Guidelines for the diagnosis and treatment of patients with polycythemia vera, essential thrombocythemia and primary myelofibrosis.

The Nordic study group on myeloproliferative disorders (NMPD) is a pan-Nordic organisation that has conducted Nordic clinical trials since 2001. NMPD decided in 2006 to write new guidelines, based on already existing national guidelines from the Nordic countries, Italy (1) and Great Britain (2). The first version was published in 2007. The aim has been to write a document that can be used in all Nordic countries. We have strived to use evidence-based medicine, i.e the conscientious, explicit, and judicious use of current best evidence in making decisions on our recommendations. The grading system employed in these guidelines is detailed on page 46. However, it should be stressed that few randomized controlled trials exist in the MPDs to support decision-making for individual patients.

The guidelines are written for health professionals with a speciality or interest in hematology. They have now been updated in 2009, this is the second update since they were first published.
We plan further updates on a yearly basis, and it is therefore recommended that colleagues use the on-line version (www.nordicmpd.org), rather than to print and copy paper versions of the document.

For the Nordic MPD Study Group April 2009

Writing committee:

- Hans Hasselbalch Herlev (chairman)
- Björn Andreasson Göteborg/Uddevalla
- Håvar Knutsen Ullevål
- Jan Samuelsson Stockholm (secretary)

Contents

General introduction

The myeloproliferative disorders (MPDs) represent a range of clonal hematological diseases with overlapping features. The main entities are polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic myeloid leukemia (CML) and some rare subtypes (3). Only the Philadelphia-negative MPD's will be reviewed here. Systemic mastocytosis and the hypereosinophilic syndrome will not be discussed in these guidelines.

The common features of PV and ET are overproduction of one or more myeloid lineages in the bone marrow and increased numbers of mature and immature cells in peripheral blood: increased production of red cells as the main feature of PV and increased production of mainly platelets in ET. This overproduction increases the bone marrow cellularity, often with fibrosis. Splenomegaly is common, often due to extramedullary hematopoiesis, which together with leukoerythroblastic anemia is the main feature of classical primary myelofibrosis (agnogenic myeloid metaplasia).

Erythroid progenitor cells from patients with PV form colonies in vitro in the absence of erythropoietin, and both erythroid and myeloid cells from these patients are sensitive to several different growth factors. Similar abnormalities are present in a proportion of patients with ET and PMF. The explanation for this hypersensitivity is a mutation in exon 14 in the gene for Janus kinase 2 (JAK2 $_{V617F}$,) on chromosome 9p24 (4-7). JAK2 is a non-membrane tyrosine kinase which is known to be involved in the signal transduction pathway for several different growth factors. The JAK2 $_{V617F}$ mutation is present in all erythropoietin-independent erythroid colonies in PV, and gives a proliferative advantage of these cells. The mutation is present in at least 95 % of PV patients and in approximately 50 percent of ET and PMF patients. It is not found in healthy persons, and is very seldom present in other hematological malignancies (8,9), except for refractory anemia with ringsideroblasts and marked thrombocytosis (about 50 % of the patients, 199). Other MPD associated JAK2 mutations in exon 12 have been described in JAK2$_{V617F}$ negative PV patients (200). Accordingly, a JAK2 mutation is therefore probably present in virtually all patients with PV and thus constitutes a sensitive diagnostic marker for the disease. This marker is therefore a decisive diagnostic test in PV, and complementary to histology for the diagnosis of ET and PMF, is included in most recently published diagnostic criteria (10,11). JAK2$_{V617F}$ has been adopted in the new WHO diagnostic criteria for PV, ET and PMF (201). Mutations in the juxtamembrane region of the thrombopoietin receptor MPL in exon 10 have also been described in PMF and ET. Retrospective analysis of the PT-1 study (218) identified MPL mutations (mostly MPLW515K) in 8.5% of JAK2 $_{V617F}$ negative patients. In vitro data support the notion that MPLW515K is a gain of function mutation, transforming hematopoietic cells into cytokine independent growth. It has been difficult to explain how the JAK2$_{V617F}$ mutation can give rise to different clinical disorders, but a growing amount of evidence points to an unknown pre-JAK2$_{V617F}$ mutation (219), at least in some patients.

It is important, however, that these new criteria are not applicable to children, in whom the JAK2$_{V617F}$ mutation occurs less frequently than in adults, and where exon 12 JAK2 mutations seem to be absent. When applying these new WHO criteria to 45 children with MPDs, Teofili et al (202) found that a significant proportion of childhood PVs were misdiagnosed, and that all familial ET, including patients carrying the hereditary MPL$_{Ser505Asn}$ activating mutation, were erroneously diagnosed as MPDs. These observations suggest that childhood MPDs require a set of specific diagnostic criteria.

