with lower survival by univariate analysis. Among the low-risk groups, patients with an abnormal karyotype (15 cases) had a median survival lesser than 50 months ($p=0.03$) compared to those with normal karyotype (112 months).[74]

In a karyotype analysis, of 81 MF cases, 37 cases (46%) had some abnormality. Among the most common defects, del (13) appears in most of them, followed by del (20) (q11q13), t (1; 6) (q21-23; p21-23), t (1; 7) (q10; p10) and (11) (q21; q21). The presence of unfavorable cytogenetic anomalies was associated with worse survival. During follow-up, none of the patients with favorable cytogenetic abnormalities progressed to acute leukemia.[75]

In a publication comprising 63 cases with karyotype analysis in PMF, cytogenetic abnormalities appeared in 16 of the 56 patients tested at the time of diagnosis (29%), and in 29 of all 63 patients (46%). The cytogenetic abnormalities found were del(13q), del (20q) and duplication 1q. Twelve patients progressed to acute leukemia, most of whom presented clonal evolution.[76]

In a series of 47 cases with karyotype analysis in PMF, 15 patients presented cytogenetic abnormalities (32%), and two patients presented from (20) (q11), (13) (q13q21), (5). The karyotype was repeated in four cases after about 1–2 years of diagnosis, with additional changes in two cases. The presence of abnormal karyotype was associated with worse survival, independent of other risk factors ($p = 0.015$).[77]

Other smaller series, with cases of PMF and post-PV MF that received alkylating agents during treatment, presented abnormal karyotype. In 28 cases, abnormal karyotype was identified in 11 patients with PMF (55%) and six patients with post-PV MF (75%). Cytogenetic anomalies in PMF included three cases involving chromosome 5, +8, +20, and other three cases, involving chromosome 6. In the MFS, abnormal karyotype was observed in three patients who received the therapy (60%), and in eight patients who did not receive the treatment (56%). There was no difference in the rate of leukemic transformation or cause of death among patients with or without abnormal karyotype.[78,79]

Polycythemia vera

In Polycythemia Vera (PV) the JAK2 V617F mutation is present in approximately 97% of patients (C).[7,9] In addition to this mutation, cytogenetic alterations also appear in about 13–35% of these patients, among which the most observed abnormalities are trisomies of chromosomes 8 and 9 and deletion of the long arm of chromosome 20 [del (20) q] (C).[66,80,81] Some reports suggest that, at the time of diagnosis, survival in patients with PV or other MPN and cells with chromosomal abnormalities is lower than in patients with normal karyotype[64,82] (C) (B).[65,83] Moreover, there is an increase in the frequency of transformation for acute myeloid leukemia, myelodysplastic syndrome, and myelofibrosis. A retrospective study found that sixteen patients (12%) underwent myelofibrosis ($n = 11$), acute myeloid leukemia ($n = 4$) and myelodysplastic syndrome ($n = 1$) during a mean follow-up of 8.5 years. Of these patients, 50% had cytogenetic abnormalities 10 (C). However, other studies did not confer a negative association between the presence of cytogenetic changes and the prognosis of the disease. A retrospective study found that association occurred only toward age. That is an isolated abnormality in the Y chromosome, trisomy of chromosomes 8, 9, del(20q), abnormalities on chromosome 1 and del(13q) del(3) (p13p21) dup (13) (q12q14), and del(11) (q21) occurred in patients older than 60 years (B).[66] Among patients with cytogenetic abnormalities only one, with abnormalities on chromosome 1, showed transformation to the disease myelofibrosis (B).[66] Thus, the incidence of abnormal cytogenetics, which is different from the Y chromosome abnormality, was evident in 4% of patients under 60 years of age, and in 15% of those aged over 60 years. In the univariate analysis, the presence of an abnormality in the Y chromosome conferred an increased risk of arterial thrombosis, hemorrhage, and leukemic transformation. However, such associations were lost in multivariate analysis after adjustment for age. The study showed that the presence of abnormal cytogenetics at the time of diagnosis did not have a significant negative impact on overall survival or leukemic transformation-free survival (B).[66]

Essential thrombocythemia

During the first two decades of the disease, 10% of patients with ET may present transformation to acute myeloid leukemia, myelofibrosis or myelodysplastic syndrome (B).[84,85] Approximately 5–10% of cases of PV, ET and PMF develop into acute myeloid leukemia, while 10–20% of cases of PV and ET develop secondary myelofibrosis. Factors that interact with the JAK2 V617F mutation to define the histological subtype of MPN and the risk of transformation are still unclear, and potentially unrelated to JAK2 gene deregulation alone (D).[71] Such factors may include recurrent cytogenetic changes as verified in a retrospective longitudinal observational study, in which 338 patients diagnosed with MPN were evaluated using conventional cytogenetics. In that study, the presence of the t(1;9)(p10;q10) translocation, in addition to the identification of two extra chromosomes (1;9) (q10;p10), observed in 40% of patients with ET ($n = 2$), when associated with the mutation in the JAK2 gene, had been associated with disease progression for acute myeloid leukemia and myelofibrosis (B).[65] However, the literature shows controversial results, such as in a cohort study with 402 patients that revealed that the presence of cytogenetic alterations was not specific, as well as presenting clinical significance and limited prognosis (B).[70]

Final discussion

The prevalence of abnormal karyotype at diagnosis varies between 30 and 35% of the cases. The abnormalities in PMF are del(20q) and del(13q). Although less frequent, the trisomies of chromosomes 8 and 9, abnormalities in chromosomes 3, 5, 7, 12 and 21 are recurrent.

Chromosomal abnormalities were the most frequent defects at diagnosis in cases of post-PV MF found in PMF. Also, such anomalies are much less frequent in chronic PV. Genotoxic therapy during the chronic phase of PV may contribute to this phenomenon. The incidence of abnormal karyotype in post-PV MF is lower in patients treated with phlebotomy alone. However, it is still unclear whether chemotherapy has led to genetic events or vice versa. Other cytogenetic abnormalities observed at the time of PV or TE diagnosis, including 8, 9 and del (20q), are also the same as those commonly seen with both post-PV MF and PMF. On the other hand, specific infrequent

translocations, such as der (6) t (1;6) (q21-23; p21.3), may be more specific for PMF.

Several PMF studies have shown reduced survival in the presence of any abnormal or unfavorable karyotype. Further, the prognostic value of the karyotype was independent of other risk factors established for PMF. The adverse effect became evident in the post-PV/ET MF scenario. Notably, more studies are necessary to validate such individual institutional observations and examine the additional prognostic diversity across more specific karyotype profiles. Finally, it is important to note that the karyotype is often the starting point for the discovery of relevant genetic mutations in the pathology of MNPs and other myeloid neoplasms.

Recommendation

Presently there is sufficient evidence to justify the inclusion of karyotype analysis during the initial evaluation of PMF. Many series have had their karyotypes performed in peripheral blood, which further favors their realization, even in cases where the aspirate is scarce due to spinal fibrosis.[71]

In patients with primary myelofibrosis, cytogenetic abnormalities other than the deletions of the long arm of chromosomes 13 and 20 have an association with a worse prognosis, both for survival and for the risk of leukemic transformation.

Although not specific for polycythemia vera, cytogenetic abnormalities such as trisomy of chromosomes 8, 9, deletion of the long arm of chromosomes 13 and 20, appear with relatively high frequency in older patients. Such anomalies have little clinical significance concerning thrombosis, hemorrhage or transformation of the disease.

Cytogenetic abnormalities at the time of diagnosis are relatively rare in essential thrombocythemia and predict neither the progression to more aggressive myeloid disorders nor lower survival rates.

PICO 5: Myeloproliferative neoplasms – prefibrotic primary myelofibrosis and essential thrombocythemia: Differential diagnosis

Myeloproliferative neoplasms (MPNS) are diseases characterized by varying degrees of bone marrow hypercellularity, which can lead to leukocytosis, erythrocytosis or thrombocytosis in peripheral blood. This guideline seeks to provide information to help distinguish between pre-fibrotic primary myelofibrosis and essential thrombocythemia. The starting point for this work was a systematic review of the literature, using the acronym PICO, where “P” stands for patients with primary fibrotic myelofibrosis, “I” stands for bone marrow biopsy, “C” stands for comparative with essential thrombocythemia, and “O” stands for differential diagnosis outcome. There was no period restriction in the medline database. We retrieved 1073 papers, six of which were selected to answer the clinical question. Details of the methodology and results of this guideline are set out in Annex III.

Introduction

The diagnosis of MPNs was traditionally based on the histopathological evaluation of the bone marrow associated with clinical and laboratory characteristics. The World Health Organization (WHO) classification defines polycythemia vera, essential thrombocythemia, and primary myelofibrosis as distinct entities based on bone marrow morphology, genotype, clinical and phenotypic data. However, those diseases tend to mimic each other phenotypically, with a trend toward evolution over time. Thus, patients who exhibit clinical features that lead to the diagnosis of essential thrombocythemia may present a diagnosis of primary myelofibrosis in the pre-fibrotic phase.[39,86]

Results

How to differentiate pre-fibrotic primary myelofibrosis from essential thrombocythemia?

The 2016 update of Myeloproliferative Neoplasms (MPNs) by the World Health Organization (WHO) incorporated molecular characteristics related to diagnostic and prognostic markers. The understanding of such characteristics has brought new insights into the differentiation of MPN subgroups. Clinical-pathological studies have already validated this integrated approach, which includes complete blood count (CBC), bone marrow aspirates and biopsy morphology, cytogenetic and molecular analysis. The standardization of MPN morphological criteria is crucial to improving diagnosis reproducibility among the different pathologists (D).[18]

Among the most relevant revisions of this 2016 update, the need to distinguish between true essential thrombocythemia (ET) and pre-fibrotic myelofibrosis (MF) deserves special attention. In addition to the high diversity of morphology, the prognosis of those diseases is notably distinct (D).[18] The clinical course of pre-fibrotic MF, when compared to ET, presents a higher risk of death, transformation to acute leukemia, and progression to advanced stages of MF, demonstrating the essential character of the early recognition of this pathology (B).[29,36,87]

A multicentric study, with seven international participating centers, developed and tested a clinicopathologic database of patients previously diagnosed as having ET ($N = 1104$). Study eligibility criteria included availability of treatment-naive BM specimens obtained within 1 year of diagnosis. All bone marrows subsequently underwent a central re-review. Diagnosis was confirmed as ET in 891 patients (81%) and was revised to early/prefibrotic PMF in 180 (16%); 33 patients were not evaluable. In early/prefibrotic PMF compared with ET, the 10-year survival rates (76% and 89%, respectively) and 15-year survival rates (59% and 80%, respectively), leukemic transformation rates at 10 years (5.8% and 0.7%, respectively) and 15 years (11.7% and 2.1%, respectively), and rates of progression to overt myelofibrosis at 10 years (12.3% and 0.8%, respectively) and 15 years (16.9% and 9.3%) were significantly worse. The respective death, leukemia, and overt myelofibrosis incidence rates per 100 patient-years for early/prefibrotic PMF compared with ET

Table 4 – Criteria for diagnosis of ET.

The diagnosis of ET requires the presence of the 4 major criteria or the first 3 major and 1 minor criterion below

Major criteria

1. Platelet count ≥450 × 10^9 L^{-1}
2. BMB with megakaryocytic hyperplasia (increase in the number of large, mature megakaryocytes with a hyperlobulated nucleus). No significant increase in granulopoiesis or immature elements of the myeloid series. Absence of erythroid hyperplasia. A rare occurrence of a slight increase in reticulin fibers (grade 1)
3. No criteria, according to WHO, of MCL BCR-ABL1, PV, PMF, myelodysplasia or another MPN
4. Presence of JAK2, CALR or MPL mutation

Minor criteria

1. Presence of a clonal marker or absence of evidence of reactional thrombocytosis

BMB: bone marrow biopsy; CLM BCR-ABL[39]: chronic myeloid leukemia; PV: polycythemia vera; PMF: primary myelofibrosis. Adapted from WHO 2016[18] (D).

Table 5 – Diagnostic criteria for pre-fibrotic MF.

The diagnosis of pre-fibrotic myelofibrosis requires the presence of all 3 major criteria and at least 1 minor criterion below

Major criteria

1. Megakaryocytic hyperplasia with atypia of megakaryocytes, with no grade 1 reticulin fibrosis, accompanied by hypercellularity (age adjusted), granulocytic proliferation and (generally) erythropoiesis reduction.
2. No WHO-compliant criteria for CML BCR-ABL1, PV, MFP, myelodysplasia or other MPN.
3. Presence of the mutation in JAK2, Cal-R or MPL or, in the absence of these mutations, presence of another clonal marker *, or absence of minor reactional fibrosis #

*ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, SF3B1

MF grade 1 due to infection, chronic inflammatory disease, autoimmune disease, hairy cell leukemia or other lymphoproliferation, metastatic malignancy or other chronic myelopathies

Minor criteria

1. Anemia non-attributable to concurrent comorbidity
2. Leukocytosis ≥11 × 10^9 L^{-1}
3. Palpable Splenomegaly
4. High LDH

CLM BCR-ABL[39]: chronic myeloid leukemia; PV: polycythemia vera; PMF: primary myelofibrosis. Adapted from WHO 2016[18] (D).

were 2.7% and 1.3% (relative risk [RR], 2.1; $p < 0.001$), 0.6% and 0.1% (RR, 5.2; $p = 0.001$), and 1% and 0.5% (RR, 2.0; $p = 0.04$). Apart from morphological findings related to bone marrow biopsy, for both situations additional risk factors for lower survival were advanced age (over 60 years), the presence of leukocytosis (leukocytes >11 × 10^9), anemia (hemoglobin <12 g/dL), and prior history of thrombosis (B).[36]

Tables 4 and 5 show the new diagnostic criteria for pre-fibrotic ET and MF by the 2016 revision of WHO classification of lymphoid neoplasms (D).[18]

Recommendation

No single specific test is sufficient to definitively establish the diagnosis of a particular myeloproliferative neoplasm or even to distinguish MPNs from other hematological diseases that mimic them. The differentiation between pathologies should be made by combining clinical, and laboratory findings with the bone marrow biopsy aspects. Clinical data on follow-up and survival analysis, molecular findings and morphological aspects identified from the bone marrow biopsy are in line with the assumption that the criteria used by the WHO 2016 review are capable of differentiating between true ET and early stages of primary myelofibrosis. This distinction is critical, given the severity of clinical evolution and poorer prognosis of prefibrotic MF when compared to ET.

PICO 6: Myeloproliferative neoplasms – what is the best prognostic scoring system for primary myelofibrosis?

Structured question

The clinical question was framed through the PICO framework components with: (P (Patient), I (Intervention), C (Comparison), O (Outcome).

P: Primary myelofibrosis
I: IPSS
C: Other prognostic scores
O: Survival

Scientific database researched

The scientific database researched was Pubmed. A manual search was performed for reviews references (narrative or systematic).

Evidence search strategy

PubMed-Medline

Strategy: (myelofibrosis OR aleukemic megakaryocytic myelosis OR myeloid metaplasia OR osteomyelofibrosis OR myelosclerosis) AND (IPSS OR DIPSS OR DIPSS-plus OR dynamic international prognosis score system OR Lilley, score) AND (survival OR death OR progression OR leukemic transformation OR acute leukemia OR AML OR acute myeloid leukemia). **N = 53**.

Results of selected evidence

Fifty-three articles were retrieved by literature search. Evidence assessment followed the Oxford classification, which establishes strength evidence from the study design of choice.

Souza et al. Med Oncol. 2013;30(2):555[88] (B)

Design: Retrospective longitudinal observational study.

Population: Data regarding clinical and hematological information of 62 patients diagnosed with primary myelofibrosis (diagnosed according to WHO criteria) were analyzed.

Outcome: Overall survival and prognostic factors were assessed by analyzing the clinical relevance of prognostic score systems (Dupriez, Cervantes, Mayo, International Prognostic Scoring System, and Dynamic International Prognostic Scoring System).

Results: Based on the Cervantes, Dupriez, and Mayo scores, most patients figured into a low-risk group (77%, 66%, and 50%, respectively). However, under the IPSS and DIPSS prognostic scoring systems, the majority of patients were in an intermediate range of risk; only 11% (IPSS) and 13% (DIPSS) of the patients, according to the system used obtained low-risk classification. Overall survival at four years was 84%. All prognostic scores – except the Dupriez score – showed a significant association with survival. Overall survival was higher in patients with lower scores compared to those with higher scores (intermediate or high risk). Through multivariate analysis, only females and Cervantes score were associated with higher survival

Scott et al. Blood. 2012;119(11):2657–64[89] (B)

Design: Retrospective and prospective longitudinal observational study.

Population: Patients ($n = 170$) diagnosed with primary or secondary myelofibrosis (mean age of 51.5 years) submitted to hematopoietic cell transplantation.

Outcome: Assessing overall survival.

Results: Mortality correlated with the category of risk verified through the DIPSS system. Among patients with intermediate-2 and high-risk, median survival rates were seven years and 2.5 years for the mean follow-up of 4.9 years (range of 0.5 to 12.2 years), and 5.7 years (range of 0.6 to 11.1 years), respectively.

Wang et al. Leuk Res. 2014;38(10):1207–11[90] (B)

Design: Retrospective longitudinal observational study.

Population: Data on 874 Chinese patients (mean age of 55 years) diagnosed with primary myelofibrosis were analyzed.

Outcome: Assessing the prognostic impact of splenomegaly on overall survival.

Results: Individuals with splenomegaly (56.6%) presented higher concentrations of hemoglobin levels, higher leukocytes, platelets, excess blood blastic lineage in peripheral blood, less dependence on red blood cell transfusion and lower scores on the Dynamic International Prognostic Scoring System (DIPSS). In the multivariate analysis, the presence of splenomegaly was a favorable prognostic factor for survival, independent of the risk established by the DIPSS.

Hussein et al. Eur J Haematol. 2009;82(4):255–9[68] (B)

Design: Retrospective longitudinal observational study.

Population: Patients ($n = 109$) diagnosed with primary myelofibrosis who presented a cytogenetic study of the bone marrow were analyzed. Cytogenetic findings were characterized as normal versus abnormal or favorable (normal or with an abnormality of 13q- or 20q- only) versus unfavorable (with all other abnormalities considered).

Outcome: Assessing the prognostic impact of cytogenetic findings.

Results: The number of patients distributed according to the international prognostic score system was 26, 31, 28 and 24 corresponding to low risk, intermediate-1 risk, intermediate-2 risk, and high-risk, respectively. Abnormal cytogenetic results show in 33% of patients; the unfavorable result was 21%. The mean follow-up time was 35 months, and unfavorable cytogenetic findings (abnormalities not included) responded for a survival-time reduction.

Cervantes et al. Blood. 2009;113(13):2895–901[91] (B)

Design: Prospective multicenter longitudinal observational study.

Population: Patients ($n = 1054$) with a diagnosis of primary myelofibrosis and mean age of 64 years. Characteristics analyzed: Period of diagnosis, institution of origin, sex, age >65 years, presence of constitutional symptoms (weight loss >10% of baseline in the year prior to diagnosis of the disease and/or unexplained fever or excessive sweating persisting for more than one month) hemoglobin levels <10.0 g/dL, leukocyte count, presence of ≥1% of circulating blasts in peripheral blood, and platelet count $<100 \times 10^9\ L^{-1}$.

Outcome: Assessing survival and impact of factors considered on the prognosis.

Results: Excluded variables: the institution of origin, gender, and period of diagnosis. Each remaining prognostic factor is assigned a point. The patient's score ranges from zero (0) to a maximum of five points (5). Four prognostic groups were considered: (1) low-risk (no factor of poor prognosis, including 22% of patients, with an average survival of 135 months); (2) intermediate-1 (presence of a prognostic factor, including 29% of patients with a median survival of 95 months); (3) intermediate-2 (two prognostic factors, 28% of patients with a median survival of 48 months), and (4) high risk (three or more prognostic factors, 21% of patients with a median survival of 27 months).

Tefferi et al. Am J Hematol. 2010;85(1):14–7[92] (B)

Design: Prospective longitudinal observational study.

Population: Patients ($n = 254$) diagnosed with primary myelofibrosis and a mean age of 59 years, monitored over time, with prognostic analysis.

Outcome: Assessing the additional impact on prognosis determined by the need for RBC transfusion (found at diagnosis or during the first year after diagnosis).

Results: Within this cohort, 62 patients required blood transfusion at the time of diagnosis; 22 patients became transfusion dependent while 170 subjects remained RBC-transfusion independent for at least one year after diagnosis.

With a mean follow-up period of 55 months, the median survival rates were 35, 25 and 117 months, respectively.

Passamonti et al. Blood. 2010;115(9):1703–8[93] (B)

Design: Prospective longitudinal observational study.

Population: Patients ($n = 525$) were analyzed over time for the following risk factors: age >65 years), hemoglobin levels <10.0 g/dL, leukocyte count greater than 25×10^9 L, blast cell count circulating ≥1% and presence of constitutional symptoms. The time of acquisition of these risk factors was recorded for each patient during follow-up. Only patients not presenting those risk factors at the study onset were included.

Outcome: Promoting a prognostic model to predict survival in patients with primary myelofibrosis (time for acquisition of selected risk factors and survival).

Results: The rate of occurrence, time to the acquisition and its association with characteristics at diagnosis were evaluated to investigate the dynamics of the acquisition of each risk factor during follow-up. Multivariate regression analysis showed that advanced age, higher leukocyte count, and low hemoglobin levels at the time of diagnosis comprised independent factors on the incidence of anemia. Through the univariate survival analysis using the Cox proportional hazards model (Cox regression), and analyzing elements such age (>65 years), hemoglobin levels (<10.0 g/dL), leukocyte count $>25 \times 10^9\ L^{-1}$, peripheral blood blasts count ≥1% and presence of constitutional symptoms, it was possible to verify that the hazard ratio was 2.6 (95% CI: 2.04–3, 45) for age, 3.57 (95% CI: 2.78–4.58) for leukocyte count, 6.74 (95% CI: 4.93–9.21) for hemoglobin levels, 3.55 95% CI: 2.77–4.56) for peripheral blood blasts, and 3.03 (95% CI: 2.60–4.20) for constitutional symptoms.

Li et al. Am J Hematol. 2014;89(11):1043–6[94] (B)

Design: Prospective longitudinal observational study.

Population: Patients ($n = 565$) diagnosed with primary myelofibrosis analyzed for the presence of cytogenetic abnormalities.

Outcome: Assessment of survival.

