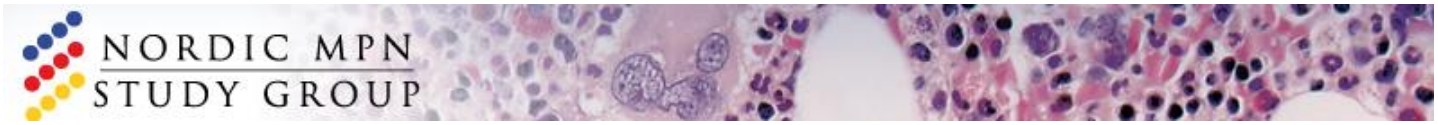

Nordic care program for patients with Essential Thrombocythemia, Polycythemia Vera and Primary Myelofibrosis

4th version March 2017

The Nordic study group on myeloproliferative neoplasms is a pan-Nordic scientific working group in Philadelphia negative neoplasms. This is the 4th update on diagnosis and treatment of Essential Thrombocythemia, Polycythemia Vera and Primary Myelofibrosis, since the first version in 2007. The program is aimed to be read by junior doctors as well as by consultants in hematology as a reference at-hand. The paper is based on the WHO 2016 criteria, and is called a “Care Program” for various reasons. It is intended to be a contemporary overview of diagnostics and evidence based practice, and to highlight the different options, which are available. However, the treatments are not equally accessible in the Nordic (or other) countries, or on the same registered indications for therapeutic options. Some pathophysiologic aspects are included, but the review is not a text-book – it is intended for daily practice. Parts of the content in the last “guideline” are maintained. The main chapters may be read quickly and separately. The treatment spectrum has similarities, and some links lead to other sections to reduce repetitions. The care program is supported by references, which are updated to 2016 – and some are directly accessible by hyperlinks in the alphabetical reference list, and illustrate some Nordic results in this exciting field of hematology.

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List of abbreviations

ALAT	alanine aminotransferase
ANA	anagrelide
ASA	acetylsalicylic acid (aspirin)
ASAT	aspartate aminotransferase
ASXL	additional sex combs like
AvWS	acquired von Willebrand Syndrome
BAT	best available therapy
BU	busulphan
CALR	calreticulin
DOAC	direct oral anticoagulants
ELN	European Leukemia Net
EPO	erythropoietin
ET	essential thrombocythemia
Hb	hemoglobin
Hct	hematocrit (erythrocyte volume fraction)
HU	hydroxyurea
IFN	interferon
JAK	Janus kinase
LDH	lactate dehydrogenase
LMWH	low molecular weight heparin
MPN	myeloproliferative neoplasm (neoplasia)
MPL	myeloproliferative leukemia virus oncogene
PMF	primary myelofibrosis
PV	polycythemia vera
PVSG	polycythemia vera study group
QoL	quality of life
S	serum (or P plasma for various parameters)
SCT	stem cell transplantation
TIBS	transferrin iron binding saturation
VKA	vitamin K antagonist
WBC	white blood cell (leukocyte)

General introduction

The Philadelphia chromosome/*BCR-ABL* negative myeloproliferative neoplasms (MPNs) represent a range of clonal hematological diseases with overlapping features. The main entities are polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) which are characterized by clonal excess hematopoiesis in one or more cell lines (Arber 2016; Saeidi 2016; Vainchenker & Kralovics 2017). They are associated with an elevated risk of arterial and venous thrombosis; many PV and ET patients have a concomitant thrombosis at the time of diagnosis. Both PV and ET can progress to myelofibrosis and all three entities can transform into acute myeloid leukemia.

Signs and symptoms of MPNs

Many patients with PV and ET are discovered due to routine blood testing for other diseases. Traditional symptoms of PV are dizziness, aquagenic pruritus, and plethora including engorged retinal veins. Other symptoms seen in some patients include headaches, light-headedness, visual disturbances, and dyspnea. The palms and feet may be red, warm and painful, sometimes with digital ischemia (erythromelalgia). ET patients are commonly asymptomatic at discovery but may present with thrombosis, fatigue and erythromelalgia like PV patients. The quality of life (QoL) at the time of diagnosis in patients with PV, ET, and PMF was evaluated in Sweden and fatigue was the most common symptom in these patients (Abelsson 2013). Patients with PV reported significantly higher mean scores for inactivity, dizziness, cough, itching, depression, and lower total QoL compared to patients with ET. Patients with PV also had significantly more headache and itching compared to patients with PMF.

Approximately 25% of PV and ET patients have had a previous or concomitant vascular complication at the time of MPN diagnosis, and of these, some 66% occurred during the two years preceding diagnosis (Enblom 2015). The majority of events were thromboembolic (95%), and included myocardial infarction, ischemic stroke, transient ischemic attack, deep vein thrombosis/pulmonary embolism, splanchnic vein thrombosis, and peripheral embolism. Bleeding was observed in only in 5% of the patients with vascular events (Enblom 2015). Therefore, sequelae due to vascular complications may also be manifest clinically at diagnosis. Almost 30 % of patients in a Danish cohort with MPN have moderate to severe impairment of kidney function at diagnosis (Christensen 2014).

Myeloproliferative neoplasms are uncommon. The annual incidence in Sweden for ET and PV has been very stable over time at 2.3 and 3 per 100,000 inhabitants. Median age at diagnosis is 68 years in ET, 69 in PV, and 72 in PMF (Abdulkarim 2017). The Danish national registry has shown similar results as in Sweden, albeit a little higher annual incidence of PV (3 / 100.000), and for PMF 1 / 100.000 habitants. The MPN-incidence increases with age (Hultcrantz 2015a; Bak 2016).

Essential thrombocythemia (ET)

All patients with unexplained and persistent (>3 months) thrombocytosis above 450x10^9/L should be investigated for the possibility of a MPN. Secondary causes of thrombocythemia like iron deficiency, inflammatory disorders, chronic infection and non-myeloid malignancy should be ruled out.
Bone marrow biopsy is necessary for the correct diagnosis of ET and distinction from early myelofibrosis (prePMF) as well as masked polycythemia. ET is diagnosed according to the WHO diagnostic criteria, which uses morphologic criteria for the classification (see also the PMF chapter, page 25). In previous classifications (i.e. PVSG), where the distinction was not made, many patients with early MF were given the diagnosis ET. The importance of this distinction lies mainly in the different prognosis with regard to transformation to overt MF and with regard to survival. True ET has a much lower MF transformation rate, and the expected survival is close to normal (Barbui 2011). At present, the goal of treatment does not differ, due to the fact that treatment is mainly aimed at reducing thrombohemorrhagic events, but in the future, with the development of new drugs, treatment choices may differ. Importantly, recently it has also been shown that some patients with ET diagnosis have a “masked polycythemia” with Hb and Hct values within the reference range (Barbui 2014a). The WHO diagnostic criteria for PV have therefore been amended (Arber 2016) with new cut-off levels for Hb and Hct (165 g/L (10.2 mmol/L) and 49% for males, 160g/L (9.9 mmol/L) and 48% for women), and bone marrow morphology has been given a more important role in PV diagnosis. If all patients with suspected ET are investigated with bone marrow biopsy the risk of misdiagnosis can be minimized. A very low S-EPO is also indicative of PV.

WHO diagnostic criteria for essential thrombocythemia (Arber 2016)

Major criteria

1. Platelet count > 450 x 10^9/L.

2. Bone marrow biopsy showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers.

3. Not meeting WHO criteria for *BCR-ABL+* CML, PV, PMF, MDS or other myeloid neoplasm

4. Presence of *JAK2*V617F, *CALR* or *MPL* mutation.

Minor criterion

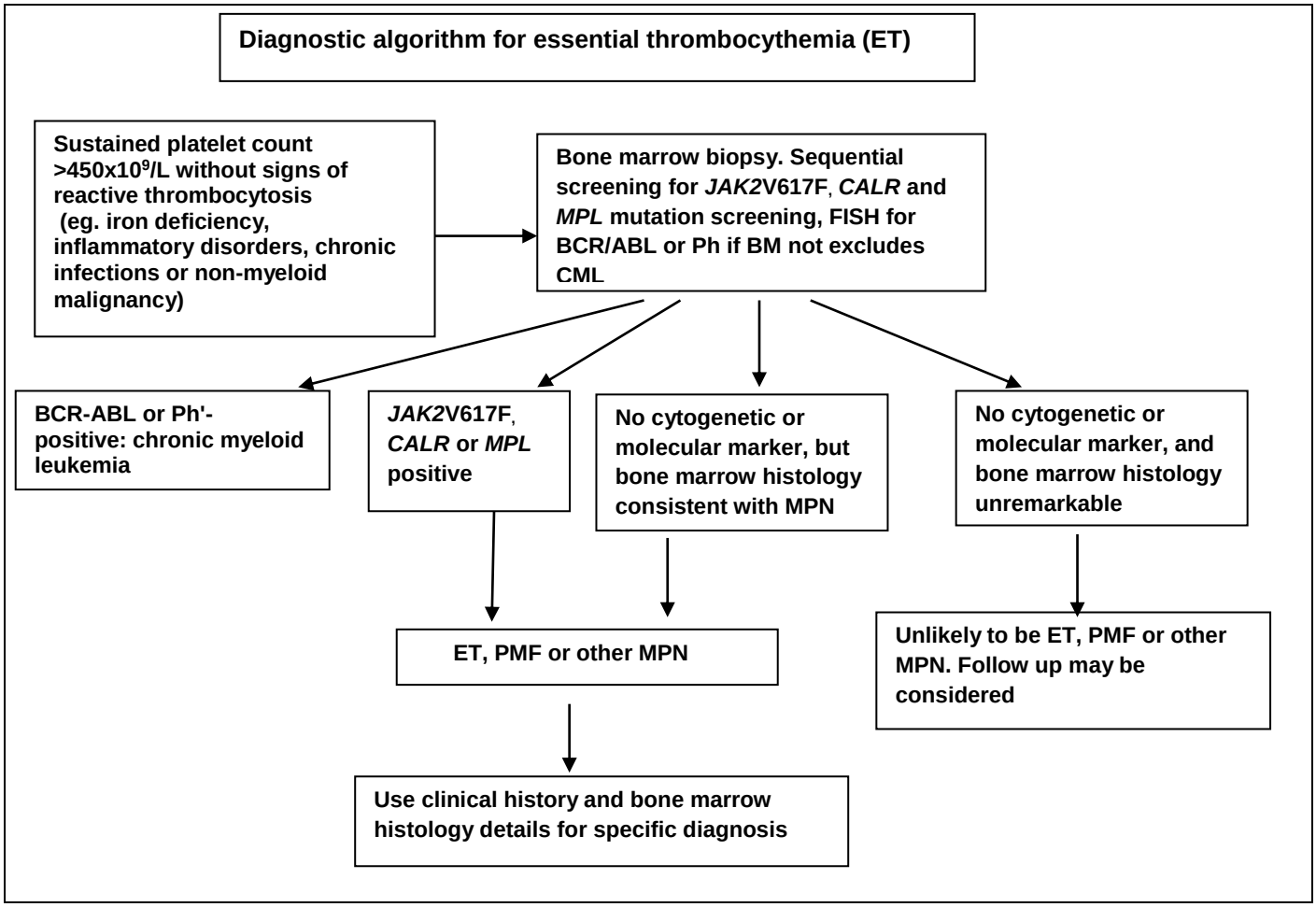
Presence of a clonal marker or absence of evidence for reactive thrombocytosis.

Diagnosis of ET requires meeting all 4 major criteria or the first 3 of major criteria and the minor criterion.

Legend: ET essential thrombocythemia, CML chronic myeloid leukemia, PV polycythemia vera, PMF primary myelofibrosis, MDS myelodysplastic syndrome

Mutations in the *CALR* gene were presented in 2013 found in about 25% of ET and 35 % of MF patients, almost exclusively in *JAK2*-negative cases (Nangalia 2013). Screening for *JAK2*V617F should be performed in all suspected ET patients (positive in 50-60%), *CALR* screening in *JAK2*V617F-negative patients and *MPL* screening in patients negative for both (*MPL* found in about 4% of ET cases). *CALR*-positive ET patients present a somewhat different phenotype with lower WBC counts, lower thrombosis incidence and lower Hb concentration. In the future, the genetic laboratories may include all known mutations in a comprehensive mutation analysis. Even in the presence of a *JAK2*V617F mutation, a diagnosis of ET requires exclusion PV and MF. Patients without any mutation (so called “triple negative”) require special consideration with regard to the possibility of a hereditary or secondary thrombocythemia.