The dominating clinical complication of these disorders is thrombosis, although hemorrhage may also occur (12). Some patients experience pruritus (typically aquagenic and mainly in PV), erythromelalgia or other symptoms of acral ischemia. In the longer term, both disorders can develop secondary myelofibrosis or transform into acute myelogenous leukemia (AML).
Previous small studies have reported familial clustering of myeloproliferative disorders including PV, ET and MF. $JAK2_{V617F}$ mutation has been assessed in multiple affected MPN families, but has been shown not to be an early germline predisposing factor for MPD but rather a facilitator of proliferative advantages. A large population-based study from Sweden showed 5- to 7-fold elevated risk of MPD:s among first-degree relatives of MPD patients, and support the hypothesis that there are common, strong, shared susceptibility genes predisposing to PV, ET, and PMF (220).

Diagnosis of erythrocytosis

Patients with a persistently raised venous haematocrit (Hct)/ hemoglobin (Hb) should, in general, be investigated. The hemoglobin/hematocrit values should be measured when the patient is normohydrated. The $97{,}5^{th}$ percentile for Hct in Nordic countries are 0.50 and 0.46 for men and women, respectively, and the 99^{th} precentile is 0.51 and 0.47 (corresponding Hb is 17.3 and 15.8 g/dl or 10,7 and 9.8 mmol/l, personal correspondence) (13). Males and females with Hct > 0.52 and > 0.48 (well above 99^{th} percentile) for more than 2 months should be evaluated. However, males and females with Hct values above 0.60 and 0.56 respectively, can be assumed to have an absolute erythrocytosis and do not require confirmatory studies (14).

The evaluation of a high Hct used to be performed by measurement of the red cell mass (RCM) by radioactive methods, with normal values between ± 25% of the mean value at any given surface area (15). The diagnosis of an absolute erythrocytosis is made when an individual's measured RCM is more than 25% above their mean predicted value. In spite of this being the gold standard for confirming an increased red cell mass, this methodology is not readily available. RCM measurement is cumbersome, time-consuming and costly, and in some countries it is not performed at all. RCM measurement does not distinguish between PV and secondary polycythemia, whereas relative polycythemia is most often appreciated from patient history and review of previous laboratory records. RCM measurement for the diagnosis of PV has by some authors been shown to be of suboptimal value (16), and the test has fallen out of favor in many countries (17), while others (221) argue that without the use of RCM measurements, many patients diagnosed as having ET according to the new WHO criteria, have in fact PV. The need for RCM measurement in the current diagnosis of PV is therefore debatable, and the issue of practical utility has also been questioned in view of the availability of more relevant biologic tests, including serum or plasma EPO level, bone marrow histology, and the $JAK2_{V617F}$ mutation (or exon 12 mutations in JAK2). As there are controversies regarding which one of the 3 red cell variables (hemoglobin level, hematocrit value, and measured red cell mass) is the most accurate in the determination of red cell volume, the new WHO criteria accept individual choice on this matter.

Terminology

The term **apparent erythrocytosis** (AP), also called pseudopolycythemia or relative polycythemia, is used for persons with a raised venous Hct due to a low plasma volume, but with a red cell mass (RCM) within the reference range. This is by far the most common cause for a mildly elevated Hct. In cases where RCM is not measured, this term is more difficult to apply, but will require the demonstration of an elevated and stable hematocrit value by serial measurements. There is no evidence in the literature supporting therapy of pseudopolycythemia with venesection. The term

idiopathic erythrocytosis is commonly reserved for cases with a strongly suspected/confirmed elevated RCM not fulfilling the criteria for PV and for which no other cause can be found (neither aquired nor congenital, see Table II).

Diagnosis of PV

The diagnostic criteria have changed during the years, and since the first version of these guidelines was published early in 2007, new diagnostic criteria for polycythemia vera, essential thrombocythemia and primary myelofibrosis have been adopted by WHO (201). Due to the very high mutation frequency of JAK2 in PV, this marker has been included as a major criterion for PV. Since JAK2 mutations are absent in familial, secondary and apparent (relative) polycytemia, the diagnostic criteria for PV have been simplified accordingly. These new WHO criteria have been adopted in these guidelines.

Table 1. Revised WHO criteria for polycythemia vera (PV)

Major criteria

1. Hemoglobin > 18.5 g/dL in men, >16.5 g/dL in women (11,5/10,2 mmol/l), or Hct > 52 in men and > 48 in women, or other evidence of increased red cell volume*
2. Presence of JAK2617V>F or other functionally similar mutation such as JAK2 exon 12 mutation

Minor criteria

1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation in vitro

Diagnosis requires the presence of both major criteria and 1 minor criterion or the presence of the first major criterion together with 2 minor criteria.