Results: Individuals with cytogenetic alterations showed reduced levels of hemoglobin and platelets. They were even more susceptible to constitutional symptoms, besides being classified as high risk by the DIPSS prognostic score system. There was no difference concerning age, sex, blast count in peripheral blood or splenomegaly. Four hundred thirty-nine patients were classified as low-risk, intermediate-1, intermediate-2, and high-risk by the DIPSS system. The median survival was 74 months (95% CI: 28–120 months), 36 months (95% CI: 20–52 months) and 21 months (95% CI: 13–29 months), respectively. According to the DIPSS-associated system, the individuals classified as low-risk, intermediate-1, intermediate-2, and high-risk by the DIPSS-plus system had a median survival of 110 months (95% CI: 27–19 months), 56 months (95% CI: 40–72 months) and 26 months (95% CI: 22–30 months), respectively. Individuals with abnormal or unfavorable cytogenetic findings showed a lower survival rate compared to other groups.

Benites et al. Clinics (Sao Paulo). 2013;68(3):339–43[95] (B)

Design: Retrospective longitudinal observational study.

Population: Patients ($n = 74$) diagnosed with primary myelofibrosis and mean age of 71.5 years, with Lille and IPSS scores calculated for risk stratification and survival analysis.

Outcome: Assessment of survival.

Results: Patients (47%) with the JAK2 V617F mutation showed lower overall survival (39% versus 77%). However, this difference is not significant. Patients classified as high-risk by the IPSS prognostic score system presented lower overall survival than those classified as low risk.

Discussion

Myelofibrosis is characterized by highly variable life expectancy, ranging from a few months to more than a decade. Therefore, the elaboration of models that allow risk stratification is necessary to estimate the long-term survival, in addition to directing the choice of therapy, always respecting the patient's preferences.[96] Several attempts have been made to develop a reliable predictor system for the prognosis of primary myelofibrosis. Such a system should comprise clinical variables verified both at the time of diagnosis and during the evolution of the disease, aiming at estimating survival, the probability of progression to acute myeloid leukemia and assisting therapeutic decisions.

Despite the heterogeneity observed in all available systems, it is possible to observe that several parameters can influence survival, even at different levels. Variables include the short time elapsed between the manifestation of symptoms and onset of signs, presence of constitutional symptoms, spleen and liver size, age, sex, anemia, thrombocytopenia, reticulocyte count, leukocytes, monocytes, increase in the percentage of immature granulocytes, circulating blasts, degree of fibrosis in bone marrow biopsy, karyotype abnormalities, transfusion needs and leukemic transformation. The reasons for this heterogeneity arise from the various criteria used at the time of diagnosis, the diversity of the parameters analyzed, and the number of individuals included in the studies. In primary myelofibrosis, several studies have shown adverse prognostic relevance due to factors such as age (>65), sex (male), anemia (usually defined as hemoglobin levels <10.0 g/dL), leukocytosis and/or leukopenia (defined as leukocyte count <4 or $>30 \times 10^9\ L^{-1}$), thrombocytopenia (platelet count $<100 \times 10^9\ L^{-1}$), circulating blasts count, constitutional symptoms, transfusional dependence, and cytogenetic abnormalities.[73,97,98]

Currently, the prognosis of patients with primary myelofibrosis comprises both the International Prognostic Scoring System (IPSS) and the International Dynamic Prognostic Scoring System (DIPSS) model. IPSS is used to estimate survival at diagnosis whereas DIPSS is applied at any stage of the disease.[91] Both IPSS and DIPSS use the same risk factors as age (>65 years), hemoglobin levels <10.0 g/dL, leukocyte count $>25 \times 10^9$/L, blast cell count circulating ≥1% and presence of constitutional symptoms.[93,99]

Table 6 – International Prognostic Scoring System (IPSS) – Cervantes et al.,[91] (B).

Risk factors		Score
Age >65 years		1 point
Constitutional symptoms[a]		1 point
Hemoglobin <10.0 g/dL		1 point
Leukocytes >25 × 10^9 L^{-1}		1 point
Circulating blasts (≥1%)		1 point
Risk category	**Score**	**Median survival**
Low	0 points	135 months
Intermediate-1	1 point	95 months
Intermediate-2	2 points	48 months
High	≥3 points	27 months

Adapted from Tefferi et al.,[92] (D).

[a] Unexplained fever or excessive sweating over one month, weight loss >10% of usual weight one year before the diagnosis of myelofibrosis.

Table 7 – Dynamic International Prognostic Scoring System (DIPSS) – Passamonti et al.,[93]

Risk factors		Score
Age >65 years		1 point
Constitutional symptoms[a]		1 point
Hemoglobin <10.0 g/dL		2 points
Leukocytes> 25 × 10^9 L^{-1}		1 point
Circulating blasts (≥1%)		1 point
Risk category	**Score**	**Median survival**
Low	0 points	Not reached
Intermediate-1	1 to 2 points	170 months
Intermediate-2	3 to 4 points	48 months
High	≥5 points	18 months

[a] Unexplained fever or excessive sweating over one month, weight loss >10% of usual weight one year before the diagnosis of myelofibrosis.

Table 8 – Refined Dynamic International Prognostic Scoring System ("*plus*") – (DIPSS plus).[99]

Risk factors		Score
DIPSS int-1		1 point
DIPPS int-2		2 points
DIPPS high risk		3 points
Thrombocytopenia <100,000		1 point
Transfusion need		1 point
Karyotype of poor prognosis[a]		1 point
Risk category	**Score**	**Median survival**
Low	0 points	185 months
Intermediate-1	1 point	78 months
Intermediate-2	2 to 3 points	35 months
High	≥4 points	16 months

[a] Unfavorable karyotype: complex karyotype or one or two abnormalities including: +8, −7/7q−, i (17q), −5/5q−, 12p−, inv (3), or 11q23 rearrangement.

The IPSS prognostic score system was developed from a cohort of 1054 patients diagnosed with primary myelofibrosis (B).[91] The disease was stratified into four prognostic categories: low-risk, intermediate-1, intermediate-2, and high-risk, with significant differences in overall survival and risk of transformation for acute myeloid leukemia (Table 6) (B).[91] The median survival in this study ranged from 135 months for patients classified as low-risk up to 27 months for those considered high-risk (B).[91]

In order to define the prognosis at any stage of the disease (not at diagnosis only), the IWG-MRT also evaluated 525 patients with MFPs regularly followed up. Next, it was verified the extent to which the acquisition of the prognostic factors identified to the IPSS over time would affect the survival. Hence, a dynamic prognostic score was created, applicable at any moment of the disease. The score allowed evaluating patients even without all the information at the time of diagnosis. Thus, it was also possible to evaluate the influence of these changes when acquired in the course of the disease. The acquisition of anemia (<10 g/dL) doubled the impact found in the IPSS. Thus, 2 points were assigned to the score for this variable. Other risk factors (age, leukocyte count >25 × 10^9/L, the percentage of blasts in peripheral blood ≥1%, and age >65 years) were also significant and scored one point in the score. Four distinct prognostic groups were defined, with survival curves significantly different from those found at IPSS, then termed as *the Dynamic International Prognostic Scoring System* (DIPSS) (Table 7).[93]

However, those models fail to consider other factors with great prognostic impact, such as transfusion dependence, thrombocytopenia, and cytogenetic abnormalities. Thus, a retrospective study involving patients seen at the Mayo Clinic between 1970 and 2009 was performed in an attempt to incorporate these three factors into the DIPSS.[73] In that study, 793 patients with available bone marrow karyotype were analyzed, and a new score was obtained. One point was assigned to the group with DIPSS Intermediate-1, unfavorable karyotype, platelet counts <100 × 10^9/L, and transfusion dependence. Patients with DIPSS Intermediate-2 and High-Risk scored 2 and 3 points, respectively. Four prognostic groups were identified as low-risk, intermediate-1, intermediate-2, and high-risk, with a median survival of 185, 78, 35 and 16 months, respectively.[73] The inclusion of these new factors allowed a more accurate stratification of the patients in those subgroups, and the effective combination of karyotype, transfusion necessity, and thrombocytopenia to DIPSS. As a result, that score was termed as DIPSS *plus* (Table 8).[99]

Numerous investigations have been carried out to try to incorporate the prognostic impact of the various mutations presented in Myelofibrosis. However, such studies have not yet been applied in clinical practice.

Recommendation

In patients diagnosed with primary myelofibrosis, all IPSS, DIPSS, and DIPSS plus – which assess the patient at any time of their evolution using the IPSS parameters – are appropriate

and validated methods to identify patients as to their prognosis.

The prognostic role of mutations and their attempt to incorporate into prognostic indexes will be described in another section.

PICO 7: Myeloproliferative neoplasms – primary myelofibrosis. The role of mutations in the prognosis

Somatic mutations in the Janus Kinase 2 gene are the most frequent mutations in BCR-ABL negative myeloproliferative neoplasms, followed by other mutations. The purpose of this guideline is to provide recommendations on the role of these mutations in the prognosis of primary myelofibrosis. This guideline was carried out from a systematic review of the literature, using the acronym PICO, where "P" stands for patients with primary myelofibrosis, "I" stands for mutations, and "O" stands for prognosis. We performed the search, without period restriction, within the MedLine database, from which we retrieved 1171 papers, of which 34 were selected to answer the clinical question. Details of the methodology and results of this guideline are set out in Annex VI.

Introduction

The V617F somatic mutation in the Janus Kinase 2 (JAK2) gene is the most frequent mutation in BCR-ABL negative myeloproliferative malignancies (MPNs). This mutation occurs in approximately 50% of patients diagnosed with primary myelofibrosis (B).[100–107] Other mutations such as the calreticulin gene (CALR) and thrombopoietin receptor gene (MPL) are described in approximately 25% and 7% of patients with primary myelofibrosis, respectively[12,103] (B) (D).[108]

Mutations in the JAK2 gene can promote a change in JAK/STAT pathway signaling, which translates signals from cytokine receptors and growth factors such as erythropoietin. The JAK2 protein, belonging to the Janus kinases family, is also a tyrosine kinase that acts as an intermediary between membrane receptors and intracellular signaling molecules. The JAK2 V617F mutation consists of the substitution of a guanine for a thymine at the exon 14 of the JAK2 gene, leading to the substitution of valine for phenylalanine at position 617 of the encoded protein. Patients with chronic myeloproliferative neoplasms positive for the JAK2 V617F mutation have higher hematocrit, higher hemoglobin, and higher neutrophil and leukocyte counts. Such patients also present an increased risk of splenomegaly and the occurrence of thrombotic events (B).[51,102,105,109] Significant differences in the percentage of mutated (quantitative) alleles and qualitative differences in the JAK2 V617F mutation may explain the heterogeneity observed between myeloproliferative disorders, suggesting different roles in MPNs.

Results

There is no single mutation responsible for the onset or development of primary myelofibrosis. However, it is a fact that somatic mutations in the JAK2, CALR, and MPL genes are the most frequently associated with the disease, being related to differences in the phenotype (B).[101,110] Mutations in the calreticulin gene are almost exclusively found in individuals diagnosed with primary myelofibrosis or essential thrombocythemia. There are differences between groups with different mutations, especially concerning overall survival. Patients with mutated CALR gene present better prognosis with longer survival. On the other hand, patients considered as "triple negatives" present the worst prognosis, with a median survival of just over two years and a higher risk of leukemic transformation (B).[102]

Results of retrospective studies, analyzing molecular parameters of patients diagnosed with primary myelofibrosis and correlating them to prognosis, verified that the simple presence of the JAK2 V617F mutation was not related to the survival or with punctuation (intermediate or more significant risk) verified in the prognostic score system (IPSS) (B).[45,91,110,111] This lack of relevance to the prognosis, concerning the presence or absence of the V617F mutation in the JAK2 gene, was also evidenced in two other studies, which did not show a reduction in overall survival even with the high presence of mutated allele loading (B).[111,112] Moreover, low mutation loading in the JAK2 gene conferred the most aggressive disease phenotype, with lower survival free of leukemic transformation (B).[111] On the other hand, separate studies have demonstrated lower overall survival and increased leukemic transformation in patients with the mutation in the JAK2 gene (B).[51,112,113]

Another somatic mutation described in 5 to 10% of patients with primary myelofibrosis is the mutation in the thrombopoietin receptor (MPL) gene, located on chromosome 1p34 (B).[108] MPL is the cytokine receptor thrombopoietin (TPO) and is expressed in the hematopoietic progenitors and the cells of the megakaryocytic lineage. The two most common MPL mutations are W515L (replacement of tryptophan by leucine) and W515K (replacement of tryptophan by lysine) and, in the same way as the mutation of the JAK2 gene, results in activation of the JAK/STAT pathway. In contrast to the JAK2 V617F mutation, the disease induced by the MPL W515L mutation is characterized by rapidly fatal evolution, marked thrombocytosis, leukocytosis and spinal cord fibrosis (B). Rare patients simultaneously present mutation in the MPL and JAK2 gene (B).[114] Mutations in the MPL gene have been associated with low hemoglobin levels at diagnosis and high reliance on red blood cell transfusion as opposed to patients with or without the mutation in the JAK2gene (B).[115]

Other mutations may coexist with mutations in the JAK2 and MPL genes – with unclear clinical significance – include LNK [Src homology 2 B3 (SH2B3)] and CB1 (Casitas B-lineage lymphoma proto-oncogene). Mutations such as ASXL1 (Additional Sex Combs Like 1), TET2 (TET oncogene family member 2), EZH2 (enhancer of zeste homolog 2), DNMT3A (DNA cytosine methyltransferase 3a), IDH1/2 (isocitrate dehydrogenase 1 and 2) and IKZF1 IKAROS family zinc finger 1), interfere in the regulation of gene expression and lead to epigenetic modifications of some pathways, including JAK/STAT. Such mutations are more rarely found in patients with primary myelofibrosis and are associated with disease progression (B).[116–118]

All calreticulin somatic mutations (CALRs) described are insertions or deletions occurring in exon 9, including the C-terminal domain, leading to loss of the ionic calcium binding site and KDEL sequence, which results in abnormal functions and abnormal proliferation of the mutated cells. In vitro studies have demonstrated that transfection of the mutant protein leads to hyperactivation of the JAK/STAT pathway. Its exact role in the development of myeloproliferative neoplasms is not yet clearly elucidated. That mutation may confer an advantage to the megakaryocytic lineage, probably occurring at the beginning of the pathogenesis of the MPN (B).[51,102,103,119]

Type 1 (52 bp deletion) and type 2 (five base pair insertion) mutations make the most frequent CALR mutations, with type 1 mutation being more prevalent in patients with primary myelofibrosis, giving them a better prognosis (B).[101] When compared to the JAK2 mutation, the occurrence of CALR mutations in patients with primary myelofibrosis is associated with lower age at diagnosis, lower hemoglobin levels and white blood cell count, higher platelet count, and lower scores on the DIPSS-plus prognostic score system, besides being associated with higher survival (B).[51,102,103,119] More recently, some investigators observed that type 1-like CALR mutations in addition to predicting better survival, alleviate the effect of other high-risk molecular mutations, such as ASXL1 and SRSF2 (B).[120]

An example of a mutation that has a significant prognostic impact is ASXL1 mutation. Patients with a diagnosis of primary myelofibrosis and non-mutated CALR and ASXL1 mutated, and the group of patients called "triple negatives" (negative for MPL, CALR, and JAK2 mutations) are those with the worst prognosis (B).[45,103,121] EZH2 and ASXL1 mutations are associated with worse overall and fail-free survival after treatment with ruxolitinib or momelotinib (B).[122] In patients with fibrotic myelofibrosis, a higher frequency of high-risk mutations (ASXL1, SRSF2, IDH1/2, EZH2) was observed. The prognosis is also unfavorable in triple negative cases and with high-risk mutations (B).[123–125]

Recommendation

The molecular characterization of patients with primary myelofibrosis can pose a substantial part of the risk stratification and establishment of the disease prognosis, allowing the definition of the best therapeutic strategy.

Verified mutations in the calreticulin gene (CALR), in particular, type1-like, are associated with longer survival and fewer thrombotic events when compared to the JAK-2 V617F mutation. Patients with high-risk (ASXL1, EZH2) and triple negative mutations have lower survival rates.

However, therapeutic decisions based on the mutational profile of patients with Myelofibrosis have not yet been well defined or standardized so far.

PICO 8: Myeloproliferative neoplasms – primary myelofibrosis: treatment with hydroxyurea

What is the role of hydroxyurea in primary myelofibrosis?

Structured question

The clinical question was framed through the PICO framework components with: (P (Patient), I (Intervention), C (Comparison), O (Outcome).

P: Primary myelofibrosis
I: Hydroxyurea
C
O: Survival morbidity, quality of life

Scientific database researched

The scientific database researched was Pubmed. A manual search was performed for reviews references (narrative or systematic).

Evidence search strategy

PubMed-Medline

Strategy 1: (myelofibrosis, primary OR myelofibrosis, primary OR primary myelofibrosis OR bone marrow fibrosis OR bone marrow fibroses OR fibroses, bone marrow OR fibrosis, bone marrow OR myelofibrosis OR myelofibrosis OR idiopathic myelofibrosis OR myeloid metaplasia OR metaplasia, myeloid OR metaplasias, myeloid OR myeloid metaplasia OR myelosclerosis OR myelosclerosis OR myelosis, nonleukemic OR myelosis, nonleukemic OR nonleukemic myeloses OR nonleukemic myelosis OR chronic idiopathic myelofibrosis OR agnogenic myeloid metaplasia OR agnogenic myeloid metaplasia OR metaplasia, agnogenic myeloid OR metaplasias, agnogenic myeloid OR myeloid metaplasia, agnogenic OR myeloid metaplasia, agnogenic OR myelofibrosis with myeloid metaplasia) AND (hydrea OR hydroxyurea OR hydroxycarbamide OR oncocarbide). **N = 296**

Strategy 2: (myelofibrosis, primary OR myelofibrosis, primary OR primary myelofibrosis OR bone marrow fibrosis OR bone marrow fibroses OR fibroses, bone marrow OR fibrosis, bone marrow OR myelofibrosis OR myelofibrosis OR idiopathic myelofibrosis OR myeloid metaplasia OR metaplasia, myeloid OR metaplasias, myeloid OR myeloid metaplasia OR myelosclerosis OR myelosclerosis OR myelosis, nonleukemic OR myelosis, nonleukemic OR nonleukemic myeloses OR nonleukemic myelosis OR chronic idiopathic myelofibrosis OR agnogenic myeloid metaplasia OR agnogenic myeloid metaplasia OR metaplasia, agnogenic myeloid OR metaplasias, agnogenic myeloid OR myeloid metaplasia, agnogenic OR myeloid metaplasia, agnogenic OR myelofibrosis with myeloid metaplasia) AND (antineoplastic agents OR antisickling agents OR enzyme inhibitors OR nucleic acid synthesis inhibitors OR hydroxyurea OR hydra OR hydroxycarbamide OR oncocarbide) AND (THERAPY/BROAD[FILTER] OR COMPARATIVE STUDY OR EPIDEMIOLOGIC METHODS). **N = 1.389**

Strategy 1 AND Strategy 2: N =236.

Results

Of the 236 articles retrieved, 2 were selected to support the evidence regarding the role of hydroxyurea in patients with primary myelofibrosis exclusively in clinical trials. Narrative reviews and case reports were excluded from the evaluation. The evidence was assessed according to the Oxford classification, which establishes strength evidence from the study design of choice

Discussion

Traditionally, PMF is referred to as the MPN with the most debilitating symptomatological profile, which includes constitutional symptoms, splenomegaly, cytopenia, extramedullary hematopoiesis, the risk of leukemic transformation and decreased life expectancy. PMF therapy seeks the evaluation of both symptoms and cytopenias and splenomegaly, as well as the impact of therapy on the disease, especially survival, is also observed, especially in patients who are candidates for bone marrow transplantation.

Highlighted therapy alternatives include the use of oral cytoreductive medications, other than hydroxyurea alone,[126,127] such as Busulfan or Melphalan (B).[128] Additional therapeutic modalities include interferon, JAK2 Inhibitors, and allogeneic hematopoietic stem cell transplantation. Therapeutic options also include splenectomy or spleen radiation for patients who are refractory to drug treatment[129–133] (C) (D).[130] Furthermore, clinical observation is often adopted in asymptomatic patients.

A prospective study with 59 symptomatic patients with PV ($n=24$), ET ($n=25$) and PMF ($n=10$) with a high platelet count and a mean follow-up of 28 months (±16 months) identified a reduction in platelet count to $<100 \times 10^9$/L within eight weeks of initiation of therapy in 60% of patients diagnosed with PMF (B).[134] Within a 12-month time frame, control of disease-related symptoms occurred in 80% of patients with myelofibrosis. No severe hematological toxicity arose in this study (B).[134]

Results of the hydroxyurea treatment of 40 patients with PMF (80%), post-ET MF and post-PV MF, with a mean age of 64 years included 100% for improvement in complaint of bone pain; 82% for improvement for constitutional symptoms, 50% for improvement in pruritus, and 40% for reduction of spleen volume. The mean duration of response was around 13.2 months (B).[135] Hematologic toxicity was identified in 18 patients (45%), consisting of the onset or worsening of pre-existing anemia or pancytopenia, which required the administration of hematopoiesis stimulating agents, like erythropoietin and/or danazol (B).[135] Regarding non-hematological toxicity, oral or lower limb ulcers were the most frequently reported events, although they were observed in a minority of patients (B).[135]

Recommendation

Although hydroxyurea is widely used in patients with PMF, information regarding its efficacy and tolerability is restricted.

Studies with hydroxyurea in PMF included a restricted number of patients. No randomized controlled trials were found. The drug confers control of erythrocytosis and thrombocytosis, but the significance of this control is unknown in this disease. Hydroxyurea use in PMF may lead to reduced splenomegaly and symptomatic control, but with variable duration. However, that therapeutic option may lead to worsening anemia.

PICO 9: Myeloproliferative neoplasms. What is the role of corticosteroids in primary myelofibrosis?

Structured question

The clinical question was framed based on the components of the **P.I.C.O.** framework:

(**P** (Patient), **I** (Intervention), **C** (Comparison), **O** (Outcome)
P: Primary myelofibrosis
I: Corticosteroids
C: ----
O: Survival, morbidity, quality of life

Scientific databases researched

PubMed-Medline was the scientific database used in the literature research. A manual search was also performed based on revision references (narrative or systematic).