Diagnostic algorithm for essential thrombocythemia



Legend: BM bone marrow examination, CML chronic myeloid leukemia, PV polycythemia vera, PMF primary myelofibrosis, MPN myeloproliferative neoplasm

Diagnostic work-up in ET

- Full blood count with differential count
- Iron status (S-ferritin, transferrin saturation)
- ASAT, ALAT, bilirubin, creatinine, LDH
- J*AK2*V617F screening, *CALR* mutation screening in negative patients
- *MPL* mutation in *JAK2*V617F and *CALR*-negative patients
- Bone marrow biopsy
- Evaluation of cardiovascular risk factors
- Physical examination including palpation of spleen
- *BCR-ABL* in triple-negative patients if differentiation against CML is not obvious
- S-EPO

Risk stratification in ET

This is based on the assessment of risk of thrombosis and bleeding, as current therapy in ET is aimed at lowering these risks. True ET, diagnosed according to the 2008 WHO criteria, and treated according to recommendations stated below (Barbui 2011c), has been shown to have a life expectancy close to normal. New prognostic scoring models have been published, using variables like WBC count, *JAK2* mutation status and cardiovascular risk factors in addition to (or excluding) the traditional risk factors age >60 years, previous thrombosis and platelets >1500 x10^9/L. The model with most documentation is the IPSET-thrombosis score (Barbui 2012a; Carobbio 2011) using age, previous thrombosis, *JAK2* mutation status and cardiovascular risk factors. It has been validated in retrospective but not in prospective studies and presently is not used as a standard. As these new score models only deal with thrombosis risk, bleeding due to extreme thrombocythemia is not included. Several of these and other studies (Lekovic 2014) have confirmed that presence of cardiovascular risk factors increase thrombosis risk.

As the *JAK2*V617F and *CALR* mutations are mutually exclusive in ET, comparison of thrombosis risk has been made, showing that *CALR*-positive patients have a lower risk of thrombosis than *JAK2*-positive (Rotunno 2014). However, this does not change the general risk stratification or treatment strategy.

In these recommendations, we use the traditional risk factors and try to use the new data in a pragmatic way, adding considerations to the basic risk stratification.

High risk

Patients with

- Age > 60 years *or*
- History of previous thrombosis *or*
- Platelets >1500 x 10^9/L

High risk patients should be treated with cytoreductive therapy with normalization of platelet levels as a treatment goal. In high risk patients with normal platelet levels a standard dose of cytoreductive therapy should be given (e.g. MPN patients with abdominal thrombosis and normal platelet counts).

Low risk
Patients with

- Age <60 years, no previous thrombosis and platelet count <1500x10^9/L

Clinical management of essential thrombocythemia

It is highly recommended that ET patients are included in prospective clinical trials if available.

Goals of therapy

- To prevent thrombotic and bleeding complications
- To reduce systemic symptoms
- To manage risk situations (e.g. pregnancy and surgery)
- To minimize the risk of transformation to leukemia and MF

Recommendations on treatment strategy in ET

Aspirin
Antiplatelet therapy with aspirin (ASA) is recommended in all high risk patients, and in low risk patients with peripheral vascular symptoms or the presence of cardiovascular risk factors. A recent study (Alvarez-Larran 2016) found no benefit but more bleedings from ASA therapy in low risk *CALR*-positive patients, which supports this rather restrictive policy. Antiplatelet therapy may also be considered in low-risk, *JAK2*V617F-positive patients. The standard dose is 75 mg daily. Aspirin should **not** be given to patients with platelets > 1500 x10^9/L (or >1000 and bleeding symptoms) due to an increased risk of bleeding caused by an acquired von Willebrand condition, instead cytoreductive therapy should be initiated (Campbell 2012). Measurement of von Willebrand factor is not recommended due to methodological problems and uncertainty about cut-off levels. Consider other contraindications for ASA such as (ASA induced) asthma and allergy, (recent) peptic ulceration or congenital hemophilia before commencing ASA.

Cytoreductive treatment is recommended to all ET patients with age > 60 years or a history of previous thrombosis or platelet count > 1500 x 10^9/L. In addition, patients with low risk criteria and additional risk enhancers like *JAK2*V617F mutation, cardiovascular risk factors and leukocytosis (especially if progressive) may be considered for cytoreductive treatment. Due to the demographic

changes in the population and the long duration of treatment even in patients < 60 years the recommendation of caution with hydroxyurea is extended to 60 years of age (Tefferi 2016).

Choice of cytoreductive therapy in ET

- < 60 years
  - First and second line: Interferon-α/Anagrelide
  - Third line: Hydroxyurea
- > 60 years
  - First-line: Hydroxyurea
  - Second-line: Interferon-α/ Anagrelide
- > 75 years or with a short expected survival
  - First-line: Hydroxyurea
  - Second-line: *intermittent* busulphan
  - Third-line: radioactive phosphorus (P^{32}) (if available)
- Combination therapy (hydroxyurea+anagrelide, hydroxyurea+interferon-α, interferon- α + anagrelide) can be an alternative second line therapy in fit patients if dose-limiting side effects occur with monotherapy.
  Grade C, evidence level IV.

Interferon-α (IFN) suppresses growth of multipotent hematopoietic progenitor cells. IFN treatment is well documented and safe in ET and is not considered leukemogenic or teratogenic. Pegylated IFN given sc weekly or even every second week has been shown to have equal efficacy as conventional IFN given three times weekly. Fatigue and mood changes are the most common side effects. Myalgia and activation of present or latent autoimmune disease including hypo- and hyperthyreosis are not uncommon (Paulsrud 2016). Liver enzymes and thyroid hormones should be monitored (page 21).
Recommendation: IFN can be used in younger patients and in older patients where long term use of HU is not suitable and in patients who do not tolerate HU. Half the standard initial dose (45 or 25-35 ug/week of Pegasys® or PegIntron®, respectively) may be tried in patients with less pronounced or no symptoms. Dose change should be considered after 8-10 weeks if the effect is insufficient. If cytoreductive therapy is indicated during pregnancy or when pregnancy is planned, IFN is the treatment of choice.
Grade B recommendation, evidence level III

Hydroxyurea (HU) is a non-alkylating, non-specific myelosuppresive drug. HU is the best documented therapy in ET (diagnosed with PVSG criteria) and is recommended as a first-line therapy in the majority of ET patients. HU is the only cytoreductive agent proven to reduce thrombotic events in a randomized controlled trial (Cortelazzo 1995). In spite of a large Swedish retrospective cohort study showing no proven leukemogenicity, caution is recommended in young patients who are to be treated for several decades (Barbui 2011a) on the basis of other studies indicating an increased rate of leukemia transformation (Kiladjian 2011). Skin ulcers, actinic keratosis and squamous cell changes

are common, fever is unusual but may be dramatic. In some patients platelet levels may fluctuate dramatically during HU therapy and make change of therapy necessary. Anemia and neutropenia may occur due to the general cytoreductive effect (page 22). International recommendations for HU resistance and intolerance are available (Barosi 2010), HU should not be used when pregnancy is planned or during pregnancy. HU should be withdrawn at least 3 months before planned conception both in men and women.
Recommendation: Hydroxyurea is recommended as a first-line myelosuppressive therapy in ET patients older than 60 years. The standard starting dose is 0.5-1.0 g daily.
Grade A recommendation, evidence level Ib

Anagrelide (ANA) has a selective effect on megakaryocytes in vitro and reduces platelet levels with the same efficacy as HU (Gisslinger 2013). The standard starting dose of 0.5 mg twice daily should not be exceeded due to risk of side effects. More details on ANA treatment is described in detail on page 23. ANA should not be used when pregnancy is planned or during pregnancy, since there is no data available concerning effects on the fetus.
Recommendation: ANA can be used as first-line alternative to IFN in patients <60 years, and as an alternative to HU or IFN in older patients with resistance or toxicity for these drugs.
Grade B recommendation, evidence level IB

Busulphan (BU) is an alkylating agent mostly given as intermittent treatment. BU is effective at controlling platelet count but associated with an increased risk of progression to acute leukemia, particularly when used sequentially with hydroxyurea. BU is a second- or third-line agent and should be restricted to patients with short life expectancy (page 23).
Recommendation: Intermittent BU treatment can be used in elderly ET patients. The dose is usually 2-4 mg daily for 4 to 8 weeks. Blood counts should be monitored closely and therapy can be stopped when platelets are < $400x10^9$/L or WBC <$3x10^9$/L. Since busulphan is an alkylating agent it should be reserved for patients where HU, IFN or anagrelide are not suitable.
Grade B recommendation, evidence level IIb.

Radioactive phosphorus (P32) is given as an intermittent treatment and is effective in controlling platelet count. P^{32} is associated with an increased risk of progression to acute leukemia, particularly when used sequentially with hydroxyurea. The response rate is high, and some patients have long response durations.
Recommendation*:* P^{32} is not recommended for routine use in ET, but could be an option in patients with short life expectancy, when other treatments have failed.
Grade C recommendation, evidence level IIB.

Response monitoring in ET

- The goal for cytoreductive therapy is normalization of platelet numbers and freedom from thromboembolic complications. Patients should also be monitored

regarding side effects of drugs and most common symptoms in ET (fatigue, concentration problems and night sweats) (Emanuel 2012).
- A more elaborate definition of response criteria, intended for clinical studies, has been published by the European Leukemia Net (Barosi 2013).

Response monitoring does not include

- Serial estimation of mutation allele burden is generally not recommended, but can be considered in interferon treated patients if a therapy free period is planned.
- Bone marrow examination (only at suspected progression/transformation)

Therapy change and second-line therapy in ET

In patients resistant or intolerant to hydroxyurea, non-leukemogenic drugs such as anagrelide or IFN should be considered. The reason is to avoid sequential use of cytotoxic agents associated with a significantly higher risk of developing acute myeloid leukemia / myelodysplastic syndrome.

Polycythemia vera (PV)

Diagnostic criteria for polycythemia vera

Males and females with hematocrit (Hct) > 0.49 and > 0.48, respectively, for more than two months should be evaluated for PV. The diagnostic work-up of PV will be reviewed here. For diagnostic work-up of erythrocytosis other than PV please read (McMullin 2012). A diagnosis of PV should be made using the newly revised WHO criteria (Arber 2016).

WHO 2016 criteria for polycythemia vera (PV) (Arber 2016)

Major criteria
1. Hemoglobin > 165 g/L in men (10.2 mmol/L), >160 g/L (9.9 mmol/L) in women, or hematocrit > 0.49 in men and > 0.48 in women, or increased red cell mass* 2. Bone marrow biopsy showing hypercellularity for age with trilineage proliferation (panmyelosis) including prominent erythroid, granulocytic and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size) 3. Presence of *JAK2*V617F or *JAK2* exon 12 mutation
Minor criterion
Subnormal S-erythropoetin level
Diagnosis requires either the presence of all 3 major criteria or the first 2 major and the minor criterion**

* More than 25% above mean normal predicted value.

** Criterion number 2 (bone marrow biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels > 185 g/L (11.5 mmol/L) in men (hematocrit >0.55) or > 165 g/L (10,2 mmol/L) in women (hematocrit >0.49) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a bone marrow biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

A diagnosis of PV can thus *no longer* be made *without* a bone marrow biopsy (Arber 2016), except for patients defined in **. A bone marrow biopsy is in addition *highly recommended* since degree of fibrosis adds valuable prognostic information (Barbui 2012b). The *JAK2*V617F mutation is present in at least 95 % of PV patients (James 2005) in later studies up to 98% (Brecqueville 2012). The *JAK2*V617F mutation is very seldom found in normal individuals (and if so at very low levels < 1 %), in patients with secondary erythrocytosis, and is rarely found in other hematologic disorders with the exception of MDS RARS-T (Malcovati 2009). If PV is suspected and the patient is negative for the *JAK2*V617F mutation, further investigation with *JAK2* exon 12 mutations should be carried out to

clarify major criterion 3. Patients with *JAK2* exon 12 mutation are characterized by isolated erytrocytosis (Passamonti 2011).