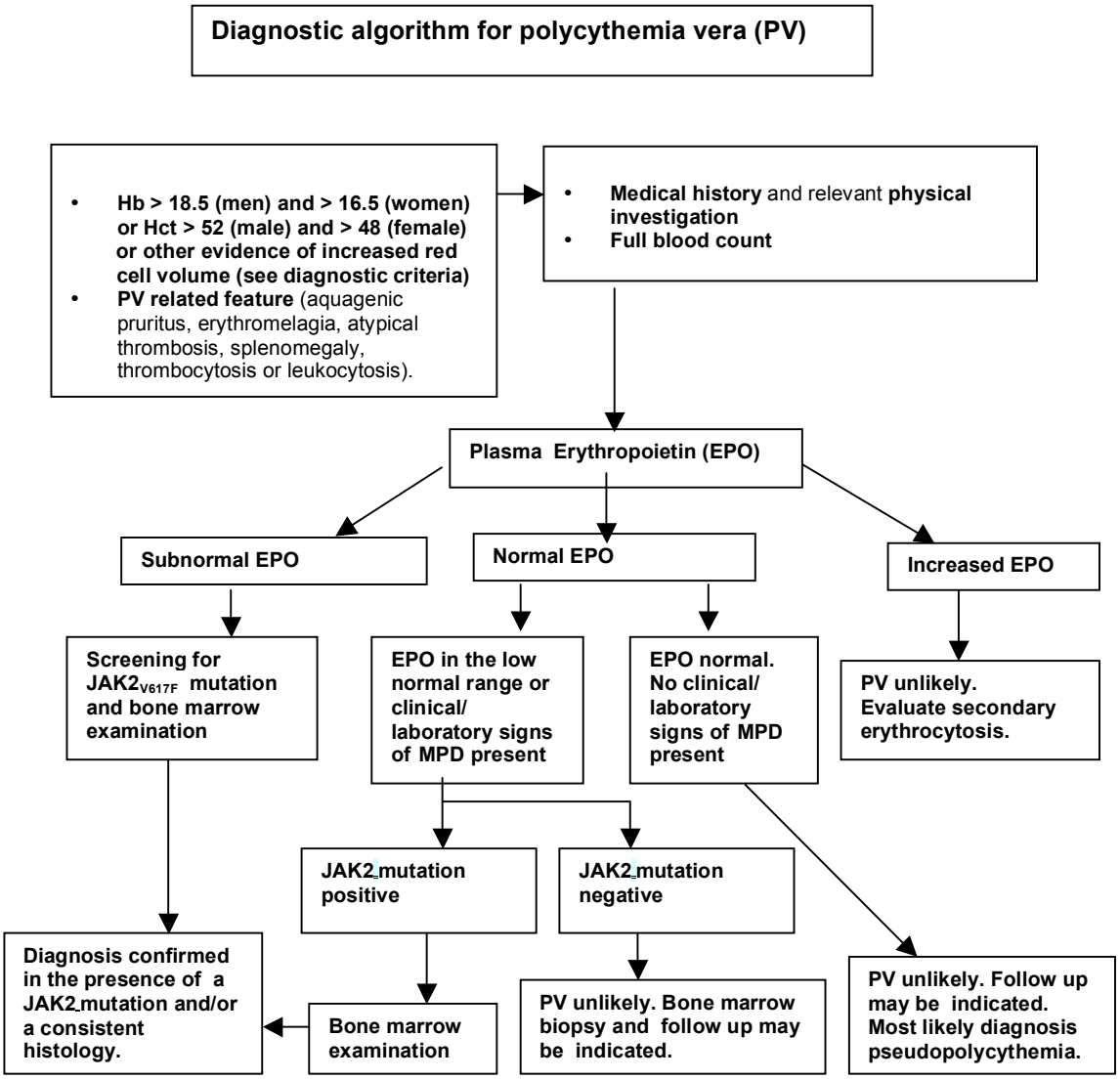
* Hemoglobin or hematocrit greater than 99th percentile of method-specific reference range for age, sex, altitude of residence or hemoglobin greater than 17 g/dL in men, 15 g/dL in women if associated with a documented and sustained increase of at least 2 g/dL from an individual's baseline value that can not be attributed to correction of iron deficiency, or elevated red cell mass greater than 25% above mean normal predicted value.

The diagnosis of PV is suspected in the presence of a sustained increase in venous hematocrit/Hb. The diagnosis should also be suspected in a patient with a lower hematocrit/Hb than the diagnostic treshhold (0.52/0.48 and 18.5/16.5 respectively) when there is a hemoglobin greater than 17 g/dL in men, 15 g/dL in women if associated with a documented and sustained increase of at least 2 g/dL from an individual's baseline value that can not be attributed to correction of iron deficiency. This should also be the case if a lower hematocrit/Hb than the diagnostic treshold if this is combined with a PV-related feature, eg. an arterial or venous thrombotic event, especially when this happens early in life or is atypical (like splanchnic vein thrombosis and Budd-Chiari syndrome, 222), aquagenic pruritus, erythromelalgia or other symptoms of acral ischemia, splenomegaly, leukocytosis, thrombocytosis or microcytosis.