Evidence-search strategies

PubMed-Medline

Strategy 1: (primary myelofibrosis OR myelofibrosis, primary OR myelofibrosis, OR primary myelofibrosis OR bone marrow fibrosis OR bone marrow fibrosis, bone marrow OR fibrosis, bone marrow OR myelofibrosis OR myelofibrosis OR idiopathic myelofibrosis OR myeloid metaplasia OR metaplasia, myeloid OR metaplasias, myeloid OR myeloid metaplasia OR myelosclerosis OR myelosclerosis OR myelosis, nonleukemic OR myelosis, nonleukemic OR nonleukemic myeloses OR nonleukemic myelosis OR chronic idiopathic myelofibrosis OR agnogenic myeloid metaplasia OR agnogenic myeloid metaplasia OR metaplasia, agnogenic myeloid OR metaplasias, agnogenic myeloid OR myeloid metaplasia, agnogenic OR myeloid metaplasia, agnogenic OR myelofibrosis with myeloid metaplasia) AND (adrenal cortex hormones OR corticosteroids OR corticoids OR steroids OR prednisone OR prednisolone OR dexamethasone OR methylprednisolone). **N = 556**

Selected evidence results

Database search retrieved 556 articles, of which 13 papers were selected to support the synthesis of evidence regarding the role of corticosteroids in patients with primary myelofibrosis in clinical trials only. Narrative reviews and case reports were

excluded from the evaluation. Evidence evaluation followed the Oxford classification, which establishes evidence strength from the study design used.

Daver et al. Leuk Res. 2014;38(9):1126–9[136] (B)

Design: Phase II clinical trial.

Population: Patients ($n = 29$) mean age 69 years, with primary and secondary myelofibrosis (post-polycythemia vera myelofibrosis and essential post-thrombocythemia), with hemoglobin levels less than 10 g/dL or RBC transfusion-dependence treated with pomalidomide (0.5 mg/day in 28-day cycles) associated with prednisone administered in the first three cycles only.

Outcome: Mean follow-up of 6.4 months, clinical improvement assessed by International Working Group criteria – Myelofibrosis Research and Treatment – IWG-MRT. Delphi method used to determine whether RBC transfusion was needed.

Results: After a mean follow-up of 18.4 months (mean of 6 cycles administered), 23 patients remained alive. After a follow-up of 11.4 months, six patients achieved a documented response; two patients showed clinical improvement (platelet levels and spleen volume reduction), and four patients became blood-transfusion independent (according to the Delphi method).

Bejanyan et al. Cancer. 2012; 118(16):3968–76[137] (B)

Design: Multicenter clinical trial conducted between 2005 and 2007.

Population: 28 patients (mean age of 66.5 years) diagnosed with myeloproliferative neoplasm, five of whom had primary myelofibrosis and received combined treatment of thalidomide, arsenic trioxide, dexamethasone and ascorbic acid for a cycle lasting 12 weeks, followed by maintenance of thalidomide alone, for an additional period of 3 months.

Outcome: Evaluating clinical response, biological response, and adverse events to therapy with thalidomide, arsenic trioxide, dexamethasone and ascorbic acid (TADA). Clinical improvement was assessed using the International Working Group criteria – Myelofibrosis Research and Treatment – IWG-MRT.

Results: Of the 28 patients, 75% ($n = 21$) completed the 12 weeks of combination therapy. 21% of the patients ($n = 6$) with a mean follow-up of 5.7 months responded to TADA. Only patients who completed the 12 weeks of combined therapy had partial remission, reduced splenomegaly, and improved anemia. A number of 15 patients obtained an extended follow-up was available for evaluation. For a mean follow up of 24.1 months, the mean progression-free survival of the disease was 14.4 months with an overall mean survival of 21.4 months.

Regarding adverse events, most of the patients presented mild to moderate severity events. 25% of subjects ($n = 7$) had hematological toxicity exhibiting thrombocytopenia, neutropenia, and leukocytosis. Concerning non-hematological adverse events, fatigue was the most commonly reported reaction, having affected 82% of patients.

Mesa et al. Blood. 2010;116(22):4436–8[138] (B)

Design: Phase II multicenter clinical trial.

Population: 48 individuals diagnosed with primary and secondary myelofibrosis (post-thrombocytosis and post-polycythemia myelofibrosis), with hemoglobin levels <10 g/dL or red blood cell transfusion dependent, were treated with lenalidomide (10 mg/day in 28-day cycles) associated with prednisone at low doses for 3 months. The response was assessed using the criteria of the International Working Group – Myelofibrosis Research and Treatment – IWG-MRT*.

Outcome: Evaluating clinical response, biological response, and adverse events to therapy.

Results: Findings report a mean follow-up of 2.3 years with a mean of six cycles of treatment administered. Combination therapy was associated with adverse events in 37 subjects (thrombocytopenia and neutropenia most commonly). Among the 42 admitted patients, clinical response appeared in 10 patients (23%), with an improvement in anemia in eight individuals (19%) and/or reduction in spleen volume in four patients (10%). In 29 patients, the disease remained stable, and in three patients, there was a progression

* The most relevant responses for analysis of clinical improvement were: increased hemoglobin levels above 2.0 g/dL above baseline for two consecutive months or no need for transfusion of red blood cells in the same time interval. Splenomegaly required identifying a 50% reduction in the palpable spleen component for a period equal to or greater than two months.

Quintás-Cardama et al. J Clin Oncol. 2009;27(28):4760–6[139] (B)

Design: Open-Label, Multicenter Phase II Clinical Trial.

Population: Forty patients (mean age 62 years) diagnosed with primary myelofibrosis received lenalidomide (10 mg/day on days 1 to 21, totaling six 28-day cycles) and prednisone (30 mg/day) for three cycles. Lenalidomide was maintained indefinitely in patients who presented clinical benefit. The response assessment applied the International Working Group – Myelofibrosis Research and Treatment – IWG-MRT criteria.

Outcome: Evaluating clinical response, biological response, and adverse events to therapy.

Results: The average time for clinical response achievement was 12 weeks – with a mean follow-up of 22 months. According to the IWG-MRT, three patients presented a partial response (7.5%) and nine patients (22.5%) presented improvement in clinical response with a duration of 18 months (mean). No patient achieved complete response according to the IWG. Overall response rates were 30% for anemia and 42% for splenomegaly. Concerning hematological complications, 23 patients (58%) presented neutropenia (grades 3 and 4) and 17 patients (42%) had anemia. Five patients presented

thrombocytopenia grade 3 and 4, and six patients presented thrombocytosis.

Mesa et al. Mayo Clin Proc. 2004;79(7):883–9[140] (B)

Design: Long-term analysis of two-phase II clinical trials conducted during the period 1999 to 2003.

Population: The initial and long-term results of 36 patients undergoing palliative treatment of myelofibrosis with myeloid metaplasia who received thalidomide ($n = 15$) or low doses of thalidomide (50 mg/day) associated with prednisone were analyzed, the latter administered for three months in decreasing doses ($n = 21$).

Outcome: Evaluating clinical response, biological response, and adverse events to therapy.

Results: Among the 36 patients analyzed (mean age of 65 years), 20 subjects (56%) presented an improvement in their clinical condition, with improvement in anemia in 15 of the 36 patients (42%), thrombocytopenia in 10 of 13 patients (77%) and splenomegaly (improvement in five of 30 patients). Therein, the association of low doses of thalidomide with prednisone, as opposed to the scheme where thalidomide was administered alone, showed higher patient tolerance and effectiveness. After a mean follow-up of 25 months, 10 of the 36 patients displayed improvement (28%).

Mesa et al. Blood. 2003;101(7):2534–41[141] (B)

Design: Clinical Trial.

Population: 21 symptomatic patients (mean age 63 years) diagnosed with myelofibrosis with myeloid metaplasia (agnogenic myeloid metaplasia, post-polycythemia myeloid metaplasia, and post-thrombocythemia myeloid metaplasia) and who had hemoglobin levels <10 g/dL or symptomatic splenomegaly received thalidomide (in low doses) associated with prednisone (over three months in decreasing doses).

Outcome: Evaluating clinical response, biological response, and adverse events to therapy.

Results: 13 patients (62%) presented improvement in hemoglobin levels. Among the ten red blood cell transfusion-dependent patients, seven (70%) improved and four (40%) became transfusion-independent. Among the eight patients with thrombocytopenia (score $<100 \times 10^9\,L^{-1}$), 74% ($n = 6$) presented an increase of more than 50% in the number of platelets. Four patients out of 21 (19%) had a greater than 50% reduction in spleen volume. Corticosteroid-related adverse events were mild and transient. Improvement in clinical response was not associated with improvement in intramedullary fibrosis or angiogenesis.

Discussion

The rationale of corticosteroid use in MF comes from the inhibition of the inflammatory process or possible immune mechanisms that could be involved in the pathogenesis of anemia.[136]

The literature review did not find prospective and designed studies to analyze the use of corticosteroids administered comparatively in patients with Primary/Secondary Myelofibrosis to PV/ET. Therefore, evaluation of efficacy, as well as its adverse events, are linked to the analysis of therapeutic regimens that also include other drugs, such as thalidomide, lenalidomide, and pomalidomide, and generally administered for no more than three months.[137–148]

The use of prednisone alone was evaluated in a retrospective study of 30 patients with primary or secondary Myelofibrosis PV/ET. In this study, other causes of anemia were excluded, based on data retrieved. The prednisone dosage was 0.5–1.0 mg/kg, the mean dose of 45 mg/day (22.5–60 mg). Hydroxycarbamide was also used at some point in seven cases. In 40% of the cases, there was a response to anemia (an increase of Hb $\geq$2 g/dL), in 36% of symptomatic patients there was a response regarding constitutional symptoms, and in 27.3% of the cases, there was improvement regarding thrombocytopenia. The mean duration of response was 12.3 months.[149]

Recommendation

No phase III studies have been found particularly analyzing the efficacy and safety of corticosteroid alone in patients diagnosed with myeloproliferative neoplasms. In a retrospective study, with few patients and lasting approximately 17 months, certain patients had clinical and/or laboratory improvement with corticosteroids alone.[149] When recovered trials evaluated the benefit of using corticosteroids (prednisone or dexamethasone), almost invariably those were associated with thalidomide or its analogs (lenalidomide or pomalidomide). Accordingly, such associations had relatively good tolerance, besides being related to the improvement in clinical responses. However, it is not possible to determine the specific role of corticosteroids in these responses.

PICO 10: Myeloproliferative neoplasms – what is the role of erythropoietin in primary myelofibrosis?

Estructured question

The clinical question was framed through the PICO framework components with: P (Patient), I (Intervention), C (Comparison), O (Outcome).

P: Primary myelofibrosis
I: Erythropoietin
C
O

Scientific database researched

The scientific database researched was PubMed-Medline. A manual search was performed for reviews references (narrative or systematic).

Evidence search strategy

PubMed-Medline

Strategy: (primary myelofibrosis OR myelofibroses, primary OR myelofibrosis, primary OR primary myelofibrosis OR bone marrow fibrosis OR bone marrow fibroses OR fibroses, bone marrow OR fibrosis, bone marrow OR myelofibrosis OR myelofibrosis OR idiopathic myelofibrosis OR myeloid metaplasia OR metaplasia, myeloid OR metaplasias, myeloid OR myeloid metaplasia OR myelosclerosis OR myelosclerosis OR myelosis, nonleukemic OR myelosis, nonleukemic OR nonleukemic myeloses OR nonleukemic myelosis OR chronic idiopathic myelofibrosis OR agnogenic myeloid metaplasia OR agnogenic myeloid metaplasia OR metaplasia, agnogenic myeloid OR metaplasias, agnogenic myeloid OR myeloid metaplasia, agnogenic OR myeloid metaplasia, agnogenic OR myelofibrosis with myeloid metaplasia) AND (erythrogenin OR erythropoietin OR erythropoietin-generating factor OR renal erythropoietic factor OR erythropoietin-generating factor OR hematopoietin OR hemopoietin OR darbepoetin OR eprex).
n = 222

Results of selected evidence

Two hundred twenty-two articles retrieved support the synthesis evidence regarding the role of Erythropoietin in patients with primary myelofibrosis exclusively in clinical trials. Narrative reviews and case reports were excluded from the evaluation. Evidence assessment followed the Oxford classification, which establishes strength evidence from the study design of choice.

Tsiara et al. Acta Haematol. 2007;117(3):156–61[150] (B)

Design: Clinical trial.

Population: Twenty patients with a mean age of 70 years and diagnosis of chronic idiopathic myelofibrosis, RBC-transfusion-dependent, treated with recombinant human erythropoietin (10,000 U dosage administered three times a week).

Outcome: Complete response characterized as the normalization of hemoglobin levels or discontinuation of RBC-transfusion-dependence. Partial response characterized as an elevation in hemoglobin levels ≥2.0 g/dL above initial treatment levels.

Result: Twelve patients (60%) (83-month mean follow-up time) displayed a favorable response to treatment (eight showed a complete response and four had a partial response). Seven patients who were in stage I of the disease (Dupriez score) responded to therapy. However, only five of those in stage II displayed a therapeutic response. There were no reports of adverse events during treatment.

Cervantes et al. Br J Haematol. 2006;134(2):184–6[151] (B)

Design: Clinical trial conducted between the years 2004 and 2005.

Population: 20 patients with a mean age of 68 years and diagnosis of myelofibrosis with myeloid metaplasia and anemia (hemoglobin levels ≤10.0 g/dL). Patients received darbepoetin alfa (a synthetic form of erythropoietin) at weekly skin doses of 150 μg. There was a dose increase to 300 μg/week if there was neither elevation in hemoglobin levels after four weeks nor a partial response after eight weeks.

Outcome: Complete response characterized as the normalization in hemoglobin levels and partial response as hemoglobin levels rise ≥2.0 g/dL in RBC-transfusion-independent patients.

Result: Eight patients (40%) (12-month mean follow-up time) displayed a favorable response to treatment (six showed a complete response, and two had a partial response).

Cervantes et al. Br J Haematol. 2004;127(4):399–403[152] (B)

Design: Clinical trial conducted between the years 2002 and 2004.

Population: Twenty patients with a mean age of 64.5 years and diagnosis of Myelofibrosis with Myeloid Metaplasia (MMM) and anemia treated with recombinant human erythropoietin (initial dose of 10,000 U given three times a week).

Outcome: Complete response characterized as normalization in hemoglobin levels or RBC-transfusion-independence while partial response as ≥50% reduction in RBC-transfusion-dependence and hemoglobin levels >10.0 g/dL maintained for at least eight weeks.

Results: Nine patients (45%) (mean follow-up period of 12.5 months) presented a favorable response to treatment (four with complete response and five with partial response). Erythropoietin therapy was well tolerated; two patients displayed an increased spleen volume throughout the treatment.

Hasselbalch et al. Am J Hematol. 2002;70(2):92–9[153] (B)

Design: Clinical trial conducted between 1997 and 2000.

Population: Thirteen patients with age range from 51 to 80 years, diagnosis of idiopathic myelofibrosis and anemia, RBC-transfusion-dependent, and treated with recombinant human erythropoietin (initial dose of 4000 U administered three times per week).

Outcome: Complete response characterized as normalization in hemoglobin or RBC-transfusion-independence levels while partial response as reduction ≥ 50% in RBC-transfusion-dependence and hemoglobin levels >10.0 g/dL maintained for at least four weeks.

Results: Eleven patients (84%) displayed a favorable response to treatment becoming RBC-transfusion-independent. Nine patients showed normal erythropoietin levels during treatment with erythropoietin. On the 12th month of treatment, four of the seven patients analyzed (57%) maintained a complete response while three patients showed a partial response.

Aloe Spiriti et al. Haematologica. 1993;78(6):371–3[154] (B)

Design: Clinical trial conducted between 1990 and 1992.

Population: Seven patients with a mean age of 68 years and diagnosis of idiopathic myelofibrosis and anemia, RBC-transfusion-dependent, treated with recombinant human erythropoietin (160 U/kg/day administered five times a week) for three months.

Outcome: Standardization in hemoglobin levels.

Results: Four patients (57%) presented a response to treatment, with three patients becoming RBC-transfusion-independent and one presenting an increase of 2.0 g/dL in hemoglobin levels. Patients displayed good tolerance to therapy, and there was no record of adverse events.

Mohr et al. Acta Haematol. 1993;90(2):65–70[155] (B)

Design: Clinical trial.

Population: Twelve subjects including ten patients with myelodysplastic syndrome (six with refractory anemia and sideroblasts), and two patients with idiopathic myelofibrosis, all treated with daily doses of recombinant human erythropoietin for 12 weeks (doses ranging from 30 U/kg to 240 U/kg for non-responders).

Outcome: Standardization in hemoglobin levels.

Results: Two patients diagnosed with myelodysplastic syndrome associated with anemia and sideroblasts showed an increase in hemoglobin levels. Regarding the other individuals, there was no register of increase in hemoglobin levels nor RBC-transfusion-dependence reduction. No former RBC-transfusion-dependent patient has benefited from the treatment as well as there was no record of hematological adverse events.

Discussion

Several clinical trials have analyzed the use of the synthetic form of erythropoietin or its recombinant human form in patients with myelofibrosis and anemia. These studies had a small number of patients, none was randomized, with no uniform response criteria, and demonstrated variable response rates between them (B).[150–155] According to those studies, the use of human recombinant erythropoietin (r-HuEPO) on patients diagnosed with idiopathic myelofibrosis (IMF) or myelofibrosis with myeloid metaplasia (MMM) associated with anemia had variable response rates, ranging from 40% to 84%, and it is a well-tolerated medication in such situation. A response rate between 40% and 50% after 6–8 weeks appears in studies with a minimum response criterion of increase of Hb >2 g/dL and/or a 50% reduction in transfusions (B).[150,152–155,156,157]

Specific factors, such as less severe anemia and lack of transfusional need, appear as positively predictive variables to the response to r-HuEPO treatment. On the other side, the correlation of this response with serum erythropoietin levels proves questionable (B).[151,152,158]

The standard dose used is 30,000 IU/week. Nevertheless, the effectiveness of such dosage requires further investigation.[150,155]

Some studies investigated the association of Erythropoietin with other agents, such as alpha-interferon, GMC-SF, thalidomide, and corticosteroids. However, the role of Erythropoietin in those situations remains unclear.[143,159,160]

Recommendation

Recombinant human erythropoietin is an eligible a treatment for anemia in Myelofibrosis, with a good safety profile. The response rate is considerably variable, apparently more effective in patients with less severe anemia and transfusion-independent. However, this analysis stems from non-randomized studies with small numbers of patients. The standard dose is 30,000 U/week, with an expected response of 6 to 8 weeks.

PICO 11: Myeloproliferative neoplasms – primary myelofibrosis the role of interferon

The action of interferon alpha in the clones of myeloproliferative neoplasms is not entirely known. However, it is recognized the suppression determined by this cytokine in the proliferation of hematopoietic progenitors, the antagonization of the action of platelet-derived growth factor, transforming growth factor beta and other cytokines that may be involved in the development of myelofibrosis. The purpose of this guideline is to provide recommendations on the role of interferon in primary myelofibrosis. This work consists of the systematic review of the literature, through the PICO acronym, where “P” addresses patients with primary myelofibrosis, “I” addresses the intervention with interferon, and “O” refers to the outcome of survival, morbidity, and quality of life. For this guideline, we retrieved 344 studies, without period restriction, in the medline database, of which 21 were selected to answer the clinical question. Details of the methodology and results of this guideline are set out in Annex V.

Introduction

Interferon, a glycoprotein produced in response to infectious agents and tumor cells, has a variety of biological properties on several cell types, including immunomodulatory effect and regulation of cell growth, acting on the induction of apoptosis and inhibition of angiogenesis.[161,162] Although their discovery dates back to the late 1950s, their availability on a large scale only occurred two decades later, from the expression of

recombinant human interferon alfa-2b in *Escherichia coli*, using recombinant DNA technology. Numerous subtypes of interferon alpha were cloned, however only interferon alpha-2a and alpha-2b are on the market. The discovery of the pegylated form of interferon brought benefits such as increased plasma half-life, which allowed the increase in the interval between doses.[163,164]

Results

What is the role of interferon in primary myelofibrosis?

The clinical use of interferon-alpha (IFN-alpha) in Myeloproliferative Neoplasms (MPNs) was the subject of some reviews (B).[161,162,165,166] Evidence for the role of this therapy in myelofibrosis (MF) is limited. Most of the original studies are retrospective and the majority of patients included have a diagnosis of polycythemia vera (PV) or essential thrombocythemia (ET) (B).[163,164,167–177] The use of IFN-alpha in its conventional or pegylated form may induce control of erythrocytosis, thrombocytosis and white blood cell count in MPNs. For example, the hematologic response is described in up to 95% of patients with PV and ET, and partial or complete molecular response may occur in a fraction of cases. Beneficial effects on splenomegaly control and on the constitutional symptoms of MPNs are also reported.

In the MFP, the responses appear to be less frequent, but there is substantial heterogeneity among distinct reports (splenic response ranging from 0 to 85%). The occurrence of response seems limited to cases without massive splenomegaly and in the pre-fibrotic phase of the disease (B).[174–177] There are small series of cases reporting a reduction in the histological changes of myelofibrosis in the bone marrow with the use of IFN-alpha[174–176] (B), as well as in the improvement of anemia associated or not with erythropoietin.[159]

The most frequently used initial doses of Interferon-alpha are 3×10^6 IU SC 3 times a week. The dose used in the pegylated formulation is quite varied (2–3 ug/kg and 45–90 ug SC per week). Adverse events are frequent, often determining discontinuation of treatment. The use of IFN-alpha is considered safe during pregnancy.[161,166]

Recommendation

The following recommendations mostly rely on retrospective studies or with few MFP patients. The use of IFN in MFP may induce a hematological response, reduction of splenomegaly and improvement of symptoms, especially among individuals who present in the proliferative phase of the disease and with non-massive splenomegaly (B), but the response rates are less frequent when compared to those obtained in the other MPNs. Discontinuation due to adverse events is common.

PICO 12: Myeloproliferative neoplasms – primary myelofibrosis – the role of immunomodulators

This guideline seeks to evaluate the role of immunomodulators in primary myelofibrosis. a systematic searchwas conductedthrough the PICO framework, where “P” stands for patients with primary myelofibrosis, “I” stands for immunomodulators, and “O” stands for survival, morbidity, and quality of life outcomes. The search paramaters into the database of choice, medline, were setwithoutdate range limits. Outof the 828 publications retrieved, 30 studies were selected to answer the clinical question. Detailed features concerning this guideline’s research methodology and results are displayed in Annex VI.