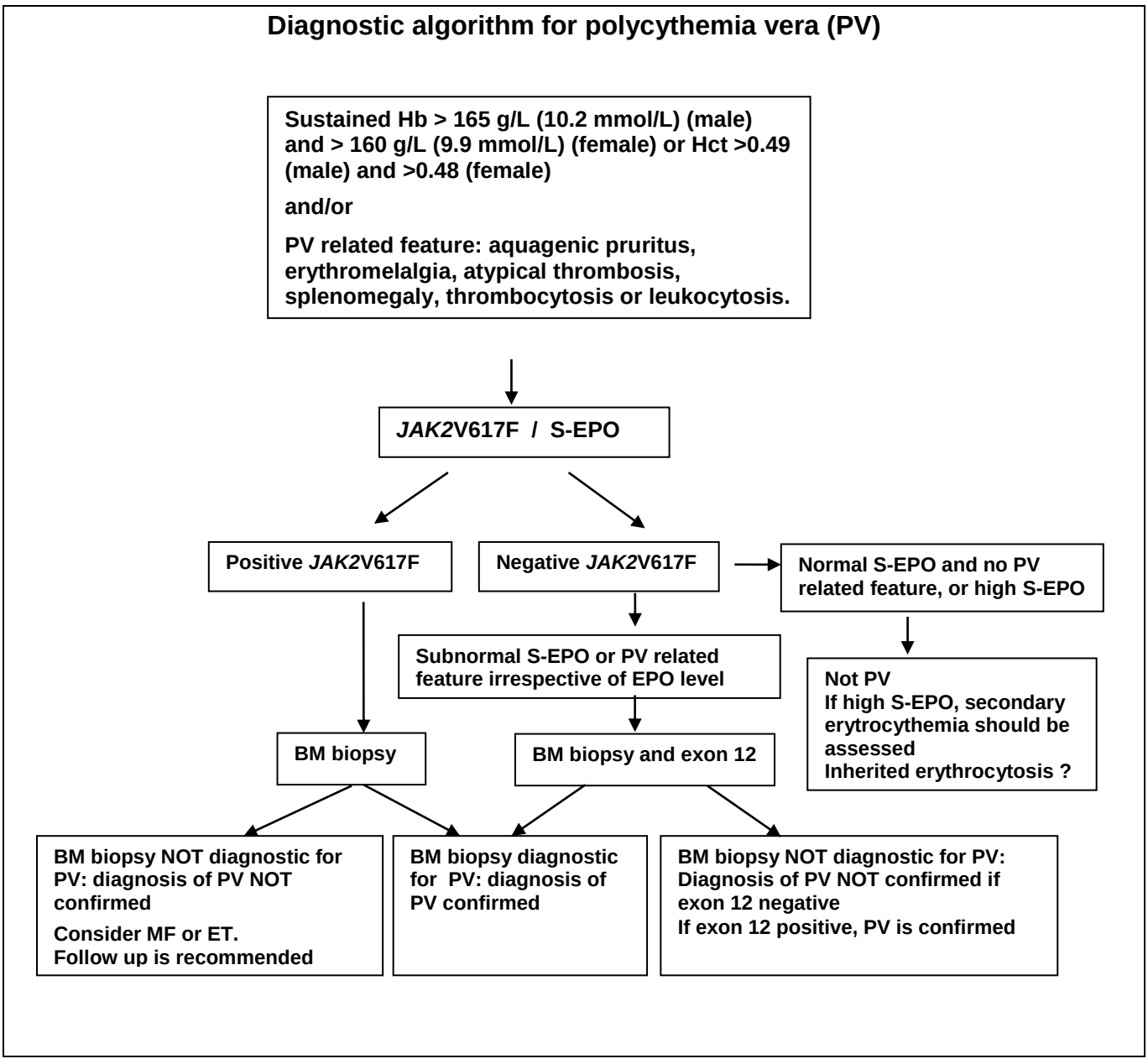
So called “masked PV” (Arber 2016, Barbui 2014a) should also be suspected in a patient with a Hct/Hb below the diagnostic threshold, if combined with a PV-related feature, e.g. an arterial or venous thrombotic event, especially in patients with an atypical thrombosis (eg. splanchnic vein thrombosis, Budd-Chiari syndrome, sinus thrombosis), aquagenic pruritus, erythromelalgia, or other symptoms of acral ischemia, splenomegaly, leukocytosis, thrombocytosis, or microcytosis. Patients with masked PV exhibit a male predominance and a more frequent history of arterial thrombosis and thrombocytosis compared to overt PV (Barbui 2014a).

Patients with PV typically have a serum erythropoietin (S-EPO) that is subnormal or in the lower reference interval. However, in later years up to 25 % of PV patients have a normal S-EPO due to substandard methods used in clinical laboratories.

Diagnostic work-up in PV

- Physical examination including palpation of spleen
- Evaluation of cardiovascular risk factors
- Full blood count with differential count
- Iron status
- ASAT, ALAT, bilirubin, creatinine, uric acid, lactate dehydrogenase (LDH)
- S-EPO
- *JAK2*V617F mutation on blood sample
- *JAK2* exon 12 mutation in *JAK2*V617F-negative patients on blood sample
- Bone marrow biopsy and aspirate (for iron staining)

Diagnostic algorithm for polycythemia vera



Legend: BM bone marrow, EPO Erythropoietin, ET essential thrombocythemia, MF myelofibrosis, PV polycythemia vera,

Risk stratification in PV referring to the risk for thrombosis and bleeding

High risk

Patients are considered high risk if they fulfill at least one of the following criteria: > 60 years, previous thrombosis, or platelet counts > 1500 x 10^9/L. (Tefferi & Barbui 2015). These patients should be treated with cytoreductive therapy. PV patients with isolated erythrocytosis i.e. normal white cell and platelet counts, seen primarily in PV patients with exon 12 mutation, can initially be treated with phlebotomy alone, until leukocytosis or thrombocytosis occurs.

Low risk

Patients < 60 years, no previous thrombosis and platelets < 1500 x 10^9/L are considered low risk patients (Barbui 2013). These patients should in general not receive cytoreductive therapy, but such therapy can be considered in certain situations (see below).

Clinical management of polycythemia vera

It is highly recommended that PV patients are included in prospective clinical trials if available.

Goals of therapy in polycythemia vera

- Avoid first occurrence or recurrence of thrombotic and bleeding complications
- Maintain good quality of life and reduce constitutional symptoms (weight loss, night sweats, fever, pruritus)
- Manage risk situations (e.g. pregnancy, surgery)
- Minimize the risk of acute leukemia and post-PV myelofibrosis

Summarized recommendations

- Vigorous treatment of cardiovascular risk factors
- Phlebotomy to maintain a Hct < 0.45 in both men and women
- Aspirin 75 - 100 mg/day unless contraindicated
- Cytoreduction should be given to high-risk patients. The goal of therapy should be normalization of peripheral blood counts.
- Cytoreduction can also be considered in low risk patients:
    - with poor tolerance/high frequency of phlebotomies
    - with symptomatic or progressive splenomegaly
    - with other evidence of disease progression e.g. weight loss, night sweats

  - with progressive leukocytosis and/or thrombocytosis
  - with several risk factors for cardiovascular diseases such as smoking, diabetes, and hypercholesterolemia
  - with a personal motivation for treatment of symptomatic PV based on an individual, balanced information regarding side effects and prognosis
- Stem cell transplantation has only been performed in small number of PV patients who have not developed post-PV myelofibrosis. Transplant prior to fibrosis development should be reserved for the very rare PV patient who does not respond to any cytoreductive therapy.

Phlebotomy

The hematocrit should be maintained at < 0.45 in all patients (Marchioli 2013).
Grade A recommendation, evidence level IB
There is currently no evidence to support a different level of Hct in men and women. A lower limit may be settled for patients experiencing a better QoL individually. Hemoglobin levels should not be used for decision-making regarding phlebotomy.

Aspirin prophylaxis

Aspirin has been shown to reduce both arterial and venous thrombosis in PV (Landolfi 2004) and should be given to all PV patients unless it is contraindicated. In Finland, 50mg tablets are available and the recommended dose is 100 mg. In the other Nordic countries, the recommended dose is 75mg per day.
Grade A recommendation, evidence level IB.

Aspirin (ASA) should **not** be given to patients with platelets > (1000-) 1500 x 10^9/L due to an increased risk of bleeding, instead cytoreductive therapy should be initiated. In case of aspirin allergy, clopidogrel has been used in small phase 2 trials, but so far there exist no data from larger trials on its efficacy in PV. The combination of aspirin and anagrelide should in general not be used due to an increased risk of bleeding. Consider contraindications of aspirin such as (ASA induced) asthma, (recent) history of peptic ulceration, hemorrhage, acquired von Willebrand disease or concomitant hemophilia, or a platelet count in excess of 1000 or a platelet count <50 x 10^9/L before ASA treatment is commenced (London Cancer Alliance 2015).

Choice of cytoreductive therapy in PV

- < 60 years: 1st line interferon-α, 2nd line hydroxyurea, 3rd line ruxolitinib
- > 60 years: 1st line hydroxyurea or interferon-α, 2nd line interferon- α or hydroxyurea, 3rd line ruxolitinib
- > 75 years or with a short expected survival 1st line hydroxyurea, 2nd line *intermittent* busulphan, 3rd line radioactive phosphorus (P^{32})

- Combination therapy (hydroxyurea+anagrelide, hydroxyurea+interferon-α, interferon- α + anagrelide) can be an alternative second line therapy in fit patients if dose-limiting side effects occur with monotherapy.

Grade C recommendation, evidence level IIIB.

Hydroxyurea

The European Leukemia Network (ELN) recommends hydroxyurea or interferon as first line therapy in PV patients at any age (Barbui 2011a). Hydroxyurea is the best documented drug in PV having been the subject of large randomized trials (Kiladjian 2006; Najean & Rain 1997). By itself it has very limited leukemogenic potential, if any (Björkholm 2011). However, long-term use of hydroxyurea in PV has not been able to prevent leukemogenic transformation in 10-20 % of patients after 20 years of therapy in some trials (Kiladjian 2011). Hydroxyurea is not recommended during pregnancy, and should be withdrawn at least 3 months before planned conception both in men and women (page 22).

Recommendation: Hydroxyurea is recommended as one possible first-line cytoreductive therapy in PV in patients > 60 years or younger patients who do not tolerate interferon.
Grade A recommendation, evidence level IB.
The documented high leukemia transformation rate despite long term hydroxyurea treatment in some trials (see above), suggests that hydroxyurea should be used with caution in patients below 60 years.
Grade C recommendation, evidence level IIIB.

Interferon-α

Interferon-α is theoretically superior to other therapies for treating PV as long-standing molecular remissions can be achieved with interferon (Kiladjian 2008a+b; Quintas.Cardama 2009a; Stauffer-Larsen 2013). Interferon does not increase the risk of leukemia (Barbui 2011a). It is along with ruxolitinib the most effective drug for PV related pruritus.

Recommendation: Pegylated interferon is the drug of choice in younger patients (<60 years) and is most likely tolerated in these patients.
Grade B recommendation, evidence level IIA.

It can also be given to older fit patients and also during pregnancy. Pegylated forms of interferon are at least equally effective as conventional interferon, and cause fewer side effects (page 21).

JAK2 inhibitors

Ruxolitinib is approved in the US and Europe for use in *hydroxyurea-resistant/refractory* patients based on a phase 3 trial (Vannucchi 2015; Verstovsek 2016) randomizing advanced PV patients with marked splenomegaly to ruxolitinib vs best available therapy, which however in 60 % of patients was a continuation of already failed therapy with hydroxyurea. The first results showed that a statistically significant greater proportion of patients receiving ruxolitinib obtained Hct control, reduction of

phlebotomies, reduction of enlarged spleen, and improvement of quality of life compared with best available therapy (BAT). A companion trial with the same design, with the exception that splenomegaly was not an inclusion criteria, has also been performed. The results are very similar with better outcome in the ruxolitinib arm compared to BAT. However, also in this trial 49 % of the HU-refractory patients went on with HU, and another 28 % did not receive any bone marrow suppressive therapy (Passamonti 2017). These data support ruxolitinib as a treatment option for *hydroxyurea-resistant or intolerant patients* with polycythemia vera. However, it should be stressed that one of the ELN criteria for hydroxyurea resistance/intolerance has been the focus of criticism, i.e. the need for supplementary phlebotomies in hydroxyurea treated patients. It has been argued that therapy with hydroxyurea and supplemental phlebotomies is an acceptable therapy. Ruxolitinib is contraindicated during pregnancy or breast-feeding.

Recommendation: Ruxolitinib is recommended as 3rd line cytoreductive therapy in PV patients < 75 years that are intolerant/refractory to both interferon and hydroxyurea.
Grade A recommendation, evidence level IB.

Busulphan

Low dose *intermittent* busulphan was more efficacious in controlling PV than radioactive phosphorus in a randomized phase III trial (Haanen 1981).
Grade A recommendation, evidence level IB.

Busulphan is an alkylating agent and can increase the risk for leukemic transformation (Björkholm 2013). Sequential treatment with hydroxyurea and busulphan has been associated with an increased risk of AML in several small studies and should be avoided if possible. However, the higher frequency of AML in these patients may also be an effect of a more aggressive underlying disease (page 23).

Recommendation*:* Busulphan should be reserved for patients 75 years or older, or for patients not tolerating hydroxyurea, interferon, ruxolitinib or anagrelide,
Grade B recommendation, evidence level IIA.

Radioactive phosphorus

Radioactive phosphorus (P^{32}) can control elevated blood counts in PV but is associated with an increased risk for leukemic transformation, especially if used in combination with hydroxyurea. Infrequent intermittent treatment is required and follow-up can therefore be minimized. It is valuable in older patients if compliance with continuous oral therapy is a problem. P^{32} is not available in Denmark.