PV can be masked in patients that present with iron deficiency and it may in rare cases be necessary to administer iron to correct this. If the serum Epo level is below the reference range or there are other strong indications of PV, iron supplementation should not be given. If iron is administered to a patient with suspected PV, it should be done extremely judiciously with at least weekly monitoring, as a rapid rise in Hct may occur, precipitating thromboembolic events. When PV is clinically suspected the first-line set of tests should include plasma or serum erythropoetin (EPO) level (see PV algoritm below). Once the EPO level is known, further evaluation for PV is warranted only if the EPO level is subnormal or in the low normal range.

The JAK2 $_{V617F}$ mutation can be detected in both bone marrow cells and in neutrophils in the peripheral blood. Detection in perpheral blood simplifies the sequence of investigations as seen in the algoritm.
In the presence of a subnormal EPO level the likelihood of PV is very high, and we recommend proceeding directly both to JAK2 mutation analysis *and* bone marrow examination, since the bone marrow can give important clinical information regarding the degree of fibrosis. These examinations should also be performed when the EPO level is in the low normal range or if clinical/laboratory signs of PV are present.

Although a bone marrow biopsy is an integral part of the diagnostic criteria, the necessity in routine clinical practice can in certain instances be questioned. In cases where the likelihood of PV is very high (eg. in a patient with erythrocytosis, subnormal EPO, and JAK2 mutation, or with the combination of eryhrocytosis, neutrophilia, thrombocytosis, subnormal EPO and palpable spleen), a bone marrow biopsy might be considered optional.



Commonly used tests in the diagnosis of PV

Full blood count

A neutrophilia is present in approximately two-thirds and thrombocytosis in 50% of PV cases (19) and provides a useful minor criterion for the diagnosis of PV (20). Smokers have significantly higher neutrophil counts than non-smokers (21), and it has been suggested that, in smokers, the upper limit of normal for neutrophil count should be taken as $12.5 \times 10^9/1$. Basophilia may be present in PV.

Plasma or serum erythropoietin (EPO) levels

Since erythrocyte production is controlled by EPO, a plasma/serum EPO level can provide information as to whether the erythrocytosis is hormonally mediated or autonomous. In patients with erythrocytosis secondary to hypoxia, EPO levels are typically elevated. In contrast, a large

majority of PV patients has an Epo level below the normal range found in healthy patients, giving a subnormal EPO value a high specificity, although a moderate sensitivity (22,23). EPO levels may remain persistingly low in the majority of cases even following adequate venesection (24). In addition, a normal EPO level excludes neither hypoxia nor PV as the cause of erythrocytosis. Nevertheless, with the availability of EPO methods that are specific, sensitive and reproducible in the lower EPO interval, a subnormal serum EPO level can now be used as a major criterion in the diagnosis of PV. When PV is suspected, erythropoietin measurement should be in the first line of investigations, and is a key factor in diagnostic algoritms.

Erythrocytosis in combination with a low EPO value in a person with a long history of high hemoglobin values and without other signs of PV, including a negative test for JAK2 $_{V617F}$ and exon 12 mutations, should prompt investigation for a congenital mutation in the EPO receptor gene.

Bone marrow examination
Although bone marrow histology is a B criterion in the WHO classification, it is in these current diagnostic criteria considered to be a very important diagnostic tool and upgraded to an A criterion. Bone marrow morphological investigation provides useful information including confirmation of the diagnosis of PV, differentiation from secondary erythrocytosis and other myeloproliferative disorders (e.g. detection of CML mimicking ET or PV), and detection of features of myelofibrosis. The characteristic bone marrow features in PV are panmyelosis with prominent erythroid and megakaryocytic proliferation. In case of JAK_{V617F} negativity, cytogenetics could be considered. The most common recurrent cytogenetic abnormalities include +8, +9, del (20q), del (13q), and del (1p).

$JAK2_{V617F}$ investigations
The $JAK2_{V617F}$ mutation can be found in at least 95% of PV patients with an allele-specific PCR. The specificity of this assay is also very high, since the mutation is not found in normal individuals or patients with secondary erythrocytosis (9). In other hematologic disorders the mutation has been found only in rare cases, except for MDS RARS-T. The mutation can be detected in both bone marrow cells and in peripheral blood. Routinely neutrophils or unfractioned peripheral blood are used. Homozygocity for the $JAK2_{V617F}$ mutation is common in PV and PMF but exceedingly rare in ET (203). Many laboratories now perform quantitative $JAK2_{V617F}$ measurements. Monitoring of $JAK2_{V617F}$ allele burden is increasingly used in clinical trials, and also in some centers to follow molecular effects of therapy.