Discussion

The use of thalidomide in MF was evaluated in a series of studies, mostly single-arm, and at doses of 50–800 mg/day. Some studies have associated corticosteroids for three months, seeking to increase drug tolerability. Patients who used lower doses of thalidomide alone or with corticosteroid combination displayed higher tolerability, although doses above 50 mg/day led to a higher dropout rate. These studies were mostly performed with a reduced number of patients, and their duration were variable but usually very short (3–36 months).[137,143–147,178–187]

In studies with a more representative number of patients the anemia response ratio was around 20%. Variable rates of increase in platelet counts and reduction of splenomegaly were also observed.[137,143–147,178–187]

The only prospective study evaluating thalidomide versus placebo used doses of 200–400 mg/day, without corticosteroids. There was no benefit of thalidomide in comparison to placebo, and the discontinuation rate due to intolerance was very high in the thalidomide arm (61.5%).[186]

The association of thalidomide, erythropoietin and methylprednisolone for six months was 100% effective in the recovery of cytopenias in a 6-month study including six patients.[143]

In a retrospective analysis involving 88 patients, the association of Danazol to thalidomide had a significantly higher response rate when compared to thalidomide alone (71 vs. 46%, $p = 0.014$), as well as increased platelet counts (50% vs. 30%, $p = 0.06$).[147]

Studies using thalidomide in Myelofibrosis are summarized in Table 9.

Lenalidomide and pomalidomide are second generation immunomodulators created from the modified thalidomide molecule, aiming to reduce toxicity and increase immunological activity. These immunomodulators have been studied in the treatment of MF because of their higher tolerance and lower neurotoxicity profile when comparing them to thalidomide.[188]

In studies with Lenalidomide, as a single agent or combination with prednisone, it was observed that the response rate for anemia ranged from 19% to 38%. In some trials, bone

Table 9

Study	Type of study	N	Dose Thalidomide (mg)	Combination medications (months)	Results (% response/n)	Dropout due to toxicity (% – months)	Mean follow-up
Barosi et al., 2001[178]	Prospective, single arm	21	100–400	–	Anemia: 43 (3 out of 7) Thrombocytopenia: 66 (2 out of 3)[3]	38.1 (1) 76.2 (3) 90.5 (6)	6
Canepa et al., 2001[179]	Prospective	10	200–800	–	Anemia, splenomegaly and Thrombocytopenia – 30	None	6.25–9.5
Pozzato et al., 2001[180]	Prospective, single arm	06	100–MDT	–	Anemia: 50	None	6
Piccaluga et al., 2002[181]	Prospective, single arm	12	100–600	–	Anemia: 75 (3 out of 4) Thrombocytopenia: 100 (2 of 2)	58.3 (7)	10
Mesa et al., 2003[141]	Prospective, single arm	21	50	Prednisone (3)	Anemia: 62 Transfusion Independence: 40 (7 out of 10) Thrombocytopenia: 75 (6 of 8) Splenomegaly: 19	5 (3)	6
Elliott et al., 2002[182]	Prospective, single arm	15	200–400	–	Anemia: 20 (3 out of 15) Thrombocytopenia: 86.6 (13 to 15) Splenomegaly: 25 (3 of 12)	80 (NA)	NA
Merup et al., 2002[183]	Prospective, single arm	15	100–800	–	No answer	36 (3)	3
Marchetti et al., 2004[184]	Prospective, single arm	63	50–400	–	Anemia: 22 (11 out of 49) Transfusion Independence: 39 (7 to 18) Thrombocytopenia 22 (4 out of 14) Splenomegaly: 50	22 (1) 24 (3) 51 (6)	6
Strupp et al., 2004[185]	Prospective, single arm	16	100–400	–	Anemia: 60 (6 out of 10) Thrombocytopenia: 71.4 (5 out of 7)	4	9
Leonidas et al., 2005[143]	Prospective, single arm	5	50	Erythropoietin (6) Methylprednisolone (6)	Anemia, Neutropenia and Thrombocytopenia: 100	0	6
Abgral et al., 2006[186]	Prospective randomized two arms (vs. placebo)	52	200–400	–	No answer	61.5 (thalido-mide arm)	6
Thomas et al., 2006[187]	Prospective, single arm	41	100–800	–	Anemia: 20 (7 of 35) Transfusion Independence: 21 (5 of 24) Splenomegaly: 31 (9 of 29) Thrombocytopenia 21 (5 of 24)	NA	NA
Weinkove et al., 2008[144]	Prospective, single arm	15	50	Prednisolone (3)	Transfusion independence: 43 (3 of 7) Splenomegaly: 50	0	8

Table 9 (*Continued*)

Study	Type of study	N	Dose Thalidomide (mg)	Combination medications (months)	Results (% response/*n*)	Dropout due to toxicity (% – months)	Mean follow-up
Hattori et al., 2011[145]	Prospective, single arm	4	50	Prednisolone (3)	Anemia: 25 (1 out of 4) Thrombocytopenia: 75 (3 of 4)	25 (1)	3
Thapaliya et al., 2011[146]	Retrospective	50	50	Prednisone (3 to 6) Cyclophos-phamide(14)Etanercept (15)	Anemia: 22 Splenomegaly: 8	NA	36
Bejanyan et al., 2012[137]	Prospective, single arm	5	100	Arsenic Trioxide Dexamethasone	Anemia: 20 (1 out of 5)	NA	24.1
Luo et al., 2018[147]	Retrospective	88	88	Thalidomide Prednisone Danazol (42)	Anemia: 58 (without Danazol: 46 vs. Danazol with 71 – $p = 0.014$) Thrombocytopenia: 39.77 (without Danazol 30 vs. with danazol: 58 – $p = 0.06$)	0	25

MTD: maximum tolerable dose; NA: not available.

marrow fibrosis regression was observed, while in others it did not. The duration of these trials was variable, ranging from 3 to 6 months.[139,148,188,189]

Although tolerability was higher than that of thalidomide (with a lower rate of drug-related withdrawal), myelosuppression was significant. About 88% of subjects had hematological toxicity with grades 3 and 4, which led to a dose reduction in several patients. Although the response rate seems to be lower than that obtained with thalidomide, tolerability was higher, and some patients maintained a response even after discontinuation of treatment.[148]

Studies using Lenalidomide with or without Prednisone in Myelofibrosis are summarized in Table 10.

A further pooled analysis of three phase 2 studies evaluated the efficacy of Lenalidomide and Thalidomide (44 patients received thalidomide as sole agent, 41 received lenalidomide, and 40 subjects received an association between lenalidomide and prednisone). In this study, it was possible to identify that therapy based on the use of lenalidomide presented high efficacy in detriment to the use of thalidomide alone (34–38% versus 16%, $p = 0.06$). Combination therapy (lenalidomide plus prednisone) showed a longer duration of response (mean 34 months) compared to the use of lenalidomide alone (7 months) and thalidomide (13 months); $p = 0.42$. A smaller number of patients discontinued Lenalidomide with Prednisone (13%), the combination therapy because of adverse events over those who were treated with a single agent (32–39%).[149]

Lenalidomide was also evaluated in association with Ruxolitinib in a phase II study in 31 patients. The proposed therapeutic plan was of 28-day cycles with Ruxolitinib 15 mg 12/12 h PO continuously, associated with 21 days of lenalidomide, 5 mg/day. The study was terminated early because of the inability to achieve pre-determined efficacy and the high dropout rate regarding lenalidomide in the initial three months.[190]

Concerning pomalidomide, beneficial effects have been reported in some clinical trials. Phase II studies are summarized in Table 11.

A prospective, randomized, double-blind study evaluated 84 patients with myelofibrosis and associated anemia, with four arms: pomalidomide (2 mg) and placebo versus pomalidomide (2 mg) and prednisone, versus pomalidomide (0.5 mg) and prednisone, versus predinisone and placebo. Pomalidomide cycles were for oral use for 28 days and prednisone was 30 mg/day with progressive dose reduction for 3 months. The response rates were 23% (95% CI: 5–41%), 16% (95% CI: 0–33%), 36% (95% CI: 16–56% (95% CI: 2–36%), respectively.[194]

Another multicenter study, evaluating patients ($n = 252$) with myelofibrosis and anemia associated with transfusional dependence, randomized (2:1) to pomalidomide or placebo. Of this group, 152 patients received pomalidomide, and 77 patients received placebo. There was no difference in response rates at six months, with both groups presenting a response rate of 16% ($p = 0.87$).[195]

Recommendation

The use of Thalidomide in Myelofibrosis presented variable response rates, usually small and with a high dropout rate due to drug intolerance. However, lower dosages (50 mg/day) or even a corticosteroid combination may increase tolerability. Concomitant use of erythropoietin with continuous corticosteroid or danazol seems to confer better response rates. However, further research with more robust design is warranted.

Lenalidomide and Pomalidomide, with or without prednisone, were more tolerable and with a lower rate of drug intolerance than Thalidomide, but also with small response rates.

Immunomodulatory therapy in myelofibrosis is associated with an increase in hemoglobin levels, platelet count and spleen reduction in selected patients (although with reduced efficacy), and may be an alternative in the treatment of anemia

Table 10

Study	Type of study	N	Dose of Lenalidomide (mg)	Combination medications (months)	Results (% response/n)	Dropout due to toxicity (% – months)	Mean follow-up
Tefferi et al., 2006[188]	Prospective, single arm (2)	68	5–10	–	Anemia: 22 Splenomegaly: 33 Thrombocytopenia: 50	44 (3)/NA	5–19
Tefferi et al., 2007[189,a]	Retrospective	3	10	–	Anemia: 22 Splenomegaly: 33	0	12–15
Quintas-Cardama et al., 2009[139]	Prospective, single arm	40	5–10	Prednisolone (3)	Anemia: 30 Splenomegaly: 42	22 (6)	22
Mesa et al., 2010[138]	Prospective, single arm	48	10	Prednisolone (3)	Anemia: 19 Splenomegaly: 10	14.5 (NA)	27.6
Chihara et al. 2016[148]	Prospective, single arm	40	5–10	Prednisolone (3)	Anemia: 32 Splenomegaly: 39	17.5 (NA)	108

[a] Myelofibrosis associated with del (5) (q31), NA: not available.

Table 11

Study	Type of study	N	Dose Pomalidomide (mg)	Combination medications (months)	Results (% response/n)	Drop out due to toxicity (% – months)	Mean follow-up
Begna et al., 2011[191]	Prospective, single arm	58	0.5–2	–	Anemia: 17[a] Plaquetopenia: 58 (14 of 24)	–	NA
Daver et al. 2013[192]	Prospective, single arm	29	0.5	–	Anemia: 10 Transfusion Independence: 20 (2 out of 10)	–	NA
Daver et al., 2014[136]	Prospective, single arm	29	0.5	Prednisone (3)	Anemia: 21 Transfusion independence (4 of 18)	–	NA
Schlenk et al., 2017[193]	Prospective, single arm, two cohorts	96	Cohort 1–0.5 Cohort 2–2	Prednisolone[b]	Cohort 1 – Anemia: 39 Cohort 2 – Anemia: 24	–	NA

[a] JAK2 + patients only.
[b] If absence of NA response – not available.

in MF, where there are no drug treatments with good efficacy and low toxicity.

PICO 13: Myeloproliferative neoplasms – primary myelofibrosis JAK inhibitor therapy

Myeloproliferative neoplasms (MPNs) are characterized by abnormal myeloid hematopoietic proliferation, which arises from the activation of signal transduction pathways caused by genetic rearrangements or mutations. This guideline aims to gather information about treatment of patients with primary myelofibrosis withinhibitors of the JAK/STAT pathway. This guideline made use of the systematic review of the literature, through the PICO acronym, where "P" stands for patients with primary myelofibrosis, "I" stands for intervention with ruxolitinib, jak, jakavi, momelotinib, pacritinib or fedratinib inhibitors, and "O" stands for the outcome of survival, morbidity. The search in the medline database had no period restrictionand retrieved 253 papers, out of which we selected 13 were to answer the clinical question. Details of the methodology and results of this guideline are set out in Annex VII.

Introduction

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) that originates from the neoplastic transformation of a pluripotent hematopoietic cell, characterized by medullary fibrosis, abnormal blood counts (anemia, thrombocytosis or thrombocytopenia and leukocytosis or leukopenia) and non-specific constitutional symptoms, including persistent fever, weight loss, night sweats, fatigue, and weakness. Additionally, extramedullary hematopoiesis (EH), mobilization of hematopoietic progenitor cells and splenomegaly may be observed, the latter also having a negative quality of life impact when accompanied by symptoms such as abdominal pain (D).[196]

Abnormal myeloid hematopoietic proliferation in MPNs arises from the activation of signal transduction pathways caused by genetic rearrangements or mutations, such as the Val617Phe mutation in the JAK2 gene (JAK2 V617F) (D).[8] Janus-activated kinases (JAK) are a family of proteins composed of four members (JAK 1, JAK 2, JAK 3 and tyrosine kinase 2) with tyrosine kinase activity, located in the intracellular domain of cytokine and growth factor receptors and involved in the transduction of signals by the JAK/STAT signaling pathway, regulating hematopoiesis, immunity, inflammation and cell growth (D).[9]

Results

The central involvement of the JAK/STAT pathway in the molecular pathophysiology of PMF justified the development of inhibitory drugs of this pathway. Among JAK inhibitors, ruxolitinib (a selective inhibitor of both JAK1 and JAK2) was the first approved drug to treat patients with PMF.[197,198]

This drug was evaluated in two multicenter clinical trials (Comfort-I and Comfort-II) for efficacy and safety in the treatment of patients with primary myelofibrosis, post-polycythemia vera myelofibrosis, and post-thrombocythemia essential myelofibrosis (A).[199,200] In both studies, inclusion criteria were patients older than 18 years who had intermediate or high-risk disease according to the International Prognostic Scoring System (IPSS), with an average life expectancy of 48 and 27 months, respectively. ECOG performance status equal to or less than 3, spleen at 5 cm or more of the left costal border, and platelet counts above $100 \times 10^9\,L^{-1}$ were also mandatory. In the COMFORT-I study, patients were randomized to treatment with ruxolitinib or placebo (A).[199] With regard to secondary outcomes, defined as (a) duration of therapeutic response, (b) reduction of Symptom Score (assessed by of MFSAF – modified Myelofibrosis Symptom Assessment Form) greater than or equal to 50% from baseline to 24th week, (c) change in total symptom score (TSS)from baseline to 24th week and (d) overall survival, it was found that:

- Reduction in spleen volume was maintained in patients treated with ruxolitinib, with 67% of subjects having a sustained response for 48 weeks or longer;
- Improvement of 50% or more in the total score of constitutional symptoms at week 24 was identified in 45.9% of patients treated with ruxolitinib versus 5.3% of those receiving placebo (OR = 15.3 with 95% CI: 6.9–33.7);
- Thirteen deaths (8.4%) occurred in the ruxolitinib group compared to 24 (15.6%) in the placebo group (hazard ratio = 0.50 with 95% CI: 0.25–0.98), over a follow-up period of 51 months.

Regarding non-hematological adverse events (ecchymosis, dizziness, headache), similar rates were identified in both groups. However, hematological adverse events such as anemia and thrombocytopenia were more common among patients receiving ruxolitinib (A).[199]

The 5-year follow-up analysis of the COMFORT I study showed that 27.7% of patients randomized to ruxolitinib and 25.2% of patients who underwent placebo crossover remained on treatment. No patient remained in the placebo arm. Patients randomized to ruxolitinib had a median duration of splenic response of 168.3 weeks and median survival greater than the placebo group (unreached versus 200 weeks in the placebo group, HR, 0.69, 95%, 0.50–0.96, $p = 0.025$), regardless of crossover for ruxolitinib. The toxicity profile did not change concerning previous analyzes (A).[201]

In the COMFORT-II study (Controlled Myelofibrosis Study with Oral JAK Inhibitor Treatment-II), 229 subjects were randomized to treatment with ruxolitinib ($n = 146$, out of which 75% were positive for the JAK2 V617F mutation) or to the arm where the best available therapy (BAT) was given ($n = 73$, of which 67% were positive for the JAK2 V617F mutation) (A).[200] A significant increase was observed in the proportion of patients who presented a reduction of 35% or greater in spleen volume identified at week 48 (28% versus 0%, with $p < 0.001$) (A).[200]

In a secondary analysis conducted in subgroups of patients, according to sex, the subtype of myelofibrosis, prognostic category, and mutation state JAK2 V617F, a reduction of spleen volume was identified in all of them. Rates of splenic response in the subgroup positive for the JAK2 V617F mutation was 33% in the ruxolitinib arm versus 0% in the BAT arm. The corresponding rates in the negative subgroup for the mutation were 14% and 0% respectively (A).[200]

During the follow-up period, quality of life analyzed through HRQoL (health-related quality of life) and symptoms associated with myelofibrosis showed improvement, with decreased appetite loss, dyspnea, fatigue, and insomnia, compared to baseline levels for patients treated with ruxolitinib. Those parameters remained stable or worsened among subjects randomized to the best available conventional therapy (A).[202]

Adverse events of any degree requiring dose reduction or even drug discontinuation were most frequently reported in patients on ruxolitinib (63% versus 15%, respectively). Besides, thrombocytopenia and anemia were more frequent among these patients. Non-hematological adverse events were rare and mainly grade 1 or 2. Two cases of acute myeloid leukemia were reported among patients randomized to BAT (A).[200] At the end of 12 months of follow-up, ten deaths had been reported (six in the ruxolitinib group [4%] and four in the randomized group for the best available conventional therapy [5%]), out of which, (seven deaths [3%] and three deaths [4%], respectively) occurred within 28 days after treatment discontinuation (A).[200]

The 5-year follow-up of the COMFORT-II study showed that the probability of maintaining splenic response was 0.48 (95% CI: 0.35–0.60) (median of 3.2 years) in patients allocated to the ruxolitinib arm. The median overall survival in the intention-to-treat analysis was yet to be achieved for the ruxolitinib arm and 4.1 years in the BAT arm. (Hazard ratio (HR) = 0.67, 95% CI: 0.44–1.02, $p = 0.06$); HR-corrected by crossover was 0.44 (95% CI: 0.18–1.04; $p = 0.06$). Longer treatment time occurred free of unexpected adverse events. The five-year analysis revealed that a sustained reduction of the splenic volume might be associated with survival benefit (A).[203]

So, according to the 2018 update of the European Leukemia Net recommendations, ruxolitinib is indicated for patients with myelofibrosis, with splenomegaly and constitutional

symptoms. Novel therapies are on trial in clinical protocols (A).[204]

Evaluation of overall survival benefit for patients initially randomized to the ruxolitinib arm in both studies is hampered by the relatively small number of patients included and by the crossover between the study and comparator arms. Overall survival is not a primary objective in both studies. Thus, an exploratory analysis of the combined data from the COMFORT I and II studies analyzed 528 patients included in both studies to broaden the comprehension of the impact on overall survival. The risk of death dropped by 30% in patients who were initially randomized to ruxolitinib, compared to patients initially randomized to control arms (mean overall survival of 5.3 vs. 3.8 years, HR 0.70 [95% CI: 0.54–0.91], $p = 0.0065$). The use of a statistical tool to eliminate the influence of the crossover on this analysis (rank preserving structural failure time – RPSFT) made this difference more pronounced (mean overall survival of 5.3 vs. 2.3 years HR 0.35 [95% CI: 0.23–0.59]) (B).[205]

Single-arm studies with Ruxolitinib (UK ROBUST and JUMP) involved a much more significant number of patients with primary myelofibrosis or post-PV/ET MF. Those trials also allowed the inclusion of patients with platelet counts up to $<50 \times 10^9$/L and patients with symptomatic IPSS Intermediate-1 and/or with splenomegaly. Those studies have demonstrated similar efficacy in the control of symptoms, reduction of splenomegaly and safety profile found in COMFORT I and II studies (A).[206,207]

Additional inhibitors of the JAK/STAT pathway have also been evaluated in PMF in phase III studies. Fedratinib showed a significant reduction in symptomatology and splenomegaly compared to placebo in patients with PMF. However, due to its toxic effects (especially Wernicke's encephalopathy), its development was discontinued (B).[208]

Pacritinib, a specific inhibitor of JAK2, has been evaluated in two randomized phase III trials against the best available therapy, one of which is Ruxolitinib (PERSIST-1 and PERSIST-2). Despite inducing a significant reduction of splenomegaly and being more tolerated for hematological adverse events, the FDA suspended its development in the USA due to the occurrence of increased mortality due to cardiovascular events and the occurrence of intra-cranial hemorrhages (A).[209,210]

Momelotinib has also been compared to the best available therapy in patients with PMF (SIMPLIFY-1), also including patients who have failed Ruxolitinib (SIMPLIFY-2). The drug failed to show superiority in both studies, especially concerning symptom control (A).[211,212]

Recommendation

Ruxolitinib demonstrated superiority compared to placebo or the best available conventional therapy in the treatment of PMF and post-PV/ET MF, with higher rates of splenomegaly control, improvement of symptoms and quality of life and longer overall survival. The responses were sustained for a median period lower than three years, and the benefit occurred regardless of the Myelofibrosis subtype, age group, presence or absence of the JAK2 V617F mutation, hemoglobin levels, and platelet counts.

Thus, Ruxolitinib is indicated as first-line treatment in patients with primary or post PV/ET myelofibrosis with IPSS Intermediate-2 and High Risk. It is also an alternative therapy for patients with IPSS Intermediate-1 with significant splenomegaly and/or symptomatology.

PICO 14: Myeloproliferative neoplasms – what is the role of bone marrow transplantation in primary myelofibrosis?

Structured question

The clinical question was framed through the PICO framework components with: (P (Patient), I (Intervention), C (Comparison), O (Outcome).

P: Primary myelofibrosis
I: Bone marrow transplantation
C: Drug-based treatment
O: Survival, morbidity

Scientific database researched

The scientific database researched was PubMed. A manual search was performed for reviews references (narrative or systematic).

Evidence search strategy

PubMed-Medline

Strategy 1: ((primary myelofibrosis OR myelofibroses, primary OR myelofibrosis, primary OR primary myelofibrosis OR bone marrow fibrosis OR bone marrow fibroses OR fibroses, bone marrow OR fibrosis, bone marrow OR myelofibrosis OR myelofibrosis OR idiopathic myelofibrosis OR myeloid metaplasia OR metaplasia, myeloid OR metaplasias, myeloid OR myeloid metaplasia OR myelosclerosis OR myelosclerosis OR myelosis, nonleukemic OR myelosis, nonleukemic OR nonleukemic myeloses OR nonleukemic myelosis OR chronic idiopathic myelofibrosis OR agnogenic myeloid metaplasia OR agnogenic myeloid metaplasia OR metaplasia, agnogenic myeloid OR metaplasias, agnogenic myeloid OR myeloid metaplasia, agnogenic OR myeloid metaplasia, agnogenic OR myelofibrosis with myeloid metaplasia) AND (Transplantation OR Bone Marrow Transplantation OR Transplantation, Bone Marrow OR Grafting, Bone Marrow OR Bone Marrow Grafting OR Bone Marrow Cell Transplantation OR Transplantation, Bone Marrow Cell OR autologous transplant* OR Transplant, Autologous OR Transplants, Autologous OR Autotransplant* OR autograft* OR allografts OR Hematopoietic Stem Cell Transplantation OR Peripheral Blood Stem Cell Transplantation OR Stem Cell Transplantation, Peripheral OR Transplantation, Peripheral Stem Cell OR Peripheral Stem Cell Transplantation)) AND "therapy/broad"[Filter]. ***n*** **=833**

Results of selected evidence

Of the total articles retrieved, 833 were selected to support the synthesis of evidence regarding the role of bone marrow biopsy in the diagnosis of patients with primary myelofibrosis (PMF) in retrospective or prospective longitudinal studies alone. Narrative reviews and case reports were excluded from the evaluation. The evidence was assessed according to the Oxford classification, which establishes strength evidence from the study design of choice.