Recommendation: Due to the leukemogenic effect, P^{32} use should be limited to patients older than 75 years where hydroxyurea, interferon or busulphan are not suitable,
Grade A recommendation, evidence level IA.

Anagrelide

Anagrelide (Xagrid®) is megakaryocyte specific and is therefore seldom used in PV since it is only effective in controlling the platelet count, and probably does not control progression of PV (page 23).

Recommendation: Anagrelide may be used to control thrombocytosis in PV patients who cannot tolerate or do not respond to interferon, hydroxyurea or ruxolitinib, and when hydroxyurea is considered a less suitable alternative (very young patients). Anagrelide should not be used during pregnancy.
Grade C recommendation, evidence level IIIA.

Evaluation of response and follow up

The goal of therapy should be normalized peripheral blood counts. Consider changing therapy in patients with resistance or intolerance to ongoing therapy and in patients not achieving treatment goals. The ELN have published criteria for hydroxyurea resistance and intolerance (Barosi 2007, see comment above regarding the phlebotomy criterion).

Patients on phlebotomy alone should be monitored with complete blood counts at least every 4 to 6 weeks. For recommendations regarding patients on cytoreductive therapy, see p fill in later. There is no indication to use repeated bone marrow trephine biopsies for routine follow-up in PV, but is essential in assessing transformation to myelofibrosis or acute leukemia. Monitoring of molecular response, including sequential assessment of the *JAK2*V617F allele burden is at the moment not recommended for routine clinical use. Measurement of quantitative *JAK2*V617F PCR every 6 months during interferon therapy may however be used to monitor a molecular response in order to guide decisions on stopping interferon therapy (Barosi 2011).

Practial considerations regarding cytoreductive therapy in PV and ET

Interferon

Interferon suppresses growth of multipotent hematopoietic progenitor cells and may contribute to the generation of T-cell mediated anti-tumor response. Pegylated interferons, which are administered weekly, are today being used in most centers due to an increased convenience and milder side effects. Initially, in patients with high risk (or high cell counts) hydroyurea may be added for more rapid cytoreduction, and then gradually tapered during monitoring. The starting dose for pegylated interferon α-2a (Pegasys®) is 45-90 µg subcutaneously once weekly. The large majority of patients respond to a dosage of 45-90 µg once weekly. When the response is stable, controls every 6-12 weeks is sufficient. The starting dose of pegylated interferon α-2b (PegIntron®) is (25-) 35 µg subcutaneously once weekly. Most patients respond to a dose between 50 - 80 µg. Responses to pegylated interferons are seen within 2-3 months. If cell counts are still elevated after 2-3 months, the dose should be increased. When sufficient effect is reached and sustained at a stable level, one possible approach is to taper the dose of interferon to the lowest effective dose. However, if the goal of therapy is molecular

remission this must be accompanied by serial JAK2 V617F measurements to ascertain that the allele burden does not increase.

Pegylated interferons can be associated with several side effects, e.g. flu-like symptoms, mild elevation of liver transaminases, hyper- and hypothyroidism and psychiatric disorders. Therefore, interferons should be used with caution and in collaboration with psychiatrists in patients with pre-existing psychiatric conditions, especially depressive states. In uncontrolled autoimmune disease it is contraindicated. Flu-like symptoms may be transient and can be well controlled by paracetamol. Treatment should be stopped if patients develop psychiatric disorders. Interferon may be used in pregnancy but is still not recommended during breast-feeding.

Hydroxyurea

Hydroxyurea is a non-alkylating, non-specific myelosuppressive drug. The recommended starting dose is 500-1000 mg daily (15-20 mg/kg/day). Elderly patients (>70 years old) are usually started on 500 mg/day. In situations when rapid platelet reduction is needed higher starting doses (1500-2000 mg/day) are recommended. Elevated leukocyte and platelet counts may decrease within days. Follow-up with full blood count is recommended every second week initially. The dose should be adjusted in order to achieve a stable platelet count of 200 – 400 $x10^9$/L. When this is achieved, control of full blood counts every 4 to 8 weeks is usually sufficient. The average dosage needed is approximately 10-14 tablets a week, lower in elderly patients. Hydroxyurea should be administered continuously. Interruptions in the treatment can result in abrupt rise in the platelet count and risk of thrombosis.

Leukopenia with neutrophil count of 1.0 – 1.5 $x10^9$/L and anemia can occur during the first 3-6 months and is accepted when the goal is to reduce large splenomegaly. In some cases the neutropenia or anemia is dose-limiting, but if the platelet count can be kept below 600 $x10^9$/L, this can (sometimes) be acceptable.

Some patients will experience other significant side effects from hydroxyurea, including gastrointestinal disturbance, skin pigmentation and increased risk of skin malignancy, mucocutaneous and leg ulcers. The latter can be seen in up to 10% of patients, and do not heal until hydroxyurea is discontinued. As a general rule hydroxyurea should not be reinstated after wound healing. International recommendations for HU resistance and intolerance are available (Barosi 2010). Hydroxyurea is contraindicated during pregnancy or breast-feeding.

Ruxolitinib in PV (Not studied or recommended in ET)

The recommended starting dose of ruxolitinib in PV is 10 mg given orally twice daily. There is limited information to recommend a starting dose for patients with platelet counts between 50 and <100 x 10^9/L. The maximum recommended starting dose in these patients is 5 mg twice daily and the patients should be titrated cautiously. When ruxolitinib is administered with strong CYP3A4 inhibitors or dual inhibitors of CYP2C9 and CYP3A4 enzymes (e.g. fluconazole) the unit dose of ruxolitinib should be

reduced by approximately 50%. Complete blood count should be monitored every 2-4 weeks until ruxolitinib doses are stabilized, and then as clinically indicated.

The most common side effect of ruxolitinib is myelosuppression. Dose reductions should be considered if the platelet count decreases below 100 $x10^9$/L. In PV, dose reductions should also be considered if hemoglobin decreases below 120 g/L (7.4 mmol/L) and is recommended to pause if hemoglobin decreases below 100 g/L (6.2 mmol/L).

Before therapy with ruxolitinib, patients should be assessed for the risk of developing serious bacterial, mycobacterial, fungal, and viral infections due t.o the immunosuppressive effects of ruxolitinib. Herpes zoster, CMV retinitis and several other opportunistic infections have been reported in patients treated with ruxolitinib (Kirito 2016). Before starting treatment, patients should be evaluated for active and inactive ("latent") tuberculosis, as per local recommendations. In patients with chronic HBV infections taking ruxolitinib, increase in the hepatitis B viral load (HBV-DNA titer) increases, with and without associated elevations in alanine aminotransferase and aspartate aminotransferase, have been reported. Patients with chronic HBV infection should be treated and monitored according to clinical guidelines. Progressive multifocal leukoencephalopathy has also been reported (Wathes 2013).

Anagrelide

Anagrelide (ANA) has a selective effect on megakaryocytes in vitro and reduces platelet levels with the same efficacy as HU, although with a slower onset. The treatment goal of platelets < $400x10^9$/L is usually reached within 8-10 weeks. The standard starting dose of 0.5 mg twice daily should not be exceeded due to risk of side effects. Dose increase should not exceed recommended 0.5 mg/day/week (Rey 2014). The average maintenance dose is 1.5-2.5 mg daily, divided in 2-4 doses daily to reduce side effects. Side effects are most common during the first weeks of treatment and usually subside. Palpitations and tachycardia are the most common side effects and can be treated with a low dose of beta blocker. Loose stools can be reduced with loperamide. Side effects are most common during the first weeks of treatment and usually subside. ANA has no leukemogenic properties. Myelofibrosis, although rare, was more common in ANA-treated patients in a large study (Harrison 2005). ANA is second-line treatment in early myelofibrosis. ANA should be used with caution in patients with previous cardiac insufficiency or arrhythmia. Cardiac disease screening has been shown to be of little value before treatment initiation (Gugliotta 2011). Coronary disease is no contra-indication to ANA (Tortorella 2016) but such patients should be closely monitored for possible tachycardia. Combination with aspirin should be used with caution and avoided in patients with previous bleeding problems. ANA should not be used when pregnancy is planned or during pregnancy, since there is no data available concerning effects on the fetus.

Busulphan

Busulphan is an alkylating agent that is given intermittently. Start with 2-4 mg daily until response (normally 2-6 weeks). A full blood cell count should be checked every week while the patient is on busulphan therapy. The leukocyte count may increase during the first 10 to 15 days, this should not be

interpreted as resistance to the drug and the dose should not be increased. If busulphan is started at 4 mg/day, the dose should be lowered to 2 mg/d when platelets start to decrease, typically within 2-6 weeks. Busulphan should be stopped when the platelet count reaches 400 $x10^9$ as platelets will continue to fall for another 2-3 weeks. Busulphan can be repeated when the platelet count rises above 400 $x10^9$ again. High cumulative doses of busulphan have been associated with an increased risk of leukemic transformation and busulphan should therefore be used only in elderly patients or patients with a short life expectancy.

Radioactive phosphorus

Radioactive phosphorus (P^{32}) is an orally given radioactive treatment that effectively controls hematopoiesis. P^{32} is associated with an increased risk of progression to acute leukemia, particularly when used sequentially with hydroxyurea. Blood counts should be checked every other week until platelet counts have normalized, after that every 8-12 weeks. The dose of P^{32} is adjusted according to age, weight, and previous treatment and is calculated by the radiotherapists.

Primary and secondary myelofibrosis

Primary myelofibrosis (PMF) is one of the classic Ph'negative myeloproliferative neoplasms (MPN) (Arber 2016). It is not possible clinically or in paraclinical tests to separate PMF from myelofibrosis secondary to previous polycythemia vera (PV) and essential thrombocythemia (ET), unless this preceding, other MPN diagnosis is substantiated by medical records. The diagnostic work-up and treatment is included in the same care program here.

PMF is characterized by progressive accumulation of connective tissue and endothelial proliferation in the bone marrow accompanied by extramedullary hematopoiesis with enlargement of the spleen and liver. PMF is associated with a significant excess mortality and the median survival of PMF patients is 5-6.5 years with a wide range (Cervantes 2009; Hultcrantz 2012; Cervantes 2012). The concept of "prefibrotic" myelofibrosis (prePMF) is increasingly recognized as the early phase of myelofibrosis in the biological continuum from early disease stage to the advanced stage of myelofibrosis with myeloid metaplasia. Accordingly, the two forms prePMF and PMF have been assigned separate diagnostic criteria in the revised 2016 WHO classification (Arber 2016). Survival depends on the risk grade and is substantially longer in low risk patients or in patients with prePMF (Barosi 2012).

Clinically PMF is characterized by progressive hypermetabolic symptoms such as unintended weightloss, night sweats and perhaps slight fever, worsened by a growing spleen, and abdominal discomfort which also may be due to hepatomegaly. Fatigue and loss of physical functions become more manifest as anemia worsens. The leukocyte and platelet counts may convert quantitatively from a proliferative to a cytopenic pattern due to progressive marrow fibrosis and splenic pooling, accompanied by symptoms of infection and bleeding, which also may be facilitated by qualitative defects. Gastrointestinal vascular complications also occur due to bleeding from a hypertensive gastropathy and eosophagus varices, and stagnation of circulation may lead to episodes of splenic infarction (Hasselbalch 1990a+b; Tefferi 2016).

PrePMF is characterized by a hypercellular bone marrow with megakaryocytic, and in contrast to true ET, also granulocytic proliferation. Megakaryocytes show extensive tight clustering and condensed nuclei with clumped chromatin and abnormal nuclear-cytoplasmic ratio. Reticulin fibrosis is absent or minimal $\leq$ MF-1 (Arber 2016). It is important to recognize that prePMF exhibits the three major criteria of the WHO classification, but leukoerythroblastosis, splenomegaly and anemia is most often not present. There may still be some controversy around this concept as a separate disease entity, and how to distinguish this from ET by distinct histopathological features (Wilkins 2008; Madelung 2013). In clinical practice the occurrence of anemia, elevated leukocyte count or elevated LDH in "ET" patients should alert the clinician to reevaluate the diagnosis and rule out PMF (Carrobio 2011; Skov 2016).