Abdominal ultrasound
In patients with elevated Hb and Hct and in the absence of liver disease, a palpable spleen is a reliable sign of PV and has been adopted as a major criterion for its diagnosis. Splenomegaly can be found in two-thirds of PV cases at diagnosis by various imaging techniques (computerized tomography, ultrasound and scintigraphy) (25). Splenomegaly, when not palpable, has been proposed as a minor criterion.

The investigation of secondary erythrocytosis

The classification of absolute erythrocytoses is shown in table 2. Once an absolute erythrocytosis is documented, and PV excluded by an elevated EPO level, it is desirable to identify the underlying aetiology. The key point is knowledge of the underlying causes of a congenital or acquired (secondary) erythrocytosis (table 2). It is important to question the patient regarding the possibility

of familial erythrocytosis, or an acquired cause (smoking, snoring, medicines etc), and do a proper physical investigation.

Table 2. Classification of absolute erythrocytosis

Polycythemia vera

Congenital erythrocytoses

- High oxygen-affinity haemoglobin
- 2,3-Biphosphoglycerate mutase deficiency
- Erythropoeitin receptor-mutations
- Chuvash erythrocytosis (VHL mutation)
- Other congenital causes

Acquired erythrocytoses

EPO-mediated

Hypoxia-driven

- Central hypoxic process
- Chronic lung disease
- Right-to-left cardiopulmonary vascular shunts
- Carbon monoxide poisoning
- Smoker's erythrocytosis
- Hypoventilation syndromes including sleep apnoea (high-altitude habitat)
- Local renal hypoxia
- Renal artery stenosis
- End-stage renal disease
- Hydronephrosis
- Renal cysts (polycystic kidney disease)

Pathologic EPO production

- Tumours
- Hepatocellular carcinoma
- Renal cell cancer
- Renal cysts
- Cerebellar haemangioblastoma
- Parathyroid carcinoma/adenomas
- Uterine leiomyomas
- Pheochromocytoma
- Meningioma

Exogenous EPO

- Drug associated
- Treatment with androgen preparations
- Postrenal transplant erythrocytosis

Idiopathic erythrocytosis

Tests used in the investigation of secondary erythrocytosis

Arterial oxygen saturation

The measurement of arterial oxygen saturation (SaO_2) is most easily achieved with the use of a pulse oximeter. A SaO_2 below 92% has been taken to indicate a causal relationship with an absolute

erythrocytosis (19). However, there are three situations causing hypoxic erythrocytosis in which the SaO_2 can be misleading: carbon monoxide poisoning, the presence of high oxygen affinity haemoglobins and sleep apnoea syndrome.

Most instruments provide carbon monoxyhaemoglobin (COHb) measurements and this value should be subtracted to give an accurate SaO_2 result. Smokers generally have higher COHb levels, although a secondary erythrocytosis because of smoking alone is uncommon (26).
High oxygen affinity haemoglobins, as well as congenitally low 2.3-bisphosphoglycerate levels, will give rise to a normal SaO_2, despite tissue hypoxia, and measurement of the p5O is important to exclude these rare conditions. Most instruments for arterial blood gas analyses calculate the p50 value. A normal daytime SaO_2 can also be seen in the sleep apnoea syndrome and supine hypoventilation because of premature airway closure. It is important to consider these conditions and to enquire about symptoms relating to nocturnal oxygen desaturation, for example snoring, nocturnal restlessness and daytime somnolence (27). The need for a respiratory sleep study should be assessed in patients with erythrocytosis of all types who are known to snore heavily and either suffer from unwanted daytime somnolence (28), or who are significantly overweight. A chest X-ray is also recommended to exclude lung pathology.

Renal and liver function tests
Erythrocytosis may be associated with both renal and hepatic disease. Serum calcium (or preferably ionized Ca^{++}) levels should be determined to exclude the very rare secondary erythrocytosis caused by parathyroid adenomas or carcinoma.

Polycythemia vera (PV)

PV is a clonal myeloproliferative disorder characterised by increased red cell production independent of normal regulatory mechanisms, resulting in an increased red cell mass, and often also an excess of myeloid cells and of platelets. Approximately 95% of PV patients carry the $Jak2_{V617F}$ mutation in exon 14, a gain-of-function mutation disturbing the homeostatic balance that keeps proliferation of bone marrow cells in order, giving rise to a proliferative advantage of these cells, and as a consequence a suppression of erythropoietin production. In $JAK2_{V617F}$ negative patients several different mutations in exon 12 of the JAK2 gene have been described.