PICO: What is the role of HSCT in primary myelofibrosis?

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only potentially curative treatment in the management of primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET). Several studies have analyzed the results concerning HSCT in the treatment of myelofibrosis, with estimates of overall survival over three years being reported between 37% and 58%.[213–222]

A clinical trial was conducted with 56 patients aged 10–66 years, and PMF or post-PV/ET MF patients submitted to HSCT from related donors ($n = 36$) or unrelated donors ($n = 20$). Of these, 44 patients received Busulfan and Cyclophosphamide (BuCY) as conditioning, and 12 patients received TBI and chemotherapy. Thirty-three patients received BM cells, and 23 patients received peripheral blood cells. Only three patients did not present engraftment. Two patients died due to recurrent or progressive disease, and 18 patients died due to other causes. 3-year overall survival estimate was 58%.[213]

A study by the Center for International Bone Marrow Transplant Research (CIBMTR) with 289 MF patients (age, 18–73 years) showed treatment-related mortality (MTR) of 27% at one year and 35% at five years. In the unrelated donor group, TRM was 43% in one year and 50% in 5 years. The five-year overall survival rate was 37% with related donors and 30% with unrelated donors.[214]

In another series, 104 patients with PMF and post-PV/TE MF, with median age of 49 years, were submitted to HSCT. Nearly 43% of the transplants used unrelated donors. Predominant cell source were peripheral stem cells. Five-year overall survival rate was 61%.[215]

In a different retrospective study, Robin et al. reported data on 147 patients (median age 53 years) diagnosed with primary (53%) or secondary myelofibrosis, who had undergone HSCT between 1997 and 2008. The conditioning was myeloablative in 31% of the patients. 59% of the patients received transplants from related donors. 10% presented graft failure. With a median follow-up of 35 months, overall survival (OS), event-free survival (EFS), and non-relapse mortality (NRM) in four years were 39%; 32% and 39% respectively. The cumulative incidence of acute GVHD was 43% at 100 days of follow-up. The cumulative incidence of chronic GVHD at four years was 39%.[216]

Recommendations

Allogeneic HSCT is indicated for PMF patients classified as intermediate-risk 2 or high-risk, and patients with PV or ET who have progressed to myelofibrosis with high-risk characteristics. Young patients with no comorbidities classified as intermediate-risk 1 who are carriers of unfavorable cytogenetic changes or transfusion-dependent may also be eligible for allogeneic HSCT (2B). The use of unrelated donors, 10/10 may be an alternative in the absence of a HLA-identical related donor (2B).

What is the role of JAK1/2 inhibitors before transplantation?

In a retrospective study, 100 cases classified according to the clinical stage and the response to JAK1/2 inhibitors at the time of transplantation were investigated. Two-year overall survival was 61% for those who improved with the use of the JAK1/2 inhibitor versus 32% for those in leukemic transformation. In the multivariate analysis, the response to the inhibitor ($p = 0.03$), the Dynamic International Prognostic Scoring System (DIPSS) ($p = 0.003$) and the donor type ($p = 0.006$) were independent survival factors. Adverse events were typical in patients who abruptly reduced dosage or discontinued use of the inhibitor over six days before transplantation. In conclusion, the previous use of the JAK1/2 inhibitor did not impair transplant results; therefore, it should be continued until close to conditioning regimen.[217]

Recommendation

The use of Ruxolitinib may improve the clinical conditions of pre-transplant patients, but it should not delay referral to allogeneic HSCT. The long-term outcome of the combination of ruxolitinib and transplantation still requires more data on efficacy and safety, preferably performed within clinical trials (C4).

Should splenectomy be performed before HSCT?

An additional study showed that prior splenectomy reduced neutrophil binding time in the group receiving reduced-intensity conditioning (13 versus 20 days, $p = 0.008$). Splenectomy risk should be considered. Data show a risk of death of 9% and morbidity of 31%.[219]

An EBMT study showed that prior splenectomy was significantly associated with a higher risk of relapse.[220]

Both splenectomy and splenic irradiation influence on HSCT outcomes were evaluated in a study with 9683 cases, in which 472 patients had previously been splenectomized, 300 had undergone splenic radiation, 1471 had splenomegaly, and 7704 had a normal spleen. The results showed delayed spinal recovery in patients with splenomegaly, a faster spinal recovery in patients undergoing splenectomy, but with no impact on survival.[221]

A retrospective analysis investigated 85 patients were transplanted in a single center with PMF, including 39 patients who underwent splenectomy before transplantation. Half of the splenectomized patients presented morbidity whether in surgery or the postoperative period, often thrombosis or bleeding. There was no significant difference regarding acute or chronic GVHD between non-splenectomized patients (NS) and splenectomized patients (S). In 5 years, event-free survival (EFS) was 41.6% in NS, and 58.1% in S ($p = 0.062$). Multivariate analysis showed an increased risk of failure for unrelated

donors and improved EFS in S. The cumulative incidence of relapse in two years was 16.6%. The cumulative incidence of transplant-related mortality (TRM) was similar in the groups. In 5 years, overall survival (OS) was 40.6% and 61.2% in NS and S, respectively ($p = 0.071$). Multivariate analysis showed that unrelated donor and splenectomy were associated with better survival.[222]

Recommendations

The pre-transplantation splenectomy is not routinely thought-out. However, this procedure can be an alternative in patients with splenomegaly above 20 cm. (C4)

What should be the conditioning regimen intensity in allogeneic HSCT for Myelofibrosis?

In a current study, from the Nordic countries, 92 patients with PMF received allogeneic HSCT with myeloablative conditioning (40 patients) or reduced intensity (52 patients). The median age was 46 and 55, respectively. Patients who received reduced-intensity conditioning were significantly better, with age adjustment, especially for patients less than 60 years of age (10-year survival rate close to 80%). There was no difference in TRM between the two groups. The group treated with reduced intensity conditioning had less acute GVHD. Five-year overall survival was 70, 49 and 51% for patients with Lille scores of 0.1 and 2, respectively.[223]

In another study, the myeloablative-conditioning scheme with cyclophosphamide and busulfan, adjusted for the serum level, provided a significant improvement in survival.[215]

In a British clinical trial, 51 patients with MP received allogeneic HSCT, predominantly related and with myeloablative conditioning (age 19–54 years) or reduced intensity (age 40–64 years). The 3-year overall survival was 44% for the myeloablative group and 31% for the low-intensity group. Relapse mortality was 15% and 46%; the non-relapse mortality was 41% and 32%; and chronic GVHD 30% and 35%, in myeloablative conditioning or reduced intensity conditioning, respectively.[224]

A CIBMTR study with 289 patients with myelofibrosis displayed no significant difference between the types of conditioning.[214]

A phase II clinical trial included 103 patients (median age 55 years) with a diagnosis of MFP/secondary myelofibrosis, submitted to related and unrelated HSCT, after reduced intensity conditioning regimen using busulfan 10 mg/kg, fludarabine 180 mg/m^2 and ATG. Only two patients did not present engraftment. The cumulative incidence of RRT was 16% for the 12-month period. The 5-year cumulative recurrence incidence estimation was 29%. With a mean follow-up of 33 months, overall survival in five years was 67% and EFS 51%. 27% of the patients had chronic GVHD, and 47% had chronic GVHD.[225]

Retrospective analysis of 160 PMF patients undergoing HSCT compared two common low-intensity conditioning regimens: Fludarabine and Melphalan (FM) or Fludarabine and Busulfan (FB). The incidence of GVHD-A was 62% in the FM group and 31% in the FB group, and for GVHD-C the rates were 49% and 53%, respectively. The 7-year progression-free survival (PFS) was 52% in the FM group versus 33% in the FB group, and the overall 7-year survival was 52% in the FM group versus 59% in the FB group, NRM was 43% in the FM group versus 31% in the FB group. In the multivariate analysis, there was no difference in PFS between the two groups, but the relapse rate was lower in the FM group, and a trend toward lower NRM was seen in the FB group. Both regimens were effective and produced similar overall survival when used in the conditioning of patients with myelofibrosis.[226]

Recommendations

Offering myeloablative allogeneic HSCT to patients below the age of 45 years and non-myeloablative/intensity-reduced for patients aged over 45 years seems reasonable.

What are the risk factors for HSCT in MFP, and which risk score should be used?

A set of 170 patients were evaluated with a median age of 51 who received HSCT related donors ($n = 86$) or unrelated ($n = 84$). Relapse, relapse-free survival, overall survival, and non-relapse mortality in 5 years for all patients were 10%, 57%, 57% and 34%, respectively. Among high-risk DIPSS patients, the risk of post-transplant mortality and non-relapse-related mortality were significantly higher when compared to low-risk patients. After a median follow-up of 5.9 years, median survival was not achieved for the low-risk and intermediate-1 groups. For the intermediate-2 and high-risk groups, it was 7 and 2.5 years, respectively.[89]

The Dupriez score, cytogenetic abnormalities, degree of medullary fibrosis were the critical risk factors for post-transplant mortality in another series. Patients conditioned with busulfan, plasma levels between 800 and 900 ng/mL, and cyclophosphamide had a higher probability of survival (76%) (1). The same result was observed in an additional study, where in the multivariate analysis, the use of BUCY (myeloablative, serum level-adjusted), high platelets at transplantation, younger patients and low comorbidities score were significant for better overall survival.[215]

According to the DIPSS score, the correlation between disease risk and benefit from HSCT, or conventional treatment, was assessed in another retrospective study of 438 patients with PMF, under 65 years, who received HSCT (190) or conventional therapy ($n = 248$). Among patients with low-risk DIPSS, the risk of death after HSCT versus conventional therapy was 5.6 (95% CI, 1.7–19; $p = 0.0051$); for those with intermediate-risk 1 was 1.6 (95% CI, 0.79–3.2; $p = 0.19$), for intermediate 2, 0.55 (95% CI, 0.36–0.83; $p = 0.005$), and high risk, 0.37 95% CI, 0.21–0.66, $p = 0.0007$). Thus, patients at intermediate-risk 2 and high-risk pointedly benefited from HSCT while low-risk patients should receive conventional therapies. Patients with intermediate-risk 1 should receive treatment on an individual basis.[227]

Another analysis, with 233 patients, sought to identify whether the DIPSS-plus score would better at predicting outcomes after HSCT. Multivariate analysis showed that each parameter incorporated in DIPSS-plus contributed to predicting mortality, relapse-free survival, and mortality not related to relapse. Overall 5-year survival, relapse, and transplant-related mortality (TRM) for low/intermediate-risk 1 patients were 78%, 5%, and 20%, respectively. An index of comorbidity

of 3 or more was associated with greater overall and non-relapse-related mortality, as well as less relapse-free survival. The incidence of relapse was higher in older patients.[228]

Recommendations

Although most studies have used either Dupriez or Cervantes risk classification, it is recommended to use the DIPSS-plus classification, which has incorporated the need for red blood cell transfusion, the presence of platelet counts $<100 \times 10^9\ L^{-1}$ and unfavorable karyotype. (C4)

Busulfan, in conditioning regimen, should be used in the intravenous or serum dosing formulation (2B).

PICO 15: Myeloproliferative neoplasms polycythemia vera prognostic system

Polycythemia Vera is an idiopathic chronic myeloproliferative disorder characterized by increased erythrocyte mass (erythrocytosis), with elevated HCT, posing a risk of thrombosis, progression to acute leukemia and myelofibrotic transformation. The goal of this guideline is to provide recommendations, which may assist in prognosis assessment of polycythemia vera patients. This investigation sought a systematic review of the literature, through PICO, where "P" addresses patients with polycythemia vera, "I" refers to indicator score, prognosis, and "O" refers to the outcome of survival, death, progression, leukemia, thrombosis, ischemia. The study had no period restriction, explored the Medline database, retrieving 1979 papers, of which 11 were selected to respond the clinical question. Details of the methodology and results of this guideline are set out in Annex VIII.

Introduction

Patients with polycythemia vera (PV) have survival rates lower than the average population. The most frequent causes of death in PV are thrombotic complications, hemorrhagic episodes, solid tumors appearance, and progression to acute myelofibrosis or leukemia.[229–232]

Results

What is the best prognostic system in polycythemia vera?

Regarding the specific risk of developing thromboembolic complications, comprehensive studies have shown that age above 65–70 years and previous history of thrombosis[230,233] (B), as well as leukocytosis[234–236] (B), were the main risk factors for that complication.

A study that followed two population cohorts, totaling 327 patients, showed relative survival rates (RSR) of 72% and 46% after 10 and 20 years of diagnosis, respectively. Multivariate analysis identified age above 70 years, leukocytes above 13,000 mm^{-3} and history of prior thromboembolism as independent risk factors. Patients with 0, 1 or 2–3 of these factors presented SRS at ten years of 84%, 59% and 26%, respectively (B).[231]

Another study followed 1545 patients diagnosed with PV based on the WHO diagnostic criteria. In addition to demonstrating a lower survival rate relative to the paired general population for sex and age, a multivariate analysis verified that advanced age, leukocytosis, previous venous thrombosis, and abnormal karyotype affected survival independently. Based on these findings, a prognostic model included the first three risk factors: age >67 years (5 points), age 57–66 years (2 points), leukocytes >15,000 mm^{-3} (1 point) and venous thrombosis (1 point). Hence, three risk groups stand as low (0 points), intermediate (1–2 points) and high (≥3 points) with median ST of 27.8 years, 18.9 years and 10.9 years, respectively (B).[232]

Leukocytosis was also an independent risk factor for leukemic and myelofibrotic transformation. Regarding the correlation between the use of hydroxyurea and increased risk of leukemic transformation, the follow-up studies with higher numbers of patients failed to demonstrate this association (B).[235] Transformation and lower survival rates also occurred in patients with PV with cytogenetic alterations, which are present in about 20% of the cases (B).[237,238] Further data obtained by genetic sequencing directed to genes of interest show that the presence of mutations in cooperative genes to the driver mutations confer lower survival free of leukemia, myelofibrosis and total (B).[239]

Recommendation

It is not possible to state which is the best prognostic system for PV due to the lack of comparison among the current scores. However, studies exploring that topic point that advancing age, especially above 57 to 70 years, previous history of thrombosis and leukocytosis are risk factors for both thromboembolic complications and increased mortality (B).

PICO 16: Myeloproliferative neoplasms – polycythemia vera target hematocrit

The present guideline brings the structured form of formulating the question synthesized by the acronym PICO, where "P" corresponds to patients with polycythemia vera, "I" refers to hematocrit level indicator and "O" stands for the outcomes morbidity, survival, thrombosis, and quality of life. After reflecting on the relevant clinical issues related to the proposed theme, and from the structured question, we identified the descriptors used as the basis for the evidence search within the Medline-Pubmed database. Following the scrutiny of the abstracts of each study, we applied the eligibility criteria (inclusion and exclusion), resulting in the selection of six papers to answer the clinical question (Annex IX).

Introduction

Polycythemia vera (PV) is a myeloproliferative neoplasm originate in a multipotent hematopoietic stem cell, causing proliferation and accumulation of red blood cells, white blood cells, platelets and their progenitors in the absence of an identifiable stimulus and to the exclusion of non-clonal hematopoiesis.[240] Patients with PV are at high risk of arterial

and venous thrombotic events. Cardiovascular events comprise the main cause of death in the first years after the diagnosis of this disease.[240,241] Therefore, the reduction of thrombotic risk is a central strategy in the management of patients with PV.

Reducing the risk of thrombosis requires two distinct but complementary strategies: hematocrit control (through therapeutic bleeding and/or cytoreductive agents) and platelet antiaggregation.[242–244] In this section, we will discuss reaching the hematocrit target, followed by the use of cytoreductive agents and then antiplatelet agents in patients with PV.

Results

Traditionally, patients with PV are recommended to steadily keep hematocrit below 45% and the platelet count below 400,000 per cubic millimeter (D).[240,242] The intensive management of these hematological variables has always been widely practiced, despite the lack of robust data in support of this recommendation (B).[245]

The CYTO-PV collaborative group published the phase III study that confirmed the benefits of intensive hematocrit control in 2014. The researchers assorted 365 adults with PV into two arms to receive either more intensive or less intensive treatment. The low-hematocrit group ($n = 182$) had a target hematocrit <45%, whereas high-hematocrit group ($n = 183$) had target hematocrit from 45 to 50%. All patients received phlebotomy, cytoreductive drugs, or both (B).[245] The groups had a median follow-up of 28.9 ± 10.9 months. The mean hematocrit (±SD) at baseline was similar in the groups ($47.2 \pm 5.1\%$ versus $47.5 \pm 4.4\%$). During the investigation period, the median hematocrit level maintained in the low-hematocrit group was 44.4%, compared to 47.5% in the high-hematocrit group. The white cell count remained significantly higher in the high hematocrit group than in the low hematocrit group ($p < 0.001$) (B).[245]

The groups had a median follow up of 28.9 ± 10.9 months. The mean hematocrit (±SD) at baseline was similar in the groups ($47.2 \pm 5.1\%$ versus $47.5 \pm 4.4\%$). Throughout the investigation period, the median hematocrit level maintained in the low-hematocrit group was 44.4% in contrast with 47.5% in the high-hematocrit group. The white cell count remained significantly higher in the high hematocrit group than in the low hematocrit group ($p < 0.001$). No significant difference was noted in the platelet count between the groups[245] (B) he incidence of death from cardiovascular causes or thrombosis was 1.1 per 100 person-years in the low hematocrit group and 4.4 per 100 person-years in the high hematocrit group. The total cardiovascular events occurred in 4.4% of the patients in the low hematocrit group and 10.9% in the high hematocrit group (HR: 2.69, 95% CI: 1.19–6.12, $p = 0.02$) (B).[245]

A further study, which combined the data from the CYTO-PV and MPN-10 trials studied the symptomatic burden of PV patients treated between the two arms. The results were analyzed to evaluate if possible changes in the symptoms burden (MPN-10 questionnaire) were observed when controlling for HCT levels. The mean MPN-10 score in the CYTO-PV cohort for the 6-month follow-up was 18, 4/100 (SD 16.6) for those with a target hematocrit <45% and 17.4/100 (SD 15.1) for those with a hematocrit between 45 and 50%. Correspondingly, the mean MPN-10 sum score for the 12-month follow-up was 13.3 (SD 12.6) for those with a target hematocrit <45% and 15.7 (SD 16.2) for those with a hematocrit between 45 and 50%. In the MPN-10 cohort individuals who met the criteria of a MPN-10 individual item score of greater than 5/10 had a significantly lower mean hematocrit (HCT = 44.9%) than those who did not meet this cutoff (HCT = 46.7%) ($p = 0.0376$). A second cutoff of a MPN-10 total score of greater than or equal to 20 was analyzed; however, this cutoff did not meet statistical significance (B).[246]

Recommendation

For patients with polycythemia vera, managing a hematocrit target lower than 45% compared to a target of 45–50% was associated with a significantly lower rate of cardiovascular death and thrombosis, without increased severe complications of treatment. Maintaining a target hematocrit of less than 45% compared to a 45–50% target may be also associated with improvement in the symptoms of patients with polycythemia vera; however, patients with a high symptom burden may have a greater benefit from other therapies. Thus, the target hematocrit for all patients with polycythemia vera should be less than 45%.

PICO 17: Myeloproliferative neoplasms – polycythemia vera – platelet antiaggregation

We adopted the structured way of formulating the question synthesized by the acronym PICO, where the "P" stands for patients with polycythemia vera, "I" stands for intervention with AAS, clopidogrel, Ticlopidine, and "O" stands for the morbidity and survival outcomes. Through the elaboration of relevant clinical issues and related to the proposed theme, from the structured question, we identified the descriptors that were the basis of the search for evidence in the databases: Medline-Pubmed, Cochrane library, of these. Thus, the studies had their abstracts reviewed and after the eligibility criteria (inclusion and exclusion), eight papers were selected to answer the clinical questions (Annex X).