In a large retrospective trial, more than 1100 patients diagnosed as having ET by pre-WHO criteria were reanalyzed. In 81 % the diagnosis was confirmed as ET, whereas 16 % were reclassified as

prePMF and 3 % were not evaluable. Clinical follow-up of these patients showed no differences in thrombosis rates. In contrast, 10 and 15 year overall survival was markedly lower in prePMF, 76 vs. 89 % at 10 years and 59 vs. 80 % at 15 years, respectively. Rates of transformation to acute leukemia and progression to overt PMF were clearly increased in prePMF (Barbui 2011c). Further analyses of this cohort have shown that major bleeding was more common in prePMF compared to ET, especially in patients treated with aspirin (Finazzi 2012). Finally, in a sub-study where 178 ET patients below 40 years of age at diagnosis were compared to 35 patients with prePMF, progression to overt PMF was more common in patients with prePMF and there was a trend towards more arterial thrombosis. Transformation to leukemia was not observed during a median follow-up of 7.6 years (Barbui 2012c).

2016 WHO diagnostic criteria for prePMF and PMF (Arber 2016)

Prefibrotic Primary Myelofibrosis (PrePMF) criteria

Major criteria

1. Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1*, accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis
2. Not meeting the WHO criteria for BCR-ABL positive CML, PV, ET, 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,† or absence of minor reactive bone marrow reticulin fibrosis‡

Minor criteria

Presence of at least 1 of the following, confirmed in 2 consecutive determinations:

a. Anemia not attributed to a comorbid condition
b. Leukocytosis $\geq$11 x 10^9/L
c. Palpable splenomegaly
d. S-LDH increased to above upper normal limit of institutional reference range

Diagnosis of prePMF requires meeting all 3 major criteria, and at least 1 minor criterion

Primary Myelofibrosis (PMF) criteria

Major criteria

1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3*
2. Not meeting WHO criteria for ET, PV, BCR-ABL-positive CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,† or absence of reactive myelofibrosis‡

Minor criteria

Presence of at least 1 of the following, confirmed in 2 consecutive determinations:

a. Anemia not attributed to a comorbid condition
b. Leukocytosis $\geq$11 x 10^9/L
c. Palpable splenomegaly
d. S-LDH increased to above upper normal limit of institutional reference range
e. Leukoerythroblastosis in blood smear

Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion

†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.

‡ *Minor (grade 1) reticulin fibrosis or higher grades of* 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.

Grading of myelofibrosis

- MF-0 Scattered linear reticulin with no intersections (crossovers) corresponding to normal BM
- 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 focal bundles of thick fibers mostly consistent with collagen, and/or focal osteosclerosis*
- MF-3 Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of thick fibers consistent with collagen, usually associated with osteosclerosis*
- Semiquantitative grading of bone marrow fibrosis (MF) with minor modifications concerning collagen and osteosclerosis
- Fiber density should be assessed only in hematopoietic areas

**In grades MF-2 or MF-3 an additional trichrome stain is recommended*

Legend: CML Chronic Myeloid Leukemia, ET Essential Thrombocythemia, MDS Myelodysplastic Syndrome, MF Myelofibrosis, MPN Myeloproliferative Neoplasia, S-LDH serum lactate dehydrogenase, PV Polycythemia Vera.

Differential diagnosis includes other Ph'negative MPN, chronic myeloid leukemia, myelodysplastic syndrome, acute leukemia and hairy cell leukemia, as well as reaction to other malignancy, inflammation, infection or vitamin D deficiency.

Diagnostic work-up of PMF

The clinical picture of PMF varies form asymptomatic disease diagnosed due to abnormal blood tests to sometimes severe symptoms relating to bone marrow failure (anemia, infection, bleeding) or progressive splenomegaly (constitutional symptoms, abdominal or bone pain).

The diagnostic work-up includes:

- Physical examination including palpation of the liver and spleen
- Evaluation of cardiovascular risk factors (smoking, blood pressure, fasting blood glucose, HbA1c, cholesterol, triglyceride)
- Hematology: Full blood count including differential count of white blood cells, blood smear for leukoerythroblastosis, and hematocrit
- Iron status (s-Fe, -TIBC, -Ferritin)
- Biochemisty: ASAT, ALAT, bilirubin, alkaline phosphatase, LDH, creatinine, uric acid, plasma calcium, phosphate, 25-hydroxyvitamin D
- S-EPO
- Molecular genetics
    - *JAK2*V617F mutation. *CALR* mutation in *JAK2* negative patients. *MPL* in *JAK2*V617F and *CALR* mutation negative patients
    - BCR-ABL in *JAK2*V617-negative patients if differentiation against chronic myelogenous leukemia is not clear
- Bone marrow biopsy with staining for evaluation of fibrosis

In patients eligible for allogeneic SCT

- Bone marrow cytogenetics
- Other mutations e.g. *ASXL1, EZH2, IDH1, IDH2, SRSF2, SF3B1, TET2, TP53*

Prognosis and risk stratification in PMF

Complications of PMF are common and contribute significantly to morbidity and mortality. Common complications are infections (20-60 %), cardiovascular events (20-50 %), thromboembolic (10-40 %), and hemorrhagic events (30 %) (Barbui 2010; Hasselbalch 1990a+b). Transformation to acute leukemia is seen in about 10-30 % of the patients (Cervantes 2009; Passamonti 2010).

Molecular genetic markers

The *JAK2*V617F mutation is found in 50-60 % of PMF patients (Baxter 2005; Kralovics 2005; Levine 2005; Rumi 2014; Tefferi 2016). A low *JAK2* mutation allele burden in such cases was found associated with poor prognosis (Tefferi 2008; Guglielmelli 2009).

A calreticulin (*CALR*) mutation is found in most patients with PMF who are *JAK2* mutation negative (Klampfl 2013). More than 50 *CALR* mutations, all in exon 9, have been described in MPN. The two

most frequent *CALR* mutations in PMF correspond to a 52 nucleotide deletion (also called type 1) and a 5 nucleotide insertion (type 2) respectively (Tefferi 2014; Vainchenker & Kralovics 2017). In PMF, presence of *CALR* type 1 mutation was associated with superior survival as compared to *JAK2*V617F mutated patients, whereas *CALR* type 2 mutation was found unfavorable. Lower WBC and blast cell counts, higher platelet counts and lesser anemia were associated with the *CALR* type 1 mutation (Tefferi 2014).
A myeloproliferative leukemia virus oncogene (*MPL*) mutation is found in 4-10% of PMF (Rumi 2014, Tefferi 2016). Approximately 10% of PMF patients are "triple negative" (no *JAK2, CALR* or *MPL* mutation), a situation reported to be prognostically unfavorable (Rumi 2014).

In selected "triple negative" cases a search for other clonal markers may be warranted (Arber 2016; Passamonti & Maffioli 2016; Tefferi 2016). Among such mutations associated with PMF, *ASXL1, EZH2, IDH1, IDH2* and *SRSF2* were reported to have negative prognostic significance regardless of DIPSS or DIPSS-plus scores (Guglielmelli 2014; Saeidi 2016; Tefferi 2016).

Cytogenetic markers

Leukemic transformation is predicted by the presence of unfavorable karyotype, which encompasses complex karyotype or sole or 2 abnormalities including +8, -7/7q-, i(17q), -5/5q-, inv(3), 12p-, or 11q23 rearrangement (Gangat 2011; Tefferi 2012).

Prognostic scoring systems

International Prognostic Scoring System (IPSS)

The International prognostic scoring system (IPSS) was introduced by the International Working Group for Myelofibrosis Research and Treatment in 2009 (Cervantes 2009). The IPSS is used for risk stratification at diagnosis. Patients are divided into four prognostic groups: low risk, intermediate-1, intermediate-2, and high risk, based on five risk factors, see Table 1. These prognostic scores do not include prePMF and neither post-PV/post-ET MF even though in clinical practice and in studies the two latter are also classified according to these systems.

Dynamic International Prognostic Scoring System (DIPSS) and DIPSS-plus

The IPSS has been modified to dynamic IPSS (DIPSS) that can be used at any time during the course of the disease (Passamonti 2010). Recently DIPSS has been upgraded to DIPSS-plus by the incorporation of three additional independent risk factors, see Table 1 (Gangat 2011). The eight DIPSS-plus risk factors define low (no risk factors), intermediate-1 (1 risk factor), intermediate-2 (2-3 risk factors), and high (≥ 4 risk factors) risk groups with median survivals of 15.4, 6.5, 2.9, and 1.3 years, respectively (Gangat 2011). Leukemic transformation was predicted by the presence of unfavorable karyotype (complex karyotype or sole or 2 abnormalities including +8, -7/7q-, i(17q),

-5/5q-, inv(3), 12p-, or 11q23 rearrangement) or platelet count < 100 x 10^9/L (Gangat 2011; Tefferi 2012).

We recommend the use of IPSS at diagnosis and DIPSS during follow-up. In order to identify patients with progressive disease, the patient's DIPSS score should be evaluated at every visit. The DIPSS-plus is not yet in broad clinical use but can be helpful in younger patients if there are insecurities regarding risk group and indication for allogeneic stem cell transplantation.

Table 1. Prognostic scoring systems IPSS, DIPSS and DIPSS-plus.

Scoring system	To be used	Prognostic factors	Risk score	Risk score and median survival (MS) (months)
IPSS	At diagnosis	Age > 65 years	1	Low risk (score 0), MS 135 Intermediate-1 risk (score 1), MS 95 Intermediate-2 risk (score 2), MS 48 High risk (score ≥ 3), MS 27
		Anemia (Hb< 10g/dL)	1	
		Leukocyte count >25x10^9/L	1	
		Circulating blasts ≥ 1 %	1	
		Constitutional symptoms (fever, excessive sweats, weight loss)	1	
DIPSS	During follow up	Age > 65 years	1	Low risk (score 0), MS not reached Intermediate-1 risk (score 1-2), MS 170 Intermediate-2 risk (score 3-4), MS 48 High risk (score 5-6), MS 18
		Anemia (Hb < 10g/dL)	2	
		Leukocyte count > 25x10^9/L	1	
		Circulating blasts ≥ 1 %	1	
		Constitutional symptoms	1	
DIPSS-plus*	During follow up	DIPSS low risk	0	Low risk (score 0), MS 185 Intermediate-1 risk (score 1), MS 78 Intermediate-2 risk (score 2-3), MS 35 High risk (score ≥4), MS 16
		DIPSS intermediate-1	1	
		DIPSS intermediate-2	2	
		DIPSS high risk	3	
		RBC transfusion-dependent	1	
		Unfavorable karyotype¶	1	
		Platelet count < 100x10^9/L	1	

* Calculate first the DIPSS score, and then add the score for transfusion dependency, cytogenetics and thrombocytopenia to calculate the final DIPSS-plus score.

¶ Prognostic unfavorable karyotype: complex karyotype or sole or 2 abnormalities including +8, -7/7q-, i(17q), -5/5q-, inv(3), 12p-, or 11q23 rearrangement.

Clinical management of prefibrotic and primary / secondary myelofibrosis

It is highly recommended that prePMF, PMF or patients with myelofibrosis secondary to ET or PV are included in prospective clinical trials if available.

Patients with post-PV or post-ET MF should be treated according to the guidelines given for PMF below.

Treatment of prePMF

There is currently no evidence that treatment of prePMF is associated with prolonged survival. The majority of patients in former studies received hydroxyurea (HU) therapy. Whether other treatment modalities such as interferon can prevent or prolong time to overt PMF progression remains unknown, but there are ongoing studies to address this question (Mascarenhas 2015; Koschmieder 2016). For the time being patients with prePMF without risk factors should not be treated with cytoreductive therapy outside study protocols. An exception may be patients with a personal motivation for treatment of prePMF. This decision must be based on individual, balanced information regarding side effects, any contraindications, and prognosis, applicable e.g. regarding IFN treatment (Hasselbalch 2011; Gowin 2012; Gowin 2017) which may possibly be most effective in *JAK2* mutated patients (Pizzi 2015). In patients with risk factors and an indication for cytoreductive treatment for platelet reduction we recommend the same treatment and treatment goals as for ET (page 11).

Goals of therapy in PMF

- Cure if possible, the only curative treatment is allogeneic stem cell transplantation which should be considered when indicated and based on prognostic scoring systems or prognostic mutational status when more established
- Alleviate anemia and other cytopenias when indicated
- Reduce symptomatic splenomegaly
- Reduce constitutional symptoms (weight loss, night sweats, fever, pruritus, fatigue)
- Avoid first occurrence or recurrence of thrombotic and bleeding complications
- Minimize the risk of acute leukemia
- Manage risk situations (e.g. surgery).

It is important to emphasize that treatment options are different in the Nordic countries, and that National recommendations or regulations have to be respected.

Curative treatment of PMF by stem cell transplantation

Allogeneic hematopoietic stem cell transplantation (SCT) is the only curative treatment in PMF, and SCT should be considered in all PMF patients at diagnosis. It is recommended in transplantable patients with intermediate-2 or high risk at diagnosis, and during follow-up of younger low/intermediate-1 patients who progress to a higher risk DIPSS (or DIPSS-plus) (Barbui 2011a; Kröger 2015; Tefferi 2016).