PV presents at a median age of 70 years. The incidence is similar in males and females. The largest epidemiological study (performed in the Gothenburg area) showed an annual incidence of PV of $1.97/10^5$/year (29). Untreated PV has a dismal prognosis as shown by an early retrospective cohort study (30), where the median survival in untreated patients was 18 months, with thrombosis being the dominant cause of death. In a large prospective study of 1638 PV patients the overall mortality rate with modern treatment principles was 3.7 deaths/100 persons/year (31). Compared to age- and sex-matched controls total mortality, mortality from cardiovascular disease, and leukaemia were 1.2, 1.4, and 36.1 times higher in PV. 45% of deaths were due to cardiovascular disease. The aims of treatment of PV are therefore to reduce the risk of thrombosis and haemorrhage, minimize the risk of transformation to acute leukaemia and myelofibrosis, and manage complications which may occur.

Clinical management of PV

Phlebotomy
A retrospective analysis of 69 patients followed over 15 years covering 332 patient-years showed that the incidence of vascular episodes was 0.2/10 years in patients with a hematocrit (Hct) of 0.40-0.44. The corresponding figures for a Hct of 0.45-0.49, 0.50-0.54, 0.55-0.59 and >0.6 were 0.92, 2.29, 3.33 and 7.5 respectively (32). It has also been demonstrated that the cerebral blood flow was significantly below normal in PV patients with a raised Hct and improved by 73% when the Hct was less than 0.45 (33). The PVSG-01 study showed an increased risk of thrombosis in the phlebotomy arm compared to ^{32}P and chlorambucil, which was predominantly observed during the first three years when the target Hct was 0.50 (34).

All patients on regular phlebotomy treatment develop iron deficiency with microcytosis, and during long term phlebotomy treatment there is often a discrepancy between a low Hb and a normal Hct due to this suboptimal hemoglobinization of the red blood cells. There is no study indicating that patients are symptomatic because of this iron depletion, it helps to reduce the need for phlebotomy and should not be a reason for iron treatment.

Recommendation: The Hct should be maintained at less than 0.45 by phlebotomy. There is currently no evidence to support a different level of Hct in males and females. Hemoglobin levels should not be used for decision making regarding phlebotomy.
Grade B recommendation, evidence level IIa.

Aspirin therapy
The ECLAP study established the therapeutic benefit of aspirin in PV (35). 518 patients neither having an indication nor a contraindication for aspirin therapy were randomised between aspirin 100 mg daily and placebo. Aspirin reduced the risk of the combined end-point of nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes (relative risk 0.41, 95% confidence interval 0.15-0.91, p=0.09). The other combined primary endpoint of nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes was significantly reduced (relative risk 0.40, 95% confidence interval 0.18-0.91, p=0.03). Aspirin was significantly more effective than placebo irrespective of duration of disease, Hct level, platelet count, and whether the patient was receiving cytoreductive therapy or not. The incidence of major bleeding was not significantly increased.

In case of contraindications to aspirin and a strong indication for therapy, clopidogrel can be introduced but no data on it´s efficacy has been documented in PV. Ruggeri et al retrospectively analyzed 84 MPD pts in whom ticlopidine (250 mg twice day) was administered to patients with a previous history of gastric ulcer, gastritis or allergy to ASA. Thrombotic rates for patients in primary prophylaxis was somewhat higher and bleeding rates slightly higher, compared to patients treated with ASA, but the differences were not statistically significant (223).

Recommendation: Aspirin should be given to all PV patients unless it is contraindicated.
In Finland, 50mg tablets are available and the recommended dose is 100mg. In the other Nordic countries the recommended dose is 75mg per day. The combination of aspirin and anagrelide should be used with some caution.

Grade A recommendation, evidence level Ib.

Cytoreductive therapy in PV

In the PVSG-01 study 91% of the patients randomised to phlebotomy had changed to alternative treatments by ten years in the French subgroup of patients in the trial (36). Thus, the role of purely using phlebotomy as treatment for PV is unclear, and in most PV patients cytoreductive therapies will be employed during the course of the disease. Such treatment should ideally not increase the risk of acute leukemia. Details of each therapeutic modality with regard to AML progression is given on pages 15-19. In order to avoid this complication, and other side effects of different therapies, an assessment of the thrombotic risk in the individual patient is mandatory. Several risk factors other than an increased Hct are important in the pathogenesis of thrombosis in PV (see below).

Additional risk factors for thrombosis

Age
In the PVSG-01 study age above 60 years was an independent risk factor for thrombosis, resulting in a two-fold higher thrombotic risk than in younger patients (37). In the prospective study of 1638 patients performed by the ECLAP project (31), age older than 65 years was one of the two most important prognostic indicators of a cardiovascular events (RR=2.1) and mortality (hazard ratio 14.3, $p < 0.0001$).

Previous thrombosis
The PVSG-01 study documented a previous thrombotic event as an independent risk factor for thrombosis. This was also found in the study by Marchioli et al (31), in which previous thrombosis conferred an increased risk of both cardiovascular events (RR=2.1) and death (hazard ratio 1.93, $p=0.0003$).