Introduction

Thrombotic complications are the main cause of morbidity and mortality in polycythemia vera (PV), occurring in more than a third of patients and causing 35–45% of deaths.[247–251]

The antiplatelet agents are inhibitors of thrombus formation, without interfering significantly in the other segments of the coagulation. They promote inhibition of platelet functions such as adhesiveness and aggregation, inhibit the platelet release or secretion reaction, reduce circulating platelet aggregates, and inhibit platelet-induced thrombus formation.[252]

Antiplatelet agents hold a well-established role in preventing arterial thrombosis, and the assembled evidence infers that those drugs may be efficacious in preventing venous thromboembolism in high-risk patients.[247]

Several molecules that inhibit platelet aggregation are currently available in clinical practice, including aspirin, dipyridamole and the former (ticlopidine, clopidogrel) and

new (prasugrel, ticagrelor) thienopyridines. Aspirin has an antiplatelet effect inhibiting the production of thromboxane, the thienopyridines act by the inhibition of adenosine diphosphate (ADP) receptor inhibitors/inhibitors of P2Y12, and dipyridamole by inhibition of thromboxane production and by inhibition of phosphodiesterase enzymes that typically break down cyclic adenosine monophosphate (cAMP). In addition to increased risk of bleeding, the primary adverse events reported include the following: for aspirin, gastric ulcer, and allergic reactions; for thienopyridine are neutropenia, thrombotic thrombocytopenic purpura and dyspnea (ticagrelor); and for dipyridamole, headache and dizziness.[252]

Results

The benefit of using platelet antiaggregation with aspirin was evaluated in the randomized phase III study published by ECLAP (European Collaboration on Low-Dose Aspirin in Polycythemia Vera) in 2004.[247] In this study, 518 patients diagnosed with PV were randomized to aspirin at low doses (100 mg per day) or placebo. The use of aspirin in low doses reduced the risk of the combined primary outcome of acute myocardial infarction, stroke, pulmonary embolism, deep vein thrombosis or death from cardiovascular causes by 60% (95% CI: 9–82% $p = 0.03$). There was a significant reduction in the rate of nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism, deep venous thrombosis, or death from any cause (relative risk reduction, 53% percent, 95% CI, ($p = 0.049$) and reductions in the rates of minor thrombosis and any thrombosis, with relative risk reductions of 53% ($p = 0.049$) and 58% ($p = 0.003$), respectively. Accordingly, the rates of major strokes, non-hemorrhagic stroke, transient ischemic attack, peripheral thrombosis, deep venous thrombosis and pulmonary embolism in the aspirin group were not significantly different from the rates of these complications in the placebo group. In the fully adjusted model, the rate of nonfatal myocardial infarction, nonfatal stroke or death from cardiovascular causes was reduced by 77% ($p = 0.02$) and the rate of nonfatal myocardial infarction, stroke cerebral infarction, pulmonary embolism, deep venous thrombosis or death from cardiovascular causes was reduced by 71% ($p = 0.008$) (B).[247]

From the statistical point of view, there was a non-significant increase in the incidence of hemorrhagic phenomena in the group of patients taking aspirin, with a relative risk for developing any bleeding of 1.82 (95% CI: 0.94–3.53, $p = 0.08$). Almost the entire incidence of bleeding was due to an 83% increase in the rate of minor bleeding episodes. Side effects were reported as the reason for discontinuation of therapy in 7.1% of patients in the aspirin group and 6.4% in the placebo group (gastrointestinal intolerance (2.8% and 4.5% respectively) and bleeding (4.4% and 1.5%, respectively) (B).[247]

Evidence from a systematic review and meta-analysis of low dose use including 630 patients with myeloproliferative neoplasms suggests that in patients with polycythemia vera and no clear indication or contraindication to aspirin therapy, low-dose aspirin compared to placebo may reduce the risk of fatal thrombotic events (Odds Ratio (OR) 0.20, 95% CI: 0.03–1.14, $p = 0.07$). Data on mortality from bleeding episodes were not provided. Statistically, the use of aspirin in doses has a non-significant benefit in mortality (OR 0.4, 95% CI: 0.21–1.01, $p = 0.05$). There is no increased risk of severe bleeding events (OR 0.99, 95% CI: 0.23–4.36, $p = 0.99$). There is a non-significant increase in the risk of low-OR bleeding episodes OR 1.85, 95% CI: 0.90–3.79, $p = 0.09$) (A).[248]

Recommendation

We recommend low dose of ASA (100 mg/d) for all patients with PV without contraindications to this treatment, in order to prevent thrombotic complications. In patients allergic to acetylsalicylic acid, we suggest another antiplatelet agent such as Clopidogrel.

PICO 18: Myeloproliferative neoplasms – polycythemia vera – cytoreductive agents

The goal of this guideline is to evaluate the role of the cytoreductive agents in the treatment of polycythemia vera. We used the structured form of formulating the question through the PICO acronym, where "P" stands for patients with polycythemia vera, "I" stands for intervention with cytoreductive agents (hydroxyurea, busulfan, interferon ruxolitinib, JAK inhibitors, phlebotomy, anagrelide) and "O" stands for outcomes morbidity, survival quality of life. The search within the MedLine database had no restriction period, and then we selected 20 studies to answer the clinical question. The details of the methodology and the results of this guideline show in Annex XI.

Introduction

In order to reduce the risk of thrombosis in polycythemia vera (PV), two distinct but complementary strategies should be used: hematocrit control (which can be done through therapeutic bleeding and/or cytoreductive agents) and platelet antiaggregation.[241] In this section, we will discuss the use of cytoreductive agents in patients with PV.

Results

The use of cytoreductive agents in PV

Among the cytoreductive agents used in PV, hydroxyurea stands out as one of the most widely used drugs in clinical practice, as well as other efficacy medications such as busulfan, interferon-alpha and the inhibitor of JAK2 ruxolitinib. Cytoreductive therapy in patients with PV is the frequent option of choice for phlebotomy-intolerant patients or also for those with high-risk diseases. The primary goal of cytoreductive therapy is to reduce the risk of thrombotic phenomena. Secondary goals include symptom control, reduced visceromegaly, decreased risk of PV progression to acute myeloid leukemia or myelofibrosis, and improved overall survival. Scarce high-quality studies delve into those outcomes in patients with PV.

Regarding hydroxyurea, evidence regarding treatment efficacy derives from assays conducted by the

PVSG – Polycythemia Vera Study Group and FPSG – French Polycythemia Study Group[241,253] (B)[254] (C) groups.

A PSVG phase II study, published in 1997, analyzed 51 patients treated with hydroxyurea with PV diagnosis. Results showed that in comparison to 134 historical controls, patients treated with hydroxyurea had a lower incidence of thrombotic events (10% vs. 33%) (D).[255]

In the randomized phase III study conducted by FPSG, 285 patients were randomized between hydroxyurea therapy (initial dose of 25 mg/kg/day followed by a maintenance dose of 10–15 mg/kg/day) or pipobroman (not available in Brazil) with an initial dose of 1.25 mg/kg/day, followed by maintenance at a dose of 0.4–0.7 mg/kg/day. After a 17-year median follow-up, the median overall survival was 20.3 years for patients treated with hydroxyurea compared to 15.4 years for patients treated with pipobroman ($p = 0.008$). The cumulative incidence of acute myelogenous leukemia with 10, 15 and 20 years of treatment was 6.6%, 16.5% and 24% in the hydroxyurea arm, compared to 13%, 34% and 52% in the pipobroman arm ($p = 0.004$). Additionally, the evolution for myelofibrosis in the hydroxyurea arm was 15%, 24%, and 32%, compared to 5%, 10% and 21% in the pipobroman arm, respectively ($p = 0.02$). The cumulative incidence of thrombotic and hemorrhagic cardiovascular events was similar in both groups (approximately 40% at 20 years of follow-up, $p = 0.61$) (B).[241,253]

Thus, these two studies demonstrate the efficacy of hydroxyurea as a cytoreductive agent for hematocrit control and reduction of thrombotic risk in patients with PV.

However, the potential for oncogenicity and hematological and non-hematological toxicities related to prolonged exposure to hydroxyurea are grounds for questioning and concern (B).[256]

Therefore, the use of interferon is a recurrent alternative for patients under 40 years of age, or with intolerance/resistance to hydroxyurea. In phase II studies, interferon demonstrated clinical efficacy, evidenced a reduction in hematocrit values, platelet count and visceromegaly reduction, and with no hemorrhagic event reported, (B).[164,257,258] A reduction in the JAK2 V617F mutation load has also appeared, with variable values (B).[259,260] Despite its effectiveness, interferon often leads to adverse events and a significant discontinuation rate, thus limiting its use (B).[261]

The pegylated formulation of Interferon presents greater dosage convenience, being applied only once a week and greater tolerability. In a study including 83 patients with polycythemia vera ($n = 43$) and essential thrombocythemia ($n = 40$) who received subcutaneous pegylated interferon alfa-2a once a week, the response rate in the 43 patients with PV was 79%, with a mean duration of 65 months (IQR 43–87 months). In addition, 33 patients with PV (77% of the cohort) achieved complete response, with normalization of all hematological parameters, with a 65-month median duration (33–82 months). There was also a significant reduction of the allelic charge of the JAK2 V617F mutation. About 22 patients (63%) presented some molecular response, with a complete response in seven cases (20%), partial in 14 cases (40%), and minimal in one case (3%). The incidence of thrombotic events was 1.22 cases/person-years of follow-up. Approximately 22% of patients discontinued treatment due to toxicity, and although the incidence of side effects declines over time, some patients have significant side effects even after five years of treatment. There was no difference in the incidence of evolution for AML/MF when compared to a historical cohort of patients (B).[262]

There are ongoing, randomized phase III studies designed to assess the safety, toxicity, and tolerability of pegylated interferon alfa-2b versus hydroxyurea (B).[263] There is also another ongoing study on the use of mono-pegylated interferon alfa-2b as compared to hydroxyurea (B).[264] Both studies included patients with PV that were not yet treated or on treatment with hydroxyurea.

The therapy with the alkylating agent busulfan is available for patients with PV as the first line or for those who are resistant/intolerant to hydroxyurea, particularly for those with low life expectancy. One study evaluated 15 patients with PV and 21 patients with ET. At the time of analysis, 27 patients discontinued busulfan; 18 for having reached complete hematologic response (RHC), eight due to hematological toxicity, and one for presenting acute leukemia. Seven patients were already on RHC before starting treatment. The likelihood of RHC at one year of busulfan treatment was 99% higher in patients receiving the initial dose >14 mg/week and 59% in those receiving <14 mg/week. An analysis of PV patients only showed that four out of five high-hematocrit patients achieved a response after a 85-day median (range 35–532), with a probability of maintaining the response without phlebotomies at one and two years of 77% and 64%, respectively (B).[265]

In 2009, the European LeukemiaNet consortium defined the criteria to identify and classify patients with PV who were resistant and/or intolerant to the use of hydroxyurea[266] (D) – Table 12.

The intolerance/resistance to hydroxyurea is of great clinical importance. The frequency and prognostic value of these criteria in PV patients was reported in a cohort of 890 patients. The presence of resistance/intolerance to hydroxyurea was documented in 15.4% of these patients, and the development of cytopenias was associated with an increased risk of death

Table 12 – Definition of resistance/intolerance to Hydroxycarbamide in patients with Polycythemia Vera.

1. Need for phlebotomy to maintain hematocrit <45% after three months of at least 2 g/day of Hydroxycarbamide, or
2. Uncontrolled myeloproliferation (platelet count >400 × 10^9 L^{-1} and leukocytosis >10 × 10^9 L^{-1}, after 3 months of at least 2 g/day of Hydroxycarbamide, or
3. Failure to reduce massive splenomegaly (>10 cm RCE) by 50%, assessed by palpation, or fail to completely relieve symptoms related to splenomegaly, after three months of at least 2 g day of Hydroxycarbamide, or
4. Neutrophil count <1.0 × 10^9 L^{-1} or platelet count <100 × 10^9 L^{-1} or hemoglobin <100 g/L at the lowest dose of hydroxycarbamide required to achieve a complete or partial clinical-hematological response,[a] or
5. Presence of leg ulcers or toxicities related to hydroxycarbamide such as mucocutaneous manifestations, gastrointestinal symptoms, pneumonitis or fever at any dose of the medication.

[a] *Complete* response defined as hematocrit <45% without need for phlebotomy, platelet count <400 × 10^9 L^{-1}, leukocyte count ≤10 × 10^9 L^{-1}, and no disease-related symptoms. *Partial* response defined as hematocrit <45% without the need for phlebotomies or response in three or more of the other criteria.

(HR – 3.5, $p = 0.003$) and a higher risk of progression to AML (HR 20.3, $p = 0.001$). The evolution for myelofibrosis was also higher in this group of patients with cytopenias (HR 5.1, $p = 0.001$), as well as in those with massive splenomegaly (HR 9.1, $p = 0.002$) (B).[267] Thus, early identification and adequate conduction of intolerance/resistance are essential.

The development of the inhibitor of JAK2 ruxolitinib was a new therapeutic option for patients diagnosed with resistance/intolerance to hydroxyurea. A phase II study of 34 patients intolerant or resistant to hydroxyurea tested the doses of 10 mg twice a day, 20 mg twice daily and 50 mg once daily. The results revealed a symptomatic improvement in the first four weeks. In 97% of the cases, the hematocrit fell to less than 45% without phlebotomy, and 63% of the patients with palpable spleen became non-palpable at week 144, and 59% of patients had complete remission. The occurrence of hematological adverse events ≥CTCA and grade 3 occurred in 9% of the cases and received a dose adjustment. From this study, the initial dose of 10 mg twice daily of Ruxolitinib was determined for PV patients resistant or intolerant to hydroxyurea (B).[268]

A phase III clinical trial (RESPONSE trial) investigated the use of ruxolitinib 10 mg twice daily compared to the best available therapy (BAT), which comprised hydroxyurea, conventional or pegylated interferon, thalidomide, lenalidomide, anagrelide, phlebotomies, observation, among others (B).[269] Twenty-two PV patients resistant or intolerant to hydroxyurea, who were phlebotomy dependent and with splenomegaly, were randomized. The primary composite endpoint comprised the hematocrit maintenance below 45% and spleen reduction by 35% (measured by magnetic resonance imaging) in the 1:1 ratio. The primary composite endpoint was reached in 21% of patients in the ruxolitinib arm versus 1% in the BAT arm ($p < 0.001$). Complete hematologic remission was achieved in 24% of patients with ruxolitinib versus 9% in the BAT setting ($p = 0.003$). Spleen reduction occurred in 38.2% versus 0.9%, and hematocrit control occurred in 60% versus 19.6% of patients with ruxolitinib versus BAT, respectively. During the review of MRI data for week 80, two additional patients randomized to ruxolitinib appeared as primary responders, which raised the total number of primary responders to 25 (22.7%). About 6% of Ruxolitinib patients developed Herpes Zoster, compared with 0% in comparator arm (B).[269]

In the RESPONSE trial, significantly higher proportion of patients in the ruxolitinib group had improved quality of life and symptomatology indexes when evaluated by the European Organization for Research and Treatment of Cancer Questionnaire-Core 30 (EORTC QLQ-C30), the Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF), the Pruritus Symptom Impact Scale (PSI), and the Patient Global Impression of Change (PGIC) (B).[270] Treatment with ruxolitinib was associated with improvements in all five PSIS components quickly and durably, and treatment with standard therapy led to worsening or minimal improvements in all PSIS components. This benefit was evident even in the presence of iron deficiency (B).[271] The probability of maintaining the long-term response in the arm of ruxolitinib was 87%, in an 80-week analysis (B).[272]

The phase III RESPONSE 2 study evaluated 173 PV patients who were intolerant or resistant to hydroxyurea, phlebotomy dependent, but now without splenomegaly. Patients were randomized into two arms, 1:1, for the BAT setting (hydroxyurea, interferon alfa-2, pegylated interferon, anagrelide, immunomodulators, among others), versus ruxolitinib 10 mg orally twice daily. The primary endpoint was hematocrit control until week 28. Hematocrit control achieved 16% of patients in the BAT arm, versus 62% in the ruxolitinib arm (OR: 7.28 [95% CI: 3.43–15.45]; $p < 0.0001$). No CTCA and grade three or four hematological events occurredin the ruxolitinib group, versus 4% of patients in the BAT group (B).[273]

About 3% of patients with ruxolitinib had angina pectoris. However, there was no significant difference concerning any serious adverse events between the two groups (B).[273] At week 80, 93% of patients in the arm of Ruxolitinib continued with medication, while in the BAT arm 77% of patients had already migrated to the Ruxolitinib arm, with no patient remaining on the BAT arm. The probability of maintaining the hematocrit response was 78% in this analysis (B).[273]

Recommendation

Hydroxyurea, busulfan, interferon (conventional or pegylated), and ruxolitinib are associated with higher rates of hematologic remission, reduction in spleen volume and, in some patients, with molecular remission.

Currently, resistance and intolerance to hydroxyurea it is a well-defined criterion that must be applied to all patients diagnosed with PV who are using the medication. This situation has a direct correlation with leukemia and myelofibrosis transformation ratios and with thromboembolic complications.

Pegylated interferon alfa-2a is a viable treatment option, especially for younger patients who wish to avoid prolonged cytotoxic therapy, or in those patients who are hydroxyurea intolerant or resistant. Lower doses minimize side effects while maintaining efficacy, suggesting an initial dose of 45 μg weekly to limit adverse events and maximize response. A more rigorous positioning on the usefulness and safety of interferons in the therapeutic arsenal of PV should await results of phase III studies that are underway.

Busulfan is a viable option for older patients with low life expectancy.

PV patients hydroxyurea intolerant or resistant treated who were treated with ruxolitinib achieve satisfactory responses for hematocrit control, spleen volume reduction and complete hematologic remission, and it is the only medication which was studied in phase III trials for this situation so far. Treatment with ruxolitinib for up to 4 years proved to be efficacious and safe.

PICO 19: Myeloproliferative neoplasms – essential thrombocythemia – prognosis mutations

The aim of this guideline is to evaluate the role of mutations in the prognosis of essential thrombocythemia, a chronic myeloproliferative neoplasm. To this end, we used the structured method of formulating the question through the acronym PICO, where “P” stands for patients with essential thrombocythemia, “I” stands for the mutations indicator, and “O”

stands for prognostic outcomes. Through the elaboration of relevant clinical propositions related to the proposed theme, we identified the pivotal descriptors of the search evidence within the MedLine-Pubmed database. We then proceeded to the scrutiny of the abstracts of the retrieved papers and, following the eligibility criteria (inclusion and exclusion), we selected 28 papers to answer the clinical question (Annex XII).

Introduction

Essential thrombocythemia is a chronic myeloproliferative neoplasm with relatively benign behavior, although its clinical course is characterized by the occurrence of microvascular disorders and/or thrombotic/hemorrhagic complications.[72] During the first two decades of the disease, 10% of patients may present transformation to acute leukemia, to myelofibrosis, or to myelodysplastic syndrome (B).[84,85,105,274]

Results

The V617F mutation in the JAK2 gene occurs in 23% to 75% of cases of essential thrombocythemia, but the impact of this mutation on the phenotype is the subject of much debate. Higher hemoglobin levels, higher leukocyte counts, and lower platelet count[275–278] (B) characterize patients with the V617F mutation. Mutations of the calreticulin region (CALR) arise in about 67% of the cases, with the mutations CALR, JAK2 and MPL being mutually exclusive (B).[12] Despite the controversial results, studies in the JAK-2 positive cases indicate a greater progression to leukemic transformation, myelofibrosis, as well as a higher risk of progression to polycythemia vera (B).[51,274,279] A longitudinal observational study analyzed clinical and prognostic aspects of patients with a diagnosis of essential thrombocythemia (B).[279] Accordingly, carriers of the JAK2 V617F mutation were more likely to become polycythemia vera, but not to acute myeloid leukemia or myelofibrosis (B).[279] In the multivariate analysis, the presence of the JAK2 V617F mutation did not represent an independent factor for the reduction of survival (B).[279]

Another study, including 105 patients with ET, displayed an evolution trend to myelofibrosis when the allelic load of mutated JAK2 was >50% ($p = 0.09$), but not for AML (B).[105]

Recent studies with New Generation Sequencing have shown new genes involved in the evolution to MF or leukemic transformation. Transformation to myelofibrosis is prevalent in patients with additional mutations in SF3B1, IDH1/2, and with the increased allelic loading of JAK2. Additional mutations in genes like DNMT3A, SRSF2, SF3B1, IDH1/2 and RUNX1 are related to a worse prognosis (B).[280] Survival is higher in cases with mutated CALR, which presents a lower risk of transformation to AML/MDS. Mutations of JAK-2, MPL, and triple negative, besides evolution for MF, are independent factors for leukemic transformation (B).[281]

Patients with ET with a higher allelic load of mutated CALR have a more proliferative phenotype, with lower levels of Hb, higher LDH and a higher rate of myelofibrotic transformation, as well as greater splenomegaly (B).[84]

Thromboembolic events also yielded conflicting results. Several clinical evidence suggests an association of the JAK2 V617F mutation with thrombotic events[275,282,283] (B) (A).[284]

A meta-analysis showed a positive correlation between the presence of the JAK2 V617F mutation and a higher risk of both arterial and venous thrombosis (OR = 1.68 with 95% CI: 1.31–2.15 and OR = 2.5 with 95% CI: 1.71–3.66) respectively[279,285] (B) (A).[284]

Fu et al. assessed the impact of CALR, MPL and JAK2 mutations on the risk of thrombosis (B).[282] Results reveal that, out of a total of 105 thrombotic events reported in 95 patients, most individuals (75%) had the JAK2 V617F mutation compared to those with the mutation in the calreticulin gene and patients considered to be triple negative 17 (B). Patients who were positive for the mutation in the calreticulin gene had more favorable thrombosis-free survival than the patients who were positive for the JAK2 V617F[282] (B) mutation.

In a recent study on the risk of thrombosis and hemorrhage in patients with MPN, patients with mutated JAK2 with an allelic load >75% had a higher incidence of thrombosis and hemorrhage (B).[286]

Conversely, additional retrospective baseline studies have not established an association between JAK2 V617F mutation and thromboembolic events (B).[276,287,288]

In a study that evaluated risk factors associated with thromboembolic events in 532 patients with a diagnosis of essential thrombocythemia, the authors did not verify the association of the V617F mutation in the JAK2 gene during the follow-up period of 7.6 years (mean) and risk of thromboembolism or even reduction in overall survival (B).[287]

Within the Swedish population register (2008–2015), out of the 1284 patients with ET who were evaluated, and 35% had vascular complications. Factors considered at risk for thromboembolic events at diagnosis included age >65 years, leukocytes $>12 \times 10^9\,L^{-1}$, and presence of the JAK2 V617F mutation (B).[289]

The disparity toward thrombotic events and the JAK2 V617F mutation found in the literature may be explained by the retrospective nature of most of the studies performed, as well as by the small number of patients included in these series. Notably, advanced age and prior history of thrombotic episodes are well-established risk factors for the occurrence of thrombosis in patients with myeloproliferative neoplasms.

In a prospective cohort of patients with a diagnosis of essential thrombocythemia, advanced age, leukocytosis, smoking, and the presence of diabetes mellitus were found to be predictors of worse survival (B).[85]

Studies on the contribution of inherited and thrombotic prothrombotic factors to thrombosis in patients with myeloproliferative neoplasms provided few significant data. A historical cohort study involving patients diagnosed with polycythemia vera and essential thrombocythemia showed a significant difference in the prevalence of Leiden's Factor V in patients with and without a history of thrombotic events (B).[290] Furthermore, a retrospective observational study showed that, among the factors analyzed (Factor V Leiden, G20210A mutation in the prothrombin gene and C677T mutation in the methylenetetrahydrofolate reductase gene), only Factor V of Leiden was the most significant contributor to

occurrence of thromboembolic events, both in patients with essential thrombocythemia and in those with a diagnosis of polycythemia vera (B).[291]

Recommendation

Among the Patients with ET, those with mutated CALR are younger, predominantly males, and have a higher platelet count and lower levels of Hb and leukocytes, as well as a lower risk of thrombosis comparing those with the mutated JAK2. The presence of the JAK2 V617F mutation appears to be associated with a higher risk of thrombosis and evolution to myelofibrosis and leukemia transformation in contrast to patients with a CALR mutation. Risk factors for thrombosis include age, history of prior thrombosis, mutation of JAK2, leukocytosis $>11 \times 10^9\,L^{-1}$ and cardiovascular risk factors.