The mortality after SCT in PMF is significant and 5-year survival has been between 30% and 60% in different studies (Abelsson 2012; Ballen 2010; Guardiola 1999). Outcome is better for patients with low risk disease, but due to the high toxicity, transplants should only be performed in patients with an expected survival of less than 5 years with IPSS, DIPSS, or DIPSS-plus risk score of intermediate-2 or high risk (Bacigalupo 2010; Ballen 2010; Gupta 2012; Kröger 2009, Kröger 2015; Patriarca 2008).

Patients over 45 years have a poor survival on myeloablative conditioning. Introduction of reduced-intensity conditioning has significantly improved results in the higher age groups but results are still poorer for patients above 60 years of age due to the high transplant-related mortality (Abelsson 2012). Sorror index can be of value in selection of patients (Armand 2012; Sorror 2005; Sorror 2013 incl link to calculator). Results are similar for sibling donors and matched unrelated donors in the Nordic countries (Abelsson 2012). However, the conditioning regimen may vary.

Recommendation: Allo-SCT with myeloablative or reduced intensity conditioning is indicated in young (< 40 years of age) intermediate-2 or high-risk patients with PMF. Reduced intensity transplantation should be considered for patients aged 40-60 (65) years with intermediate-2 or high risk at diagnosis or later during the course of the disease. SCT should also be considered in intermediate-1 patients with high-risk mutations (*ASXL1* and other, page 28), for whom close monitoring and preparedness is an alternative.
Grade B recommendation, evidence level IIB.

Treatment of anemia

Anemia in PMF is multifactorial and deficiency of iron, vitamin B12 and folic acid should always be ruled out before considering other therapies. As a general guideline, pharmacological treatment of anemia should be initiated at Hb levels approximately < 110g/L (6.6 mmol/L) in symptomatic patients and should be considered in asymptomatic patients with Hb levels < 100g/L (6 mmol/L).

Danazol

Androgens stimulate bone marrow function and have been shown to improve Hb in about 40 % of the patients, in particular those patients with only moderate splenomegaly and normal cytogenetics. In general, treatment with danazol (Danol®) is well tolerated (Cervantes 2005). Side effects include generally modest increases in liver enzymes (in < 20 % of patients), and androgenic side effects in female patients. Danazol is contraindicated in the presence of prostate cancer. Male patients should be screened for prostate cancer (serum PSA) before initiating treatment with danazol (Cervantes 2005).

Danazol is administered at an initial dose of 600 mg/day, divided in three doses. Median time to response has been 5 months (range 1-9 months). After response has been achieved, the dose should be progressively tapered to the lowest effective, often 200 mg/day (Cervantes 2005).

Synergistic effects between steroids and danazol and between human recombinant erythropoietin and danazol have been recorded (Hasselbalch 2002). It is recommended to combine danazol and prednisolone (20-30 mg daily) if no response to monotherapy is seen. Regular monitoring of liver function tests, and in males PSA, is recommended (Cerquozzi 2016). Prophylaxis against osteoporosis must be considered in patients using glucocorticoids.

Danazol is easily available only in Denmark among the Nordic Countries. Individual licence agreements may be applied for from other countries.

Recommendation: Danazol (+/- prednisolone) is, if available, recommended as one of the first-line therapies in the treatment of anemia in PMF.
Grade B recommendation, evidence level IIIB.

Erythropoietin (EPO)

Recombinant human erythropoietin (EPO) has been shown to effectively increase the Hb-concentration in 20-60 % of PMF patients in non-randomized studies (Huang & Tefferi 2009). The starting dose is 30.000 U once weekly, and may be increased to twice weekly in patients not responding after 6 weeks of therapy. Darbepoietin-α administered once a week is equally effective and the recommended dose is in the range 150-300ug/week subcutaneously. If no response is seen after 8 weeks of full dose EPO therapy should be discontinued. A treatment trial with EPO may be suggested at Hb-levels < 100 g/L (6 mmol/L) (Tefferi 2016). The therapy goal in other hematological malignancies is Hb-levels around 120 g/L (7 mmol/L), reasonable also in PMF. A higher Hb-level should be avoided in order to minimize the risk of thrombosis.

A S-erythropoietin below 125 U/L has been clearly associated with a higher probability of response. However, higher S-EPO levels do not preclude patients from responding (Huang & Tefferi 2009). Unfortunately EPO is often ineffective in transfusion dependent patients, and could exacerbate splenomegaly, limiting its usefulness (Tefferi 2016).

Recommendation: Erythropoietin is recommended as alternative to danazol, when unavailable for treatment of anemia in PMF in patients. Combination regimens for anemia may be effective. The treatment benefit on Hb and transfusion dependency should be assessed after 4 months.
Grade B recommendation, evidence level IIB.

Glucocorticoids

Treatment with glucocorticoids is indicated in patients with Coombs positive immune hemolysis, but may also be effective in patients with anemia without overt hemolytic activity. In the latter situation, a staring dose of 30 mg prednisolone is recommended, with individual tapering.
Grade C recommendation, evidence level IV.

Thalidomide and thalidomide analogues

Thalidomide can increase the Hb-level and decrease spleen size in PMF patients. Low-dose thalidomide (50mg/day) in combination with prednisolone can improve anemia in 20-30 % of patients (Thapaliya 2011). However, thalidomide is associated with non-hematological toxicity including peripheral neuropathy, constipation and rash. Lenalidomide and prednisolone have similarly been reported with an improvement in spleen size and anemia in PMF (Quintas-Cardama 2009b).

Recommendation: Low-dose thalidomide (50 mg/day) + prednisolone (1 mg/kg for 2 weeks and afterwards tapering to the lowest dose maintaining an adequate Hb-concentration) is recommended for patients not responding to danazol or erythropoietin. Prophylaxis against osteoporosis must be considered in patients using glucocorticoids. In the rare patient harboring del(5q) lenalidomide should be considered.

Grade B recommendation, evidence level IIB.

Treatment of symptomatic splenomegaly and constitutional symptoms

Hydroxyurea (HU)

The efficacy and safety of HU (0.5 g/2d – 2 g/d) in the treatment of PMF has been reported in several studies (Löfvenberg & Wahlin 1988; Martinez-Trillos 2010). Whereas HU lowers elevated leukocyte and platelet counts within days, regression of an enlarged spleen may take several months. HU can also alleviate constitutional symptoms. In some patients bone marrow fibrosis may regress during treatment with HU (Löfvenberg 1990), although this finding has not been reproduced in most recent larger studies.

Recommendation: Hydroxyurea is recommended as first-line cytoreductive therapy in older PMF patients not eligible for transplantation, unless the patient is candidate for ruxolitinib by National recommendations. Dosages and side effects are commented above and previously (page 22).

Grade B recommendation, evidence level IIB.

Interferon-α (IFN)

Several studies have shown that interferon-α (IFN) may be efficacious in PMF, in particular for patients in the hyperproliferative stage of the disease (Gowin 2012: Gowin 2017; Hasselbalch 2011; Ianotto 2009; O´Neill 2016; Pizzi 2015; Silver 2011). In addition, it is assured that IFN is not leukemogenic. IFN-α treatment may also be associated with regression of bone marrow fibrosis, especially in patients with lower grades of fibrosis (Pizzi 2015; Silver 2011). However, in PMF patients with advanced fibrosis treatment with IFN is associated with significant side effects and a high degree of discontinuation.

Recommendation: IFN-α is recommended as first-line therapy in patients < 60 years who are not immediate candidates for transplantation. IFN-α may be considered to patients > 60 years old, who have good performance status and no contraindications for IFN-α therapy. Patients should be in the hyperproliferative phase of the disease and not have extensive fibrosis. Dosages and side effects are

commented previously (page 21).
Grade B recommendation, evidence level IIB.

Jak2 inhibitors

So far, ruxolitinib is the only *JAK2* inhibitor approved in the USA and in Europe (Jakavi®). Ruxolitinib has been shown to reduce spleen volume by at least 35 % in 40 % of patients with intermediate-2 or high risk disease (Harrison 2012; Verstovsek 2012a+b). Most patients experience very rapid relief in constitutional symptoms within a few days from start of therapy and the reduction of spleen size is usually seen within 2-6 weeks of therapy. Responses have been durable in the course of the two randomized phase 3 clinical trials comparing ruxolitinib to placebo (Verstovsek 2012a) or best available therapy (Harrison 2012). So far there is no clear evidence that ruxolitinib can slow disease progression. A survival benefit has been suggested in patients on ruxolitinib when compared to patients on placebo or best available therapy (Harrison 2012; Harrison 2016; Verstovsek 2012a+b). However, a Cochrane Review concluded that the evidence was insufficient to allow any conclusion regarding the efficacy of the drug in myelofibrosis, mainly due to lack of statistical potency of the phase 3 trials to measure a possible survival gain (Martí-Carvajal 2015).

Ruxolitinib is approved in symptomatic patients with myelofibrosis. It has not been studied thoroughly in patients with low and intermediate-1 risk disease and is not recommended for patients in these disease stages at the present time. In the phase 3 studies, the ruxolitinib dose was 15-20 mg x 2 daily. Ruxolitinib can be given with caution to patients with platelet counts below $50x10^9$/L but should be withdrawn at levels <20-$25x10^9$/L. It is recommended to start with lower doses e.g. 5 mg x2 in thrombocytopenic patients. Also in anemic patients, it may be recommended to start with lower doses e.g. 10 mg x2 in patients with moderate symptoms and without massive splenomegaly, to reduce the negative effect on anemia. The dose may thereafter be escalated depending on the response (Cervantes 2014).

Most common adverse events include anemia, thrombocytopenia, headache and diarrhea. Increased risk of respiratory, urinary and gastrointestinal tract infections as well as herpes zoster infections has been reported. The immunosuppressive effect has also manifested as an increased risk of non-melanoma skin cancers (Harrison 2016).

In one center occasional patients experienced rebound symptoms on discontinuation and the drug may therefore be tapered during a period of a week if discontinuation is not immediately necessary due to severe side effects (Tefferi & Pardanani 2011; Tefferi 2011). This rebound phenomenon has not been seen in a larger patient population (Verstovsek 2012b).

Recommendation: Ruxolitinib is indicated in patients with symptomatic splenomegaly and /or constitutional symptoms due to intermediate-2 and high-risk PMF, and in patients with postET/PV myelofibrosis.
Grade A recommendation, evidence level IB.

Data are emerging, but until ongoing trials in patients with low and intermediate-1 risk patients (in DIPSS) have been completed, ruxolitinib can not be generally recommended in these disease stages.

Ruxolitinib may also be considered in patients in need of fast relief of splenomegaly and symptoms prior to stem cell transplantation. The drug is currently being investigated in this setting in several trials.

Splenectomy

In addition to mechanical discomfort and early satiety, a massively enlarged spleen is associated with portal hypertension and a hyperdynamic portal flow, implying an increased risk of bleeding from the upper gastrointestinal tract. Furthermore, the enlarged spleen contributes to the development of anemia and thrombocytopenia consequent to pooling and sequestration of red blood cells and platelets. All these features of hypersplenism may be alleviated by splenectomy with symptomatic improvement in most patients and a rise in Hb-concentration in about half of the patients. Thrombocytopenia is also improved in approximately 50% of patients (Mesa 2006; Taner 2013).

Accordingly, before the ruxolitinib era the main indications for splenectomy in PMF were in addition to pronounced mechanical discomfort episodes of upper gastrointestinal bleeding secondary to portal hypertension (varices), and transfusion-dependent anemia.
However, ruxolitinib effectively reduces splenomegaly in most patients and may actually also be associated with regression of portal hypertension and thereby esophagus varices. Therefore, splenectomy is only indicated in those patients with huge splenomegaly being refractory to treatment with ruxolitinib.

Since the procedure is associated with significant morbidity (25-30 %) and mortality (7-10 %), conditioning and timing of the patient and expertise of the surgeon are of utmost importance (Mesa 2006). There is no evidence in the literature to support the contention that splenectomy is followed by an increased risk of leukemic transformation (Barosi 1998). Splenectomy prior to SCT in patients with huge spleens is a matter of debate.

Recommendation: Splenectomy should be considered in the rare patients with marked splenomegaly associated with repeated upper gastrointestinal bleeding episodes due to portal hypertension and/or cytopenias secondary to hemodilution, splenic pooling and sequestration of blood cells, not responsive to HU, IFN-α, or ruxolitinib and fit for the operative procedure.
Grade B recommendation, evidence level IIIB.