Thrombocytosis
The role of thrombocytosis as a cause of vascular events in PV remains controversial. The weight of the evidence in the literature fails to demonstrate a clear correlation between platelet counts and the incidence of thrombosis (38). In the PVSG-01 trial, there was no correlation between a raised platelet count and thrombotic events (37). Neither was any correlation found in the observational study performed by the ECLAP group (31).

On the other hand, circumstantial evidence for the benefit of reducing the platelet count is provided by studies in ET, where reducing the platelet count significantly reduced the incidence of vascular events (39). In this trial, a platelet count $> 1500 \times 10^9$/l was used as a trigger level to start therapy. In addition, aspirin reduces the incidence of thrombosis in PV (35), suggesting a key role for platelets in thromboembolic events. Finally, a potential role for leukocyte-platelet aggregates as a cause of thrombosis has ben suggested (40,41).

Two other issues are important when discussing the impact of thrombocytosis in PV. Hemorrhage has been reported in association with high platelet counts and an acquired von Willebrands disease (42). In the PVSG-05 study comparing ^{32}P to phlebotomy plus high doses of anti-platelet agents, aspirin (300mg tid) and dipyridamole (75mg tid), the hemorrhage and death rate was significantly

increased in the phlebotomy and antiplatelet arm and the trial was therefore stopped (43). In the majority of cases a high platelet count was found at the time of hemorrhage.

Different treatments used in PV vary little in the frequency of myelofibrotic transformation, although one randomised trial did show a greater incidence for hydroxyurea compared to pipobroman (44). In this trial, poor control of the platelet count was strongly associated with progression to myelofibrosis. This may suggest that controlling the platelet count will influence the rate of transformation to myelofibrosis. Very long follow-up of French patients entered into PVSG trials has been reported (36). Twelve out of 60 patients treated for more than 3 years with phlebotomy alone developed myelofibrosis within 10 years, compared to none of 60 patients treated with ^{32}P. However, the patients in the phlebotomy arm who developed myelofibrosis had a very long survival.

Leukocytosis

Leukocytosis is a common feature of PV, especially during the later stages of the disease. Besides leukocyte activation and interaction with platelets, other possible mechanisms behind a prothrombotic property of leukocytes include microparticles containing tissue factor, interactions with the endothelium and acceleration of arteriosclerosis. Landolfi et al.has analyzed the ECLAP control population and found that patients with leukocytes $>15x10^9$/l had an increased risk of arterial thrombosis, HR 1.71 (p=0.013), compared to patients with leukocytes $<10x10^9$/l (45). In another study of 459 patients with PV multivariate analysis identified age >60 (p <0.0001), leucocytosis (leucocyte count $>15x10^9$/l; p=0.0006) and arterial thrombosis at diagnosis (p=0.01) as independent predictors of inferior survival (204). In the absence of the first two risk factors, median survival was projected at 272 months as opposed to 108 months in the presence of both risk factors (P <0.0001). Leucocytosis was also identified as an independent predictor of both leukaemic transformation and venous thrombosis during follow-up. However, no study has yet been performed aimed at investigating whether lowering of neutrophil counts reduces thrombosis incidence.

Degree of $JAK2_{V617F}$ positivity

Vannuci et al studied 135 PV patients within 6 months of diagnosis and followed them for a median of 23 months. The degree of $JAK2_{V617F}$ positivity was 1-25% of cells in 34% of patients, 26-50 in 23%, 51-75 in 15%, and 76-100 % in 21% of patients. 7% were wild-type. In patients with greater than 75 % mutant allele, the risk of requiring chemotherapy (RR 1.8; P=0.001) or developing major cardiovascular events (RR 7.1; P=0.003) during follow up were significantly increased (46). However, other studies failed to find any correlation between allele burden and thrombosis (205,206)

Other prothrombotic factors

A prevalence of FV Leiden carriership in PV and ET of 4,6% has been reported, i.e. comparable to that observed in the general population (47). The prevalence was significantly higher in patients with a history of venous thromboembolism (VTE) before or at diagnosis, 5/27 (16%), compared to asymptomatic carriers, 9/263 (3%, p=0.003). It was also suggested that FV Leiden may be more common in MPD patients with recurrent VTE, 3.6% in asymptomatic carriers, 6.9% in patients with a single episode of VTE, and 18.1% in patients with recurrent VTE.

The association between a prothrombin gene mutation and the risk of VTE was analyzed in 214 patients with PV and ET. The rate of VTE was 14.7/100 patient-years in patients with the prothrombin mutation compared to 0.8 in patients without the mutation (rate ratio 17.5) (48).

Since both these trials were retrospective, the question whether all patients with PV and ET should be tested for prothrombin gene and Factor V Leiden mutations can only be answered by large prospective clinical trials.