PICO 20: Myeloproliferative neoplasms – essential thrombocythemia therapeutics

The treatment of essential thrombocythemia (ET) aims at preventing thrombotic and hemorrhagic complications and relieving vasomotor symptoms. Thus, this guideline intends to provide recommendations that may help in choosing the best therapeutic option for essential thrombocythemia. This guideline made use of the systematic review of the literature, through the PICO acronym, where "P" stands for patients with essential thrombocythemia, "I" stands for intervention with cytoreductive treatment, anagrelide, hydroxyurea, interferon, JAK inhibitors, platelet antiaggregants, and "O" stands for survival, morbidity, quality of life outcomes. We performed the search in the Medline database without period restriction, and retrieved 80 papers, out of which we selected 18 articles to answer the clinical question. Details of the methodology and results of this guideline appear in Annex XIII.

Introduction

The purpose of essential thrombocythemia (ET) treatment is to prevent thrombotic and hemorrhagic complications and relieve vasomotor symptoms (headaches, dizziness, visual disturbances, burning dysesthesia). The available treatment options are non-curative, having failed to demonstrate the ability to prevent transformation to AML or post-ET myelofibrosis.

The definition of the need to initiate treatment for a patient with ET requires the evaluation of the following items:

- History of prior venous or arterial thrombosis, spontaneous abortion, and hemorrhagic complications;
- Presence and intensity of vasomotor symptoms;
- Presence of cardiovascular risk factors, including Hypertension, Diabetes, Smoking, and Dyslipidemia;
- Mutational status of the JAK2 gene and calculation of prognostic scores, such as IPSET-thrombosis[292] and modified IPSET-thrombosis.[293]

Results

Cytoreductive therapy

The mainstay of treatment is the use of a cytoreductive agent (such as Hydroxyurea (HU), Anagrelide or Interferon), usually associated with a low dose of aspirin (B).[294]

Indications for cytoreductive treatment include:

- Previous history of thrombosis
- Age over 60 years
- Acquired von Willebrand disease

Treatment target

Although no study has compared different targets for platelet counts, the major clinical studies on cytoreduction aimed at maintaining platelets in the normal range (150,000 to 450,000/mm^3) or close to normal (450,000 to 600,000/mm^3 (B).[295–297]

Anagrelide vs. hydroxyurea

A meta-analysis including two randomized controlled trials (RCTs)[296,297] (B) compared Anagrelide with Hydroxyurea in reducing the rate of thrombosis, bleeding, and death among patients with ET.

The overall rates of thrombosis were not different between the two groups (RR 0.86, 95% CI 0.64–1.16). However, there was a significant difference between arterial thrombosis (AT) and venous thrombosis (VT) events (RR 0.64 95% CI 0.45–0.90 and RR 2.67, CI 1.26–6.11, respectively), favoring HU for reducing ATs and Anagrelide for reducingVTs (B).[296,297]

The incidence of severe bleeding was lower in patients treated with HU (RR 0.37, 0.18–0.75). Low-risk bleeding rate, however, was not different between groups (RR 0.82, 0.59–1.15), although there was a trend towards lower frequency in patients treated with HU (B).[296,297]

The risk of transformation to AML was similar in both groups (RR 1.50, 0.43–5.29), whereas the transformation to Myelofibrosis was less frequent in patients receiving HU (RR 0.33, 0.13–0.83). There was no significant difference in mortality rates in both groups (B).[296,297]

In one of the two randomized studies, the frequency of serious adverse events was more common in the Anagrelide group, leading to definitive discontinuation in almost double of the cases (148 vs. 79 patients) (B).[297]

Interferon

Interferon alfa (IFN-α), in the conventional or pegylated form, can reduce thrombocytosis and reduce the risk of thrombotic complications in ET, at the expense of greater toxicity potential, parenteral need, and higher cost. Unlike Anagrelide and HU, it can be used safely in pegnancies (B).[167,298] In most cases, the response is achieved within the first three months of treatment.

Cytoreductive drugs dosing:

- Hydroxyurea: Start with 15 mg/kg/day and adjust for obtaining platelet control
- Anagrelide: 0.5–1 mg twice daily (maximum unitary dose = 2.5 mg, max. daily dose = 10 mg)
- Interferon alfa: 3–4 MUI/day Sub-Q
- Peg-Interferon: 90 ug/week Sub-Q

JAK inhibitors

Ruxolitinib was tested in a phase-2 randomized trial for patients with HU failure or intolerance. Compared to BAT, ruxolitinib did not show improvement in response rates. Thus, despite relative symptom improvement, the drug did not reduce the rates of thrombosis, hemorrhages or transformation (B).[299]

Toxicity of cytoreductive treatments

Prolonged treatment with cytoreductive agents may be followed by side effects, often leading to dose reduction, with potential impact on efficacy. Since HU is the most frequently used cytoreductive therapy, the criteria for defining intolerance or resistance to this drug in ET have been defined (B).[300] As a method to overcome failure or toxicity with monotherapy, combined treatment with two drugs, HU + Anagrelide or HU + IFN can be considered as a practical option for the management of selected patients (B).[301]

Antiplatelet agents for the prophylaxis of thromboses and control of vasomotor symptoms

In a systematic review on the use of antiaggregants (APT) in PV or ET, no RCT included patients with ET12 (A).[249]

A retrospective study evaluated 300 patients with classical low-risk ET (age < 60 years and no previous history of thrombosis), of whom 198 received APT monotherapy, and 102 were kept under observation. The rates of thrombotic events were 21.2 and 17.7 per 1000 patients/year, respectively ($p = 0.6$). However, JAK2 V617F positive patients not receiving APT showed an increased risk of VT (incidence rate ratio ([IRR]: 4.0; 95% CI: 1.2–12.9; $p = 0.02$) and those with cardiovascular risk factors showed IRR: 2.5; (95% CI: 1.02–6.1; $p = 0.047$). Increased risk of major bleeding was observed in patients with platelets above 1,000,000/mm^3 receiving APT (B).[302]

APTs are effective in controlling vasomotor symptoms associated with ET (B).[303]

- Aspirin Dose: 81–100 mg/d;
- When there is no control of vasomotor symptoms, the dose frequency can be increased to 81–100 mg bid (B).[304]

Cytoreductive therapy + antiplatelet vs. isolated cytoreductive therapy

In an observational study, 247 patients with high-risk ET were evaluated. The indication of cytoreduction included: age > 60 years, extreme thrombocytosis, microvascular symptoms not responsive to APTs. First-line cytoreductive therapy included: HU ($n = 215$), Anagrelide ($n = 27$), IFN ($n = 4$), and Busulfan ($n = 1$). The follow-up was 763 patients/year for cytoreduction + low dose aspirin, and 685 patients/year for isolated cytoreduction. The rate of thrombosis was not significantly reduced in the cytoreductive therapy with the association of aspirin (14.4 events per 1000 person-years) compared to isolated cytoreductive therapy (24.8 events per 1000 person-years; $p = 0.2$). However, in the subgroup of patients aged > 60 years, the addition of low-dose aspirin was associated with a significantly lower rate of thrombosis (8.6 vs. 29.2 events per 1000 patient-years for combined and cytoreductive treatment alone, respectively, $p = 0.02$). The major bleeding rate was significantly higher with combined therapy versus isolated therapy (14.4 vs. 1.4 bleeding events per 1000 patient-years respectively, $p = 0.006$) (B).[305]

Therefore, low-dose aspirin therapy benefits patients with high-risk ET, aged > 60 years receiving primary cytoreductive therapy, although there is an increased risk of major bleeding events (B).[306]

Antiplatelet drugs and anticoagulants as secondary prophylaxis

Patients with PV and ET and history of thrombosis present the highest risk of presenting new thrombotic episodes, with an estimated incidence of 7.6 thrombotic events per 100 patient-years.[306] There are no RCTs in patients with ET which have explored the role of antiplatelet agents in the secondary prevention of thrombosis.

A retrospective study with 494 patients (235 with PV and 259 with ET) with a prior history of ATs (67.6%), VTs (31%) or on both territories (1.4%) showed that cytoreduction reduced the recurrences. Significant prevention of rethrombosis in VT subjects was obtained with an oral anticoagulant (OAC) (HR 0.32, 95% CI 0.15–0.64) and antiplatelet therapy (APT) (HR 0.42 C 95% 0.22–0.77). The authors conclude that contemporary use of OAC after VT and APT after AT enhances the protective effect of cytoreduction, and that prospective studies are needed to determine whether strategies based on the affected territory can improve secondary prophylaxis (B).[306]

In another study, 150 patients (79 with PV and 71 with ET) who had at least 1 episode of thrombosis were treated with coumarin (AVK) and followed for a period of 4.3–13.9 years (mean of 7.7). Thirty patients died during follow-up. Among the 25 patients who had the cause of death determined, six patients had thrombotic complications as the cause. 42 patients (28%) had a total of 58 recurrent thrombotic events (36 venous and 22 arterial), at an incidence of 6.0 per 100 patient-years. There was a significant correlation between the vascular territory involved in initial thrombosis and recurrences. Among patients with initial arterial thrombosis, the recurrent event also involved the arterial tree in 86% ($p = 0.004$). Patients with initial venous thrombosis had recurrence involving the same territory in 79% of the cases ($p = 0.012$). After an observational period of 963 patient-years, the incidence of re-thrombosis was 4.5 and 12 per 100 patient-years during AVK therapy and after the end of therapy respectively ($p < 0.0005$). Arterial and venous thrombotic

events were significantly reduced with the use of AVK, without a significant increase in the risk of major bleeding (B).[307]

Special situations

Pregnancy

Hydroxyurea and Anagrelide are potentially mutagenic and should be avoided in pregnancy. Pregnant women requiring cytoreduction should be treated with interferon-alpha or, in emergency situations, with apheresis. Uncontrolled studies describe benefits in the use of aspirin, heparin or interferon alfa in reducing maternal-fetal complications. Nevertheless, those studies present selection biases.[306]

Extreme thrombocytosis

A Platelet Count > 1,000,000/mm^3 is not, per se, a recommendation for cytoreductive therapy. Patients with thrombocytosis above this level and Ristocetin Cofactor activity < 30% should not receive APT. Patients with extreme thrombocytosis, bleeding, and diagnosis of acquired von Willebrand disease should undergo cytoreduction.[306]

Recommendation

Secondary prophylaxis of thrombosis is indicated in all patients with ET (A), preferably oral anticoagulant in cases of previous VTs and low dose aspirin (81–100 mg) in cases of prior ATs (C).

In patients with ET without a history of thrombosis, primary prophylaxis with low doses of aspirin is recommended as below (B):

- In patients older than or equal to 60 years regardless of mutation status JAK2V617F.
- In young patients with JAK2V617F mutation and/or with concomitant cardiovascular risk factors.
- In patients with vasomotor symptoms (headaches, acromelalgia).

There is no evidence to support the use of antiplatelet drugs in young patients (age < 60 years), without previous history of thrombosis, without JAK2 V617F mutation and with no cardiovascular risk factors (IPSET-thrombosis risk score = 0 or modified IPSET-thrombosis = 0) (B).

Cytoreductive therapy is indicated in the following situations (B):

- Previous history of thrombosis
- Age over 60 years
- In patients with extreme thrombocytosis and acquired von Willebrand disease

The therapeutic target is the normalization of platelet counts (B).

Hydroxyurea is the preferred drug in the first line for cytoreductive therapy (A). Therapeutic alternatives in case of intolerance/failure to HU are: Anagrelide (A) and Interferon-alfa (conventional or pegylated form) (B).

The purpose of essential thrombocythemia (ET) treatment is to prevent thrombotic and hemorrhagic complications and relieve vasomotor symptoms (headache, dizziness, visual disturbances, burning dysesthesia). The available treatment options are non-curative, having failed to demonstrate the ability to prevent transformation to AML or post-ET myelofibrosis.

The definition of the need to initiate treatment for a patient with ET requires the evaluation of the following items:

- History of prior venous or arterial thrombosis, spontaneous abortion and hemorrhagic complications;
- Presence and intensity of vasomotor symptoms;
- Presence of cardiovascular risk factors, including Hypertension, Diabetes, Smoking, and Dyslipidemia;
- Mutational status of the JAK2 gene and cCalculation of prognostic scores, such as IPSET-thrombosis1 and modified IPSET-thrombosis2.

The mainstay of treatment is the use of a cytoreductive agent (such as Hydroxyurea (HU), Anagrelide or Interferon), usually associated with a low dose of aspirin3 (B).

Indications for cytoreductive treatment include:

- Previous history of thrombosis
- Age over 60 years
- Acquired von Willebrand Disease

Conflicts of interest

The authors declare no conflicts of interest.

ANNEX I.

Clinical question

What is the best system classification to MPNs?

Eligibility criteria

The main reasons for exclusion were: They did not respond to PICO and intermediate outcomes.

No period limit consulted.

Studies languages include Portuguese, English, and Spanish.

Narrative reviews, case reports, case series, and randomized controlled clinical trials phases II and I not included in the evaluation.

Literature search

Database

The scientific information base consulted included Medline (via PubMed), Lilacs, SciELO, Embase, Cochrane Library, Premedline via OVID and manual search.

– Table AI.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AI.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample calculation
Reference, study design, JADAD, strength of evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result
Primary, secondary, instrument of measure of the outcome of interest	Benefit or harm in absolute data, benefit or harm on average

Critical evaluation

Relevance – the clinical importance
These Guidelines resulted from a series of questions of connection on the adequate diagnosis of these diseases, advances in the identification of molecular and cytogenetic alterations, and on the evidence on the management of patients with these diseases.

Reliability – internal validity
The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity
The level of Scientific Evidence follows the type of study according to Oxford (Table AI.1).[20]

– Table AI.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

The selected evidence comprise a randomized controlled clinical trial (RCT) submitted to an appropriate critical evaluation checklist (Table AI.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AI.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE,[22] considering cohort studies consistent with score ≥6 and inconsistent <6.

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies shall receive a specific definition, whenever possible. The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AI.4).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some display principles:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE[23]) in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

– Table AI.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

ANNEX II.

Clinical question

What is the role of mutations in the diagnosis of primary myelofibrosis, polycythemia vera and essential thrombocythemia?

Eligibility criteria

The main reasons for exclusion were: They did not respond to PICO and intermediate outcomes.

No period limit consulted.

Studies languages include Portuguese, English, and Spanish.

Narrative reviews, case reports, case series, works with presentation of preliminary results were, in principle, excluded from the selection. We used Systematic reviews and Meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included.

Literature research

a. DatabaseThe basis of scientific information consulted was Medline (via PubMed) and manual search.
b. Identification of descriptors

P: Myeloproliferative Neoplasms
I: Mutations
C
O: Diagnosis

Research strategy

i. Strategy 1: ((((essential thrombocythemia OR essential thrombocythaemia OR thrombocytosis OR polycythemia OR erythema OR polycythaemia OR erythrocytosis OR myelofibrosis OR leukemic megakaryocytic myelosis OR myeloid metaplasia OR osteomyelofibrosis OR myelosclerosis)) AND ((mutation* OR molecular abnormalities OR JAK2 OR BCR-ABL OR exon 12 OR MPL OR CALR OR calreticulin)))) AND diagnosis/broad [filter]. *N* = 1556.
ii. Strategy 2: ((essential thrombocythemia OR essential thrombocythaemia OR thrombocytosis OR polycythemia OR erythema OR polycythaemia OR erythrocytosis OR myelofibrosis OR aleukemic megakaryocytic myelosis OR myeloid metaplasia OR osteomyelofibrosis OR myelosclerosis)) AND ((mutation* OR molecular abnormalities OR JAK2 OR BCR-ABL OR exon 12 OR MPL OR CALR OR calreticulin)). *N* = 3736.
iii. Manual search – Reference of literature, reviews and guidelines.

– Table AII.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AII.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample calculation
Reference, study design, JADAD, strength of evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result Benefit or harm in absolute data, benefit or harm mean
Primary, secondary, instrument of measure of the outcome of interest	

Critical evaluation

Relevance – the clinical importance

The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

Reliability – internal validity

The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

– Table AII.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

– Table AII.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AII.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primária	
PubMed-Medline #1	1556
PubMed-Medline #2	3736

Application of results – external validity

The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AII.1).

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AII.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AII.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE,[22] considering cohort studies consistent with score ≥6 and inconsistent <6.

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AII.4).

Results

Works retrieved (05/2017) (Table AII.5).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE)[23] in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

ANNEX III.

Clinical question

How to differentiate pre-fibrotic primary myelofibrosis from essential thrombocythemia?

Eligibility criteria

The main reasons for exclusion were: They did not respond to PICO and intermediate outcomes.

No period limit consulted.

Studies languages include Portuguese, English, and Spanish.

Narrative reviews, case reports, case series, work with presentation of preliminary results were, in principle, excluded from the selection. We used Systematic reviews and

– Table AIII.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AIII.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample calculation
Reference, study design, JADAD, strength of evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result
Primary, secondary, instrument of measure of the outcome of interest	Benefit or harm in absolute data, benefit or harm mean

Meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included.

Literature research

Database

The basis of scientific information consulted was Medline (via PubMed) and manual search.

Identification of descriptors

P: Prefibrotic primary myelofibrosis
I: Bone marrow biopsy
C: Essential thrombocythemia
O: Differential diagnosis

Research strategy

1 – (myelofibrosis OR aleukemic megakaryocytic myelosis OR myeloid metaplasia OR osteomyelofibrosis OR myelosclerosis).

2 – (bone marrow OR biopsy OR pathology OR anatomopathology OR histologic OR histology OR cytology OR fibrosis OR mutations).

3 – (essential thrombocythemia OR essential thrombocythemia).

Medline – (myelofibrosis OR aleukemic megakaryocytic myelosis OR myeloid metaplasia OR osteomyelofibrosis OR myelosclerosis) AND (bone marrow OR biopsy OR pathology OR anatomopathology OR histologic OR histology OR cytology OR fibrosis OR mutations) AND (essential thrombocythemia OR essential thrombocythemia).

Manual search – Reference of references, reviews and guidelines.

Critical evaluation

Relevance – the clinical importance

The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

Reliability – internal validity

The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity

The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AIII.1).

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AIII.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AIII.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE,[22] considering cohort studies consistent with score ≥6 and inconsistent <6.

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AIII.4).

Results

Works retrieved (04/2018) (Table AIII.5).

– Table AIII.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

– Table AIII.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study Design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AIII.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline	1073

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE)[23] in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

ANNEX IV.

Clinical question

What is the role of mutations in the prognosis of primary myelofibrosis?

Eligibility criteria

- Overall exclusion criteria were failure to answer PICO framework questions and intermediate outcomes.
- Database search conducted with no period/data range limit.
- Studies published in Portuguese, English, and Spanish.
- Narrative reviews, case reports, case series, papers presenting preliminary results were, at the outset, excluded from selection. We used Systematic reviews and Meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included.

Database search

Database
The scientific Database search included Medline (via PubMed), and manual search.

Identification of descriptors. P: Primary myelofibrosis
I: Mutations
C: ------
O: Prognosis

Research strategy

Medline – (myelofibrosis OR aleukemic megakaryocytic myelosis OR myeloid metaplasia OR osteomyelofibrosis OR myelosclerosis) AND (mutations OR molecular abnormalities OR JAK2 OR BCR-ABL OR exon 12 OR MPL OR CALR OR calreticulin) AND (survival OR death OR progression OR transfusion OR leukemic transformation OR acute leukemia OR AML OR acute myeloid leukemia OR splenomegaly OR anemia OR fibrosis OR thrombosis).
Manual search – Reference of references, reviews and guidelines.

Critical evaluation

Relevance – the clinical importance
The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

Reliability – internal validity
The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity

The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AIV.1).

– Table AIV.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AIV.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample calculation
Reference, study design, JADAD, strength of evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result
Primary, secondary, instrument of measure of the outcome of interest	Benefit or harm in absolute data, benefit or harm mean

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AIV.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AIV.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE,[22] considering cohort studies consistent with score ≥6 and inconsistent <6.

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AIV.4).

– Table AIV.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study Design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AIV.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline	1171

Results

Works retrieved (04/2018) (Table AIV.5).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE)[23] in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making.

– Table AIV.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

ANNEX V.

Clinical question

What is the role of interferon in primary myelofibrosis?

Eligibility criteria

The main reasons for exclusion were: They did not respond to PICO and intermediate outcomes.

No period limit consulted.

Studies languages include Portuguese, English, and Spanish.

Narrative reviews, case reports, case series, works with presentation of preliminary results were, in principle, excluded from the selection. We used systematic reviews and meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included.

Literature research

Database

The basis of scientific information consulted was Medline (via PubMed) and manual search.

Identification of descriptors

P: Primary myelofibrosis
I: Interferon
C: ------
O: Survival, morbidity, quality of life

Research strategy

- #1 – (primary myelofibrosis OR myelofibroses, primary OR myelofibrosis, primary OR primary myelofibrosis OR bone marrow fibrosis OR bone marrow fibroses OR fibroses, bone marrow OR fibrosis, bone marrow OR myelofibrosis OR myelofibrosis OR idiopathic myelofibrosis OR myeloid metaplasia OR metaplasia, myeloid OR metaplasias, myeloid OR myeloid metaplasia OR myelosclerosis OR myelosclerosis OR myelosis, nonleukemic OR myelosis, nonleukemic OR nonleukemic myeloses OR nonleukemic myelosis OR chronic idiopathic myelofibrosis OR agnogenic myeloid metaplasia OR agnogenic myeloid metaplasia OR metaplasia, agnogenic myeloid OR metaplasias, agnogenic myeloid OR myeloid metaplasia, agnogenic OR myeloid metaplasia, agnogenic OR myelofibrosis with myeloid metaplasia).
- #2 – (interferon*).

Medline – (primary myelofibrosis OR myelofibroses, primary OR myelofibrosis, primary OR primary myelofibrosis OR bone marrow fibrosis OR bone marrow fibroses OR fibroses, bone marrow OR fibrosis, bone marrow OR myelofibrosis OR myelofibrosis OR idiopathic myelofibrosis OR myeloid metaplasia OR metaplasia, myeloid OR metaplasias, myeloid OR myeloid metaplasia OR myelosclerosis OR myelosclerosis OR myelosis, nonleukemic OR myelosis, nonleukemic OR nonleukemic myeloses OR nonleukemic myelosis OR chronic idiopathic myelofibrosis OR agnogenic myeloid metaplasia OR agnogenic myeloid metaplasia OR metaplasia, agnogenic myeloid OR metaplasias, agnogenic myeloid OR myeloid metaplasia, agnogenic OR myeloid metaplasia, agnogenic OR myelofibrosis with myeloid metaplasia) AND (interferon*).