Splenic irradiation

Several reports have documented that irradiation of the spleen may benefit symptomatic patients with huge spleens. However, the risk of ensuing prolonged and severe cytopenias is considerable, probably also due to an effect on circulating progenitor cells. The improvement of symptoms is in most patients but temporary lasting approximately 3-12 months. Irradiation prior to splenectomy is associated with an increased risk of postoperative bleeding (Elliot 1998; Federico 2009; Mishchenko 2010). Irradiation should thus be reserved for patients not responsive to conventional therapy and who are not candidates for splenectomy. Different fractionation schemes have been proposed (Elliot 1998; Federico 2009; Mishchenko 2010). Notably, low intensity (0.2 Gy daily doses x 10 up to the total dose 2 Gy) was found equally effective while causing less cytopenias than higher doses (such as 1 Gy daily up to a total dose of 10 Gy) in a small study (Federico 2009). Repeated irradiation treatment courses

(such as 3-4 courses totally) may alleviate symptoms from enlarged spleens in some patients over a prolonged time. It has also been suggested that preemptive treatment e.g. with 1 Gy as single or fractionated dose every 1-3 months might be effective in reducing problems with enlarged liver or spleen in a palliative setting in selected patients (Mishchenko 2010).
Grade C recommendation, evidence level IIIB.

Other less commonly used therapies in PMF

Busulphan (BU)

Busulphan (BU) was previously used extensively in the treatment of PMF. Low-dose (2 mg/ day) BU is administered in repeated courses of 1-2 months at intervals of 3-6 months. Busulphan is leukemogenic. The sequential use of HU and BU is accompanied by a high risk of leukemic transformation (about 30 %) (Björkholm 2011; Nielsen 2003). Combination therapy with BU and danazol has been reported to be well tolerated and can alleviate constitutional symptoms and increase Hb-levels in selected patients. Practical aspects of BU therapy have been described previously (page 23).
Grade B recommendation, evidence level IIIB.

2-Chlorodeoxyadenosine (2-CdA)

2-Chlorodeoxyadenosine (2-CdA) may be useful in symptomatic patients who do not tolerate other cytolytic agents. In particular, 2-CdA may be used in patients with progressive hepatomegaly and symptomatic leukocytosis and thrombocytosis following splenectomy. It is administered at 0.05-0.1 mg/kg for 7 days monthly for up to five treatment cycles (Faoro 2005).
Grade C recommendation, evidence level IIIB.

Anagrelide (ANA)

Anagrelide (ANA) 0.5-3 mg/d may be used in PMF patients with symptomatic thrombocytosis who do not tolerate other cytolytic agents due to side effects or the development of granulocytopenia without adequate control of the platelet count. This agent does not inhibit progression of myelofibrosis or the production of growth factors in PMF or essential thrombocythemia (Ejerblad 2013; Yoon 1999). ANA should be used with caution in patients with previous cardiac insufficiency or arrhythmia (page 23). Cardiac disease screening has been shown to be of little value before treatment initiation (Gugliotta 2011). Coronary disease is no contra-indication to ANA (Tortorella 2016), but such patients should be closely monitored for possible tachycardia. ANA should not be used during pregnancy.
Grade B recommendation, evidence level IIIB.

Irradiation of lungs and other sites

Irradiation of the lungs (whole-lung external beam radiotherapy in a single fraction of 1 Gy) may induce marked clinical improvement and decrease in pulmonary artery systolic pressure in patients with pulmonary hypertension due to myeloid metaplasia (Mishchenko 2010).

Symptomatic extramedullary hematopoiesis, other than the spleen and liver, may be seen in virtually all organs with infiltrates in the skin, peritoneum (ascites), pericardium (congestive heart failure/pericardial tamponade), pleura, lungs (pulmonary hypertension), brain, spinal cord and bone (granulocytic sarcomas). This manifestation can be treated with low-dose irradiation (Mishchenko 2010).

Avoiding thrombotic and bleeding complications

Retrospective analyses indicate that the incidence of thrombotic complications seems similar in PMF and ET (Barbui 2011c; Carobbio 2011). No prospective trials of platelet reducing agents or aspirin (ASA) have been performed in PMF. We suggest that clinicians follow the guidelines given for ET regarding thrombosis and bleeding prevention in PMF (page 10 and page 18). Aspirin as drug of choice to prevent thrombosis as 75 mg once daily is recommended, considering contraindications such as (ASA induced) asthma or allergy, (recent) history of peptic ulceration, hemorrhage, acquired von Willebrand disease or concomitant hemophilia, or a platelet count in excess of 1000 or a platelet count < $50x10^9$/L (London Cancer Alliance 2015). Low platelet counts may especially be encountered in myelofibrosis.

Evaluation of response and follow-up of PMF patients

- The evaluation of PMF patients should be focused on the major clinical problem(s). Change of therapy should be considered in patients with resistance or intolerance to ongoing therapy and in patients not achieving treatment goals.

- Evaluation of full blood count is recommended at least on a weekly basis when new therapies are started that have a potential to lower blood counts, since PMF patients are especially susceptible to marrow suppression.

- There is no indication for repeated bone marrow trephine biopsies in routine follow-up in stable PMF, but bone marrow investigation is essential in assessing transformation to acute leukemia.

- Quantitative *JAK2*V617F or other molecular marker analysis is recommended in the setting of bone marrow transplantation to monitor residual disease. Outside of the SCT setting, routine monitoring of molecular response is at the moment not validated for clinical use.

Management of complications in MPNs

Practical suggestions to guide clinical decisions in these settings remain largely empirical. Therefore, the recommendations below follow recent experts' consensus recommendations.

Thrombosis

MPN patients are at an increased risk of both thromboembolic events and bleeding, causing most of the morbidity and mortality in PV and ET (Hultcrantz 2015b; Kessler 2004, Tefferi & Elliot 2007). It is therefore, of paramount importance to actively screen for and treat risk factors for vascular events such as smoking, hypertension, hyperlipidemia, and diabetes mellitus in all patients diagnosed with MPN. Vigorous action should also be taken to encourage smoking cessation and weight loss in patients with obesity. The incidence of thrombosis in PV and ET derives mainly from observational and retrospective studies. The incidence of arterial and venous thrombosis ranges from 1.1 to 4.9% per year in PV, and from 1.3 to 6.6% per year in ET (Barbui 2014b; Patrono 2013). In patients with PV or ET not taking aspirin the risk of thrombosis is 5 to 10 fold increased (from 0.2 % per year in the general population to 1.5 and 1.0% per year, respectively in PV and ET) ([1-3]. The risk of thrombotic complications is similar in PMF and ET (Barbui 2010; Barbui 2011c, Carrobio 2011). Risk factors for thrombosis include elevated Hct, WBC count, and cardiovascular risk factors while elevated platelet count has not been linked to an increased risk of thrombosis in MPN patients (Landolfi 2007; Marchioli 2013).

In a recent population-based study of 9665 MPN patients, the absolute risk for arterial and venous thrombosis at time of MPN diagnosis +/- 30 days was 10% in MPN patients compared to controls. The odds of having a thrombosis at time of MPN diagnosis were high, indicating that thrombosis is an important first symptom of MPN. The risks of arterial and venous thrombosis were substantially elevated in MPN patients compared to matched population controls (Hultcrantz 2014). The highest risks of thrombosis were observed shortly after the MPN diagnosis and the risk then decreased with follow-up time after diagnosis. The hazard ratios of arterial thrombosis at 3 months, 1 year, and 5 years were 3.0, 2.0 and 1.5, respectively, compared to controls. The corresponding hazard ratios for venous thrombosis were 9.7, 4.7 and 3.2 in MPN patients compared to controls. The risk of thrombosis was overall similar between the MPN subtypes.

In a retrospective study in ET and PV following an arterial or venous thrombotic event, cytoreductive therapy reduced the incidence of re-thrombosis in the entire cohort by 50% due to a marked reduction of arterial events (De Stefano 2008). This study showed a significant prevention of re-thrombosis was independently achieved in patients with previous venous thrombosis by both oral anticoagulants and antiplatelet drugs. In a small retrospective study of patients with MPN and previous proximal deep venous thrombosis of the legs the rate of recurrence was compared in patients receiving six months of Vitamin K Anticoagulants (VKA) after the event, with those receiving long-term VKA. The cumulative

probability of recurrent VTE was some 23% at two years after first VTE, 34% at five years, and 64% at 10 years in those who stopped VKA after 6 months. In patients receiving long-term VKA the cumulative probability of recurrence reached a plateau at two years of 17%; however, the risk of recurrence did not differ significantly between the two groups (De Stefano 2007). Retrospective analyses indicate that the incidence of thrombotic complications is similar in PMF and ET (Barbui 2011c; Carrobio 2011). An inflammatory response appears to be important in the thrombotic events in MPN (Barbui 2011b; Hermouet 2015). Activated JAK-STAT signaling leading to inflammation is common to *JAK2*, *CALR* and *MPL* mutated patients (Vainchenker & Kralovics 2017).

Treatment of arterial vascular events

Acute arterial thrombotic events should be treated as in non-MPN patients with the addition of rapid lowering the hematocrit (Hct) and platelet levels. In emergency situations, such as acute cerebrovascular complications or severe digital ischemia, phlebotomy or acute platelet apheresis can be used in order to achieve a rapid reduction in blood counts. Since the effect is brief, cytoreductive therapy with preferably hydroxyurea should be started as soon as possible in patients not already on cytoreductive therapy. Moreover, control of the Hct and platelet count should be optimized in all patients with thrombosis.

Treatment of venous thrombosis

As in arterial thrombosis, control of the Hct and platelet count should be optimized and cytoreductive therapy with preferably hydroxyurea should be started as soon as possible. Regarding anticoagulant treatment there are no prospective studies including only MPN patients comparing currently available anticoagulant therapies: low molecular weight heparin (LMWH), vitamin K antagonists (VKA) or Direct Oral Anticoagulants (DOAC). Moreover, there are no prospective studies comparing long and short-term treatment of venous thrombosis with anticoagulant treatment. In general, patients with MPN are in a chronic hypercoaguable state and even if the overall risk of thrombosis is higher than that of bleeding, the bleeding risk is not negligible. Until prospective studies are available, warfarin is recommended as first line treatment and secondary prevention of venous thrombosis. Duration of treatment should be decided taking all risk factors for both thrombosis such as age, male gender, obesity, unprovoked thrombosis, extent, localization and inherited thrombophilia as well as bleeding in consideration.

The use of DOAC in the treatment of thrombosis or in prophylaxis in MPN is not well established.

Summarized recommendations for thrombosis in MPN patients

- Primary prophylaxis: Low dose aspirin to all patients without contraindication
- Acute treatment same as for non-MPN patients with the addition of lowering elevated blood counts

- Secondary prophylaxis: Limited evidence regarding secondary prophylaxis of venous thrombosis, most expert opinions recommended continuous warfarin
- DOAC: Limited experience of DOACs in MPN patients, until more information on efficacy and safety, warfarin treatment is recommended in MPN whenever oral anticoagulation is indicated
- In younger patients: Work-up for inherited thrombophilia according to national guidelines is recommended
- Prophylaxis in patients with platelet counts > (1000-) 1500x10^9/L must be individuallized and based on assessment of risk of bleeding versus risk of thrombosis, and combined with a cytoreductive treatment

Treatment of splanchnic vein thrombosis

MPN is one of the most common underlying causes of splanchnic vein thrombosis, including Budd-Chiari Syndrome, extrahepatic portal vein obstruction and portal vein thrombosis. Of all patients with splanchnic vein thrombosis, around 40% are positive for the *JAK2*V617F mutation (Smalberg 2012). Of all patients diagnosed with splanchnic vein thrombosis inherited thrombophilia is present in at least one-third, and the factor V Leiden or the prothrombin G20210A mutations are the most common mutations. Multiple risk factors are present in approximately one-third of the patients with Budd-Chiari Syndrome and two-thirds of the patients with portal vein thrombosis (Smalberg 2011). Immediate anticoagulation with LMWH is used to treat patients acutely. Upon clinical deterioration, catheter-directed thrombolysis or transjugular intrahepatic portosystemic shunt is recommended in conjunction with anticoagulation (De Stefano 2010). No evidence-based guidelines can be given regarding long-term therapy after splanchnic vein thrombosis, but in expert opinions continuous warfarin therapy is recommended if possible. Normalization of any elevated blood counts is also important and cytoreductive therapy is recommended unless low blood values.

Bleeding

In patients with MPN, the overall incidence of major hemorrhages (both intracranial and gastrointestinal) ranges between 0.3 and 0.8% per year which is significantly higher than in the general population (Marchioli 2005). In a large retrospective analysis of patients with ET and prefibrotic PMF major bleeding during follow-up occurred in 6% WHO-ET and 12% PMF patients, at a rate of 0.79 and 1.39% patients per year, respectively, which represents a significant difference. Risk factors for bleeding included diagnosis of PMF subtype, leukocytosis, previous hemorrhage, and aspirin therapy (Finazzi 2012). In contrast, thrombocytosis per se was not a risk factor for bleeding. However, low-dose aspirin had a synergistic hemorrhagic effect unmasking the bleeding tendency of patients with extreme thrombocytosis (Finazzi 2012).