Manual search – reference of references, revisions and guidelines.

– Table AV.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AV.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample calculation
Reference, study design, JADAD, strength of evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result
Primary, secondary, instrument of measure of the outcome of interest	Benefit or harm in absolute data, benefit or harm mean

Critical evaluation

Relevance – the clinical importance

The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

Reliability – internal validity

The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity

The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AV.1).

– Table AV.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

– Table AV.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study Design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AV.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline	334

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AV.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AV.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE.[22]

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AV.4).

Results

Works retrieved (04/2018) (Table AV.5).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE)[23] in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient

ANNEX VI.

Clinical question

What is the role of immunomodulators in primary myelofibrosis?

Eligibility criteria

- Overall exclusion criteria were failure to answer PICO framework questions and intermediate outcomes.
- Database search conducted with no period/data range limit.
- Studies published in Portuguese, English, and Spanish.
- Narrative reviews, case reports, case series, papers presenting preliminary results were, at the outset, excluded from selection. We used Systematic reviews and Meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included.

Database search

Database
The scientific Database search included Medline (via PubMed), and manual search.

Identification of descriptors. P: Primary myelofibrosis
I: Immunomodulators
C
O: Survival, morbidity, quality of life

Research strategy.

i. Strategy 1: (primary myelofibrosis OR myelofibroses, primary OR myelofibrosis, primary OR primary myelofibrosis OR bone marrow fibrosis OR bone marrow fibroses OR fibroses, bone marrow OR fibrosis, bone marrow OR myelofibrosis OR myelofibrosis OR idiopathic myelofibrosis OR myeloid metaplasia OR metaplasia, myeloid OR metaplasias, myeloid OR myeloid metaplasia OR myelosclerosis OR myelosclerosis OR myelosis, nonleukemic OR myelosis, nonleukemic OR nonleukemic myeloses OR nonleukemic myelosis OR chronic idiopathic myelofibrosis OR agnogenic myeloid metaplasia OR agnogenic myeloid metaplasia OR metaplasia, agnogenic myeloid OR metaplasias, agnogenic myeloid OR myeloid metaplasia, agnogenic OR myeloid metaplasia, agnogenic OR myelofibrosis with myeloid metaplasia) AND (thalidomide OR lenalidomide OR pomalidomide OR immunologic factors).
ii. Strategy 2: ((primary myelofibrosis OR myelofibroses, primary OR myelofibrosis, primary OR primary myelofibrosis OR bone marrow fibrosis OR bone marrow fibroses OR fibroses, bone marrow OR fibrosis, bone marrow OR myelofibrosis OR myelofibrosis OR idiopathic myelofibrosis OR myeloid metaplasia OR metaplasia, myeloid OR metaplasias, myeloid OR myeloid metaplasia OR myelosclerosis OR myelosclerosis OR myelosis, nonleukemic OR myelosis, nonleukemic OR nonleukemic myeloses OR nonleukemic myelosis OR chronic idiopathic myelofibrosis OR agnogenic myeloid metaplasia OR agnogenic myeloid metaplasia OR metaplasia, agnogenic myeloid OR metaplasias, agnogenic myeloid OR myeloid metaplasia, agnogenic OR myeloid metaplasia, agnogenic OR myelofibrosis with myeloid metaplasia) AND (thalidomide OR lenalidomide OR pomalidomide OR immunologic factors OR immunosuppressive agents)) AND "therapy/broad" [Filter].
iii. Manual search – Citation of references, revisions, and guidelines.

Critical evaluation

Relevance – the clinical importance
The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

– Table AVI.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AVI.2 Critical evaluation of randomized controlled clinical trials script.

Study data Reference, study design, JADAD, strength of evidence	Sample calculation Estimated differences, power, level of significance, total of patients
Selection of patients Inclusion and exclusion criteria	Patients Recruited, randomized, prognostic differences
Randomization Description and blindfolded allocation	Patient follow-up Time, losses, migration
Treatment protocol Intervention, control and blinding	Analysis Intent of treatment, analyzed intervention and control
Considered outcomes Primary, secondary, instrument of measure of the outcome of interest	Result Benefit or harm in absolute data, benefit or harm mean

Reliability – internal validity
The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity
The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AVI.1).

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AVI.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AVI.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE,[22] considering cohort studies consistent with score ≥6 and inconsistent <6.

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

– Table AVI.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

– Table AVI.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AVI.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline strategy 1	1167
PubMed-Medline Strategy 2	829
Strategy 1 AND Strategy 2	828

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AVI.4).

Results

Works retrieved (05/2018) (Table AVI.5).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE)[23] in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

ANNEX VII.

Clinical question

What is the role of JAK inhibitors in primary myelofibrosis?

Eligibility criteria

- Overall exclusion criteria were failure to answer PICO framework questions and intermediate outcomes
- Database search conducted with no period/data range limit.
- Studies published in Portuguese, English, and Spanish.
- Narrative reviews, case reports, case series, papers presenting preliminary results were, at the outset, excluded from selection.

Database search

Database
The scientific Database search included Medline (via PubMed), and manual search.

Identification of descriptors. P: Primary myelofibrosis
I: Ruxolitinib
C: ------
O: Survival, morbidity

Research strategy.

- # 1 – (myelofibrosis OR aleukemic megakaryocytic myelosis OR myeloid metaplasia, osteomyelofibrosis OR myelosclerosis).
- # 2 – (JAK inhibitor OR ruxolitinib OR jakafi OR momelotinib OR pacritinib OR fedratinib).

Medline – (myelofibrosis OR aleukemic megakaryocytic myelosis OR myeloid metaplasia, osteomyelofibrosis OR myelosclerosis) AND (JAK inhibitor OR ruxolitinib OR jakafi OR momelotinib OR pacritinib OR fedratinib).

– Table AVII.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AVII.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample calculation
Reference, study design, JADAD, strength of evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result
Primary, secondary, instrument of measure of the outcome of interest	Benefit or harm in absolute data, benefit or harm mean

Manual search – Reference of the references, reviews and guidelines.

Critical evaluation

Relevance – the clinical importance
The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

Reliability – internal validity
The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity
The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AVII.1).

– Table AVII.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AVII.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline	253

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AVII.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AVII.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE,[22] considering cohort studies consistent with score ≥6 and inconsistent <6.

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AVII.4).

Results

Works retrieved (04/2018) (Table AVII.5).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

– Table AVII.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE)[23] in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

ANNEX VIII.

Clinical question

What is the best prognostic system for polycythemia vera?

Eligibility criteria

The main reasons for exclusion were: They did not respond to PICO and intermediate outcomes.

No period limit consulted.

Studies languages include Portuguese, English, and Spanish.

Narrative reviews, case reports, case series, works with presentation of preliminary results were, in principle, excluded from the selection. We used Systematic reviews and Meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included.

Literature research

Database

The basis of scientific information consulted was Medline (via PubMed) and manual search.

Identification of descriptors

P: Polycythemia vera, erythremia, polycythemia, erythrocytosis

– Table AVIII.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AVIII.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample calculation
Reference, study design, JADAD, strength of evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result
Primary, secondary, instrument of measure of the outcome of interest	Benefit or harm in absolute data, benefit or harm mean

I: Score, prognostic, classification

C: ----

O: survival, death, progression, leukemic transformation, AML, acute leukemia; thrombosis, ischemia

Research strategy

(Polycythemia OR erythema OR polycythaemia OR erythrocytosis OR polycythemia vera OR Primary Polycythemia OR polycythemia rubra Vera * OR Erythremia * OR Osler-Vaquez disease OR Osler-Vaquez disease) AND ("diagnosis/narrow" [Filter] OR "prognosis/narrow" Filter]))

Manual search – Reference of references, revisions and guidelines.

Critical evaluation

Relevance – the clinical importance

The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

Reliability – internal validity

The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

– Table AVIII.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

– Table AVIII.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AVIII.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline	1979

Application of results – external validity

The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AVIII.1).

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AVIII.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AVIII.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE.[22]

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AVIII.4).

Results

Works retrieved (04/2018) (Table AVIII.5).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE[23]) in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

ANNEX IX.

Clinical question

What is the target hematocrit in polycythemia vera?

Eligibility criteria

The main reasons for exclusion were: They did not respond to PICO and intermediate outcomes.

No period limit consulted.

Studies languages include Portuguese, English, and Spanish.

Narrative reviews, case reports, case series, works with presentation of preliminary results were, in principle, excluded from the selection. We used Systematic reviews and Meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included

– Table AIX.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

Literature research

Database
The basis of scientific information consulted was Medline (via PubMed) and manual search.

Identification of descriptors
P: Polycythemia vera
I: Hematocrit Level
C:
O: Morbidity, survival, thrombosis, quality of life

Research strategy

(polycythemia OR erythema OR polycythaemia OR erythrocytosis OR polycythemia vera OR Primary Polycythemia OR Polycythemia Rubra Vera* OR Erythremia* OR Osler Vaquez Disease OR Osler-Vaquez Disease)))) AND Hematocrit)
Manual search – Reference of references, revisions and guidelines.

Critical evaluation

Relevance – the clinical importance
The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

Reliability – internal validity
The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity
The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AIX.1).

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AIX.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥ three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AIX.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE[22]

– Table AIX.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample Calculation
Reference, Study Design, JADAD, Strength of Evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result
Primary, secondary, instrument of measure of the outcome of interest	Benefit or harm in absolute data, benefit or harm mean

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AIX.4).

Results

Works retrieved (05/2017) (Table AIX.5).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE[23]) in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

– Table AIX.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

– Table AIX.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AIX.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline	1928

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

ANNEX X.

Clinical question

What is the role of platelet antiaggregation in polycythemia vera?

Eligibility criteria

The main reasons for exclusion were: They did not respond to PICO and intermediate outcomes.

No period limit consulted.

Studies languages include Portuguese, English, and Spanish.

Narrative reviews, case reports, case series, works with presentation of preliminary results were, in principle, excluded from the selection. We used Systematic reviews and Meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included.

Literature research

Database

The basis of scientific information consulted was Medline (via PubMed) and manual search.

Identification of descriptors

P: Polycythemia, Erythema, Polycythaemia, Erythrocytosis

I: Antiaggregation, Clopidogrel, Ticlopidine, Acetyl Salicylic Acid, Aspirin

C: ----------

O: Survival, Death, Quality Of Life, Thrombosis, Pulmonary Embolism, Embolic, Deep Venous, Thromboembolic, Thromboembolism, Cardiovascular Events, Transfusion, Anemia, Hemorrhage, Ischemia

Research strategy

(Polycythemia Vera OR Primary Polycythemia OR Polycythemia Ruba Vera OR Polycythemia Rubra Vera OR Polycythemia Rubra Veras OR Vera, Polycythemia Rubra OR Veras, Polycythemia Rubra OR Erythremia) AND (Antiagregation OR Clopidogrel OR Ticlopidine OR Acetil Salicylic Acid OR Aspirin) AND (Therapy/Broad [filter]).

Manual search – Reference of references, reviews and guidelines.

Critical evaluation

Relevance – the clinical importance

The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

Reliability – internal validity

The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity

The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AX.1).

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AX.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21]

– Table AX.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AX.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample calculation
Reference, study design, JADAD, strength of evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result
Primary, secondary, instrument of measure of the outcome of interest	Benefit or harm in absolute data, benefit or harm mean

considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AX.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE,[22] considering cohort studies consistent with score ≥6 and inconsistent <6.

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AX.4).

Results

Works retrieved (05/2017) (Table AX.5).

– Table AX.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study Design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AX.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline	202

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE[23]) in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

– Table AX.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcomeassessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

ANNEX XI.

Clinical question

When to use cytoreductive agents in polycythemia vera?

Eligibility criteria

- Overall exclusion criteria were failure to answer PICO framework questions and intermediate outcomes.
- Database search conducted with no period/data range limit.
- Studies published in Portuguese, English, and Spanish.
- Narrative reviews, case reports, case series, papers presenting preliminary results were, at the outset, excluded from selection. We used Systematic reviews and Meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included.

Database search

Database
The scientific Database search included Medline (via PubMed), and manual search.

Identification of descriptors
P: Polycythemia, erythremia, polycythemia, Erythrocytosis

I: Hydrea, Hydroxyurea, Hydroxycarbamide, Chemotherapy, Anagrelide, JAK Inhibitor, Ruxolitinib, Jakafi, Warfarin, Phlebotomy, Busulfan

C: ---------

O: Survival, Death, Quality of Life, Thrombosis, Pulmonary Embolism, Embolic, Deep Venous, Thromboembolic, Thromboembolism, Cardiovascular Events, Transfusion, Anemia, Hemorrhage, Ischemia, Progression, Leukemic Transformation, Acute Leukemia, AML, Acute Myeloid Leukemia, Myelofibrosis, Fibrosis

Research strategy

(Polycythemia Vera OR Primary Polycythemia OR Polycythemia Ruba Vera OR Polycythemia Rubra Vera OR Polycythemia Rubra Veras OR Vera, Polycythemia Rubra OR Veras, Polycythemia Rubra OR Erythremia) AND (hydrea OR hydroxyurea OR hydroxycarbamide OR oncocarbide OR JAK inhibitor OR ruxolitinib OR jakafi OR momelotinib OR pacritinib OR fedratinib OR thalidomide OR lenalidomide OR pomalidomide OR immunologic factors OR interferon*) AND (Therapy/Broad[filter]).
Manual search – Reference of references, reviews and guidelines.

Critical evaluation

Relevance – the clinical importance
The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

– Table AXI.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AXI.2 Critical evaluation of randomized controlled clinical trials script.

Study data Reference, study design, JADAD, strength of evidence	Sample calculation Estimated differences, power, level of significance, total of patients
Selection of patients Inclusion and exclusion criteria	Patients Recruited, randomized, prognostic differences
Randomization Description and blindfolded allocation	Patient follow-up Time, losses, migration
Treatment protocol Intervention, control and blinding	Analysis Intent of treatment, analyzed intervention and control
Considered outcomes Primary, secondary, instrument of measure of the outcome of interest	Result Benefit or harm in absolute data, benefit or harm mean

Reliability – internal validity
The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity
The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AXI.1).

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AXI.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AXI.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE,[22] considering cohort studies consistent with score ≥6 and inconsistent <6.

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

– Table AXI.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

– Table AXI.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AXI.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline	780

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AXI.4).

Results

Works retrieved (05/2017) (Table AXI.5).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE[23]) in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

ANNEX XII.

Clinical question

What is the role of mutations in the prognosis of essential thrombocythemia?

Eligibility criteria

- Overall exclusion criteria were failure to answer PICO framework questions and intermediate outcomes.
- Database search conducted with no period/data range limit.
- Studies published in Portuguese, English, and Spanish.
- Narrative reviews, case reports, case series, papers presenting preliminary results were, at the outset, excluded from selection. We used Systematic reviews and Meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included.

Database search

Database
The scientific Database search included Medline (via PubMed), and manual search.

Identification of descriptors
P: Essential thrombocythemia
I: Mutations
C: ----------
O: Prognosis

Research strategy

(essential thrombocythemia OR essential thrombocythaemia OR thrombocytosis) AND (mutations OR molecular abnormalities OR JAK2 OR BCR-ABL OR exon 12 OR MPL OR CALR OR calreticulin) AND (survival OR death OR progression OR transfusion OR leukemic transformation OR acute leukemia OR AML OR acute myeloid leukemia OR splenomegaly OR anemia OR fibrosis OR thrombosis).
Manual search – Reference of references, reviews and guidelines.

– Table AXII.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AXII.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample calculation
Reference, study design, JADAD, strength of evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result
Primary, secondary, instrument of measure of the outcome of interest	Benefit or harm in absolute data, benefit or harm mean

Critical evaluation

Relevance – the clinical importance
The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

Reliability – internal validity
The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity
The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AXII.1).

The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AXII.2). The critical evaluation of the RCT allows classification according to the JADAD score,[21] considering the JADAD tests <three (3) as inconsistent (grade B), and those with a score ≥three (3), consistent (grade A).

When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AXII.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE,[22] considering cohort studies consistent with score ≥6 and inconsistent <6.

– Table AXII.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study Design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AXII.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline	1096

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AXII.4).

Results

Works retrieved (05/2017) (Table AXII.5).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.

– Table AXII.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE[23]) in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

ANNEX XIII.

Clinical question

What is the therapy for essential thrombocythemia?

Eligibility criteria

- Overall exclusion criteria were failure to answer PICO framework questions and intermediate outcomes.
- Database search conducted with no period/data range limit.
- Studies published in Portuguese, English, and Spanish.
- Narrative reviews, case reports, case series, papers presenting preliminary results were, at the outset, excluded from selection. We used Systematic reviews and Meta-analyzes with the principle of retrieval of references that had been lost at first from the initial search strategy. Randomized controlled trials (RCTs) and/or observational comparative studies were included.

Database search

Database
The scientific Database search included Medline (via PubMed), and manual search.

Identification of descriptors
P→essential thrombocythemia, essential thrombocythemia, thrombocytosis

I→treatment, care, medical assistance

C→hydrea, hydroxyurea, hydroxycarbamide, chemotherapy, anagrelide, JAK inhibitor, ruxolitinib, jakafi, warfarin, aspirin, ticlopidine, antiaggregation, acetylsalicylic acid, anticoagulation, agrylin

– Table AXIII.1 Degree of recommendations and level of evidence.

A: Experimental or observational studies of better consistency.
B: Experimental or observational studies of lower consistency.
C: Case reports/uncontrolled studies.
D: Opinion that is not critical based on consensus, physiological studies or animal models.

– Table AXIII.2 Critical evaluation of randomized controlled clinical trials script.

Study data	Sample calculation
Reference, study design, JADAD, strength of evidence	Estimated differences, power, level of significance, total of patients
Selection of patients	Patients
Inclusion and exclusion criteria	Recruited, randomized, prognostic differences
Randomization	Patient follow-up
Description and blindfolded allocation	Time, losses, migration
Treatment protocol	Analysis
Intervention, control and blinding	Intent of treatment, analyzed intervention and control
Considered outcomes	Result
Primary, secondary, instrument of measure of the outcome of interest	Benefit or harm in absolute data, benefit or harm mean

O→death, morbidity, survival, quality of life, progression, leukemic transformation, acute leukemia, AML, acute myeloid leukemia, fibrosis, thrombosis, pulmonary embolic, deep venous, thromboembolic, thromboembolism

Research strategy
(Essential thrombocythemia OR essential thrombocythemia OR thrombocytosis) AND (Treatment, care OR medical assistance) AND (hydrea OR hydroxyurea OR hydroxycarbamide OR chemotherapy OR anagrelide OR JAK inhibitor OR ruxolitinib OR jakafi OR warfarin OR aspirin OR ticlopidine OR antiaggregation OR acetylsalicylic acid OR anticoagulation OR agrylin) AND (death OR morbidity OR survival OR quality of life OR progression OR leukemic transformation OR acute leukemia OR AML OR acute myeloid leukemia OR fibrosis OR thrombosis OR pulmonary embolic OR deep venous OR thromboembolic OR thromboembolism).

Manual search – Reference of references, reviews and guidelines.

Critical evaluation

Relevance – the clinical importance
The present guidelines were prepared through a clinically relevant question to gather information in medicine to standardize behavior to assist in decision-making.

Reliability – internal validity
The selection of the studies, the evaluation of the titles and abstracts obtained with the search strategy in the consulted information bases performed in an independent and blinded manner, strictly adhering to the inclusion and exclusion criteria, separating the works with potential relevance. Articles

– Table AXIII.3 Critical evaluation of cohort studies script.

Representativeness of exposed and selection of non-exposed (max 2 points)	Study description (max 1 point)	Demonstration that the outcome of interest was not present at the start of the study (max 1 point)	Comparability on the basis of design or analysis (max 2 points)	Outcome assessment (max. 1 point)	Appropriate follow-up time (max 2 points)	Score and level of evidence

– Table AXIII.4 Spreadsheet used to describe and explain the results of each study.

Evidence included
Study design
Selected population
Follow-up time
Considered outcomes
Expression of results: percentage, risk, odds, hazard ratio, mean

– Table AXIII.5 Number of works retrieved with the search strategies used for each scientific information base.

Database	Amount of works
Primary	
PubMed-Medline	80

were required to display enlightening titles and abstracts and received an in-depth scrutiny. Only full-text papers were eligible for critical evaluation.

Application of results – external validity

The level of Scientific Evidence follows the type of study according to Oxford[20] (Table AXIII.1).The selected evidence comprise a randomized controlled clinical trial (RCT), submitted to an appropriate critical evaluation checklist (Table AXIII.2). The critical evaluation of the RCT allows classification according to the JADAD score[21] considering the JADAD tests < three (3) as inconsistent (grade B), and those with a score ≥ three (3), consistent (grade A).When the selected evidence was defined as a comparative study (observational cohorts or non-randomized clinical trial), it was submitted to an appropriate critical evaluation checklist (Table AXIII.3), allowing the classification of the study according to the NEW CASTLE-OTTAWA SCALE,[22] considering cohort studies consistent with score ≥ 6 and inconsistent < 6.

Method of extraction and analysis of results

For results with available evidence, the population, intervention, outcomes, presence or absence of benefit and harm and controversies require specific definition, whenever possible.

The results will be presented in absolute data, absolute risk, the number needed to treat (NNT), or number to harm (NNH), and possibly in mean and standard deviation (Table AXIII.4).

Results

Works retrieved (05/2018) (Table AXIII.5).

Application of evidence – recommendation

The authors of the review will elaborate the recommendations with the primary characteristic of synthesis of the evidence and submit it for validation by all the authors participating in the elaboration of the Guideline.

The available evidence will follow some principles of display:

- It will be outcome-oriented.
- Its components must include the number of patients, type of comparison, magnitude and precision (standard deviation and 95% CI).

The global synthesis will be constructed considering the evidence described and will have its estimated strength (Oxford[20]/GRADE[23]) in 1b and 1c (grade A) or strong and in 2a, 2b and 2c (grade B) or moderate or weak or very weak.

Conflict of interests

The authors herein state no conflict of interest concerning this review.

Final statement

The Guidelines Project, an initiative of the Brazilian Medical Association, in a joint effort with the Societies of Specialties seek to reconcile medical information to standardize behaviors that aid the physician's reasoning and decision making. The information contained in this project shall be submitted to the evaluation and criticism of the physician, responsible for the conduct to be followed, in the face of the reality and clinical condition of each patient.

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