The most important cause of bleeding in ET and PV is acquired von Willebrand´s syndrome (AvWS) associated with high platelet counts (>1500 x 10^9/L). Therefore, the most important therapeutic intervention to manage acute bleeding in the thrombocythemic patient is platelet reduction, and the recommended agent is hydroxyurea. Platelet apheresis is indicated when extreme thrombocytosis is

accompanied by an urgent need to reduce platelet counts i.e. severe or life-threatening bleeding (Elliott 2005)

In case of major bleeding, all use of aspirin or anticoagulants should be stopped at once. These drugs should also be avoided in patients with previous bleeding episodes and AvWS and not administered until the platelet count is < 1000 x 10^9/L, No data are available, as to when platelet inhibitors or VKA may be administered if a bleeding has not been due to a coagulopathy (*ie* AvWS), but for instance a gastric ulcer – and if proton pump inhibitors should be used simultaneously. In view of reports of thrombotic complications among non-hematological patients at high risk for thrombosis treated with desmopressin, this agent is not recommended (Elliott 2005). The same goes for von Willebrand factor-containing plasma products (Barosi 2007; van Genderen 1997).

The combination of aspirin and anagrelide was in the PT-1 trial associated with an increased risk of bleeding. This combination should therefore be used with caution (Barbui & Finazzi 2011; Harrison 2005). Patients with PMF are at higher risk of bleeding than patients with ET (Finazzi 2012). No prospective trial of platelet reducing agents or aspirin has been performed in PMF. Clinicians are suggested to follow the guidelines given for ET regarding prevention and treatment of thrombosis and bleeding in PMF.

The principal risk of concomitant congenital bleeding disposition, like von Willebrand Syndrome, should be identified by the patient history and examined if any clinical suspicion.

Pruritus

Pruritus, typically aquagenic, can be a severe clinical problem in PV and in one study reported as “unbearable” by 15% of patients with PV (Siegel 2013). Several studies describe improvements with treatment with interferon (Hasselbalch 2011; Kiladjian 2008a). Ruxolitinib has been reported to have a good effect on pruritus in patients with myelofibrosis (Harrison 2012; Verstovsek 2012a) and PV (Vanucchi 2015; Verstovsek 2014) and should be considered in patients with severe pruritus not responding to or unsuitable for interferon therapy. Antihistamines may be of benefit. Selective serotonin re-uptake inhibitors can also lead to improvement of pruritus (Kümler 2008). Benefit has been shown with phototherapy using psoralen and ultraviolet A light.

Elective surgical interventions

Patients with MPNs have both a high risk of thrombosis and a high risk of bleeding when undergoing surgery (Barbui & Finazzi 2011). It is generally recommended to use cytoreductive agents in order to normalize blood counts before elective surgery. It has been shown that perioperative complications after splenectomy have decreased after prompt use of cytoreductive agents to counteract post-splenectomy thrombocytosis, implying the benefit of lowering elevated blood counts before invasive surgery. Patients should be monitored closely after major surgery regarding thrombotic complications, especially in the abdominal veins after splenectomy (Barbui & Finazzi 2011).

It may be recommended that all patients not already on oral anticoagulants should be given LMWH at prophylaxis dose for at least 8 weeks after major surgery.

Transformation to acute myeloid leukemia

The risk of transformation is highest in patients with PMF, followed by PV and then ET (Swerdlow 2008; Björkholm 2014). The results after conventional acute myeloid leukemia (AML) induction chemotherapy are dismal in patients developing post-MPN AML, with a very short median survival (Björkholm 2011). Results are not significantly better than palliative therapy. If possible it is recommended that patients undergo allogeneic stem cell transplantation after induction chemotherapy or azacytidine (Kröger 2015; Thepot 2010). Such patients should be discussed with the transplant team in order to choose a proper induction therapy. Patients with highly proliferative leukemia should receive initial conventional chemotherapy if medically fit and suitable for such therapy (as evaluated in *de novo* AML), whereas those characterized by cytopenia may be transplanted upfront or after induction with azacytidine. In patients not eligible for transplantation, authors of a phase II trial of azacytidine suggest that azacytidine may confer a better survival compared to conventional induction (Thepot 2010). There are also studies on ruxolitinib, azacytidine, decitabine, and panabinostat that have shown promising results, and studies on these therapies alone or in combination are ongoing. (Björkholm 2014; Eghtedar 2012; Mascarenhas 2010).

Pregnancy

There is only limited information in the medical literature about the management of MPNs in pregnancy (Griesshammer 2008). In ET, the live birth rate is about 60% due to an overall incidence of first trimester miscarriage of 31-36% (about twice that expected) and an increased risk of intrauterine death and stillbirth (8%). There is also in increased risk of abruptio placentae, pre-eclampsia, and intrauterine growth retardation (Barbui 2011a). Major maternal complications are less common and occur in approximately 8% of ET patients (Barbui & Finazzi 2011; Harrison & Robinson 2011). The number of reported pregnancies in PV are fewer but the frequency of maternal complications is higher in PV than ET. The rate of fetal complications are similar in expecting mothers with ET and PV (Griesshammer 2008). At the EHA 2106 the European Leukemia Net presented a series of 121 pregnacies in 48 PV patients. The success rate of pregnancies was significantly better (49% versus 77%, respectively) for patients in whom the diagnosis of PV was known and appropriate management performed according to current guidelines (Griesshammer 2016)

To avoid teratogenic effects, hydroxyurea, ruxolitinib, and anagrelide should be gradually withdrawn 3-6 months prior to planned conception, also in fathers to be, and may be substituted with interferon if necessary (Harrison & Robinson 2011). Pregnant MPN patients should be followed in hematology centers with experience in handling pregnancies and in close collaboration with experienced obstetricians, and especially at labor also anesthesiologists. Therapeutic strategies for PV and ET in pregnancy are influenced by the patient's disease status and prior obstetric history. The increased

plasma volume often results in a reduced Hct and platelet count during the second trimester. The levels rise again during the post-partum period contributing to an increased thrombotic risk during the first six weeks after delivery. Close monitoring of blood counts is important during this period (Harrison & Robinson 2011). If any of the following factors are present, the pregnancy is likely to be at a high risk of complication for the mother and/or fetus (Barbui & Finazzi 2011):

- Previous venous or arterial thrombosis in mother
- Previous hemorrhage attributed to MPN
- Previous or current pregnancy complication that may have been caused by PV/ET, i.e. unexplained recurrent first trimester loss (≥3), intrauterine growth restriction (>5 percentile for gestation), intrauterine death or stillbirth (no other cause identified), severe preeclampsia, significant ante- or postpartum hemorrhage, or placental abruption.
- Platelet count rising to >1500 x 10^9/L

Therapeutic options include aspirin, low molecular weight heparin (LMWH), phlebotomy in PV, and interferon. Low dose aspirin is safe and seems advantageous during pregnancy in ET. We recommend that in the absence of clear contraindications all PV and ET patients should be on aspirin 75 mg per day throughout the pregnancy. In patients not already on aspirin, this should be started before planned pregnancies to facilitate formation of placental circulation (Barbui & Finazzi 2011). On the day of the delivery, aspirin is substituted by a prophylactic dose of LMWH which is given until six weeks after delivery to lower the risk of venous thrombosis (Harrison & Robinson 2011). Blood counts should be checked weekly to detect rebound thrombocythemia or polycythemia. Treatment recommendations for low and high risk pregnancies are as follows (Barbui 2011a; Harrison & Robinson 2011).

High risk pregnancy

- If previous major thrombosis or severe pregnancy complications: In addition to cytoreduction with interferon LMWH throughout pregnancy plus aspirin (stop aspirin if bleeding complications) during the whole pregnancy and six to eight weeks after delivery.
- Doses of LMWH that have been reported in pregnancy are dalteparin 5000 U, enoxaparin 40 mg or tinzaparin 4500 U daily x 1.
- Consider interferon if platelets >1000 - 1500 x 10^9/L.
- If previous bleeding: avoid aspirin and consider interferon to lower platelets, and use LMWH as prophylaxis.

Low risk pregnancy (none of risk factors listed above):

- Low dose aspirin during pregnancy and prophylactic dose LMWH from delivery until six to eight weeks postpartum.
- If previous bleeding: avoid aspirin and consider interferon to lower platelets, and use LMWH as prophylaxis.

Thus, cytoreductive therapy is recommended in patients with 1) previous major thrombotic or major hemorrhagic complication, 2) severe complications in previous pregnancy despite aspirin and/or

LMWH, 3) an increasing platelet count > 1000-1500 x 10^9/L (Griesshammer 2006). The drug of choice is interferon (Griesshammer 2008), and pegylated forms of interferon are also considered safe.

Compression hosiery (stockings) and other symptomatic advice (motion, elevation etc) may be considered individually.

Close monitoring with frequent ultrasound including uterine artery Doppler (notch) ultrasound at week 20 and 24 have been recommended in order to identify growth retardation or impaired placental circulation which may indicate a need for a LMWH/higher dose of LMWH (Griesshammer 2008).

Breast-feeding is safe with heparin, but contra-indicated with the cytoreductive agents, for principal reasons. The precaution also includes interferon – mostly due to lack of data (Giles 2011).

Pediatric MPN

The incidence of different MPN in patients aged less than 20 years is so low that formal evidence based recommendations is impossible to give. This has recently been emphasized by the ELN (Barbui 2011a).

PV and PMF is more uncommon than ET, which in turn is 90-fold less frequent than in adults with an estimated annual incidence of 4 new ET cases per 10 million inhabitants. In children with ET a lower proportion of less than 1 in 3 *JAK2*V617F mutated patients has been found. The importance of bone marrow histology in the diagnosis of childhood ET is also undefined at the present (Teofili 2007) suggesting the need for improved and more specific criteria in childhood ET. In contrast, the WHO criteria for adult PV and PMF can be used in children (Barbui 2012)

ELN recommends that cytoreductive therapy in children with PV and ET should be given as a last possibility. Therapy in children with PV and ET should be tailored according to the risk profile of the patient (Barbui 2012; Harrison 2010). High risk in children is arbitrarily defined as previous life threatening major thrombotic or severe hemorrhagic complication. In such children therapy with interferon or hydroxyurea is recommended as first line therapy by the ELN. There are concerns of potential long-term leukemogenicity of hydroxyurea and we therefore recommend interferon as first-line therapy in PV. In ET anagrelide can be an alternative to interferon as first-line therapy.

Low risk PV patients are recommended to be treated with phlebotomy to maintain a hematocrit < 0.45. Aspirin is warranted in PV and ET if presence of microcirculatory symptoms or concomitant cardiovascular risk factors. Aspirin may also be given to patients without such problems based on the ECLAP study in adults (Landolfi 2004), but no formal studies have been performed of it´s efficacy in children. In asymptomatic pediatric patients aspirin should be used with caution due to the risk of Reye's syndrome (Harrison 2010).

The natural course of pediatric PMF seems different from that in adults. Variable outcomes with either a fulminant course rapidly evolving to acute leukemia or on the other hand a relatively indolent course have been described. It is imperative that children with PMF are evaluated for stem cell transplant using the same risk score as in adults. Other palliative treatments also follow the recommendations given for adults (page 31 and page 32 ff)

Levels of Evidence for Therapeutic Studies

http://www.cebm.net.

Type of evidence
1A Systematic review (with homogeneity) of RCTs
1B Individual RCT (with narrow confidence intervals)
1C All or none study
2A Systematic review (with homogeneity) of cohort studies
2B Individual Cohort study (including low quality RCT, e.g. <80% follow-up)
2C "Outcomes" research; Ecological studies
3A Systematic review (with homogeneity) of case-control studies
3B Individual Case-control study
4 Case series (and poor quality cohort and case-control study
5 Expert opinion without explicit critical appraisal or based on physiology bench research or "first principles"

Grade Practice Recommendations

http://www.cebm.net.

A Strong recommendation. Level I evidence or consistent findings from multiple studies of levels II, III, or IV. Clinicians should follow a strong recommendation unless a clear and compelling rationale for an alternative approach is present

B Recommendation Levels II, III, or IV evidence and findings are generally consistent Generally, clinicians should follow a recommendation but should remain alert to new information and sensitive to patient preferences

C Option Levels II, III, or IV evidence, but findings are inconsistent. Clinicians should be flexible in their decision-making regarding appropriate practice, although they may set bounds on alternatives; patient preference should have a substantial influencing role

D Option Level V evidence: little or no systematic empirical evidence. Clinicians should consider all options in their decision making and be alert to new published evidence that clarifies the balance of benefit versus harm; patient preference should have a substantial influencing role

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