Very few studies have been performed in PV regarding conventional risk factors for atherosclerosis such as smoking, diabetes, hyperlipidaemia and hypertension. In the study by Marchioli et al (31), none of these factors was an independent indicator for thrombosis. However, in a recent analysis of the same material, Landolfi et al found that smokers had an increased thrombotic rate compared to non-smokers (45). Despite the paucity of data, it seems logical to manage these risk factors in the same manner as in individuals not having PV.

Summarized recommendations for assessing risks of complications in PV

Evidence level Ib;
Age > 60-65 years, previous thrombosis and a hematocrit > 0.50 are risk factors for thrombosis.

Evidence level IIa;
An elevated platelet count is a risk factor for myelofibrosis.
A hematocrit > 0.45 is a risk factor for thrombosis.

Evidence level III;
A platelet count > 1500 x 10^9/l is a risk factor for bleeding.
A leukocyte count >15 x 10^9/l is a risk factor for venous thrombosis and leukemic transformation.
Routine thrombophilia screening is not supported by current evidence.
All patients should be encouraged to stop smoking.

Evidence Level IV;
Conventional risk factors for atherosclerosis should be managed aggressively.

Choice of cytoreductive therapy in PV

(Recommended dosages of all agents are given in the chapter on ET therapy)

Hydroxyurea (HU)
Hydroxyurea has been used in a phase II study, PVSG-08, and a number of case series. The PVSG-08 study was a Phase II efficacy study that included untreated and previously treated patients (49). One-year failure free survival was 73% in the previously untreated and 59% in the previously treated. The previously untreated patients in this study were compared to historical venesected controls randomised to phlebotomy in PVSG -01 (50) with the Hct maintained at less than 50%. There was less thrombosis including in the early period after the start of therapy and no difference in the rate of leukaemia. With prolonged follow-up, there was no statistical difference in the

incidence of myelofibrosis (spent phase) between the groups (51). Therefore, HU is efficacious using the historical comparison.

The French Polycythaemia Study Group (FPSG) published two randomised trials in 1997. The first was a two arm comparison of ^{32}P alone against ^{32}P with maintenance HU in patients over the age of 65 years (52). Significant numbers of patients crossed between the two treatment arms. Median survival was not significantly different, 9.3 years (^{32}P + HU) versus 10.9 years (^{32}P, p=0.15). No differences were observed for vascular end-points or progression to myelofibrosis. The actuarial risk of leukaemia, myelodysplastic syndrome or lymphoma was significantly greater for the ^{32}P + HU group, with the difference becoming apparent after 5 years, and the gap continuing to widen up to the 15th year. At 10 years this risk was 8% for ^{32}P and 19% for ^{32}P + HU. In addition, the actuarial risk of non-haematological malignancy was also much greater for the ^{32}P + HU arm, 19% compared to 10%.

The second trial from the FPSG was a comparison of HU therapy with pipobroman (Pi) in 292 patients under the age of 65 years (44, 53). The final analysis of this trial was made after a median follow-up of 16.3 years (224). After randomization in the trial (136 pts in the HU arm, 149 in the Pi arm), 94 (33%) pts had received only HU, 130 (46%) only Pi, and 61 (21%) both drugs. Median survival was 20.3 years (95% CI: 16.4 – 25) in the HU arm, and 15.4 years (95% CI: 13.4 – 17) in the Pi arm (p=0.008). 95 pts had died, the 3 main causes of death being evolution to AML/MDS in 51 pts (54%), vascular events in 19 pts (20%), and solid tumor in 11 pts (12%). Analyses according to the main treatment actually received by pts showed cumulative incidence of AML/MDS of 7%, 14%, and 22% with HU, and 12%, 37%, and 56% with Pi at 10, 15, and 20 years, respectively (p=0.008). A significantly higher incidence of MF was found in pts who had received HU as main treatment: 15%, 24%, and 32% at 10, 15, and 20 years, compared to 5%, 10%, and 21%, respectively, in patients who received mainly Pi (p=0.02).

Hydroxyurea thus generally gives good disease control in PV. It is the most common treatment for MPD-associated thrombocytosis, and has recently shown superior activity over anagrelide in ET with regards to arterial thrombosis and development of myelofibrosis (54), whereas the protection against venous thrombosis was inferior. The most important point of concern is the potential leukemogenicity of HU. The literature does not present conclusive evidence for increased leukemogenicity of HU given as a single agent. In a series of reports the incidence of acute leukaemia varies from 0% to 6% in patients treated with HU alone (55), with the exception of two studies who reported an actual rate of 12% (53) and 10.5% (56). In the largest cohort study performed thus far no excess risk of leukemia was observed in HU treated patients (57), but the short median follow-up of 2.5 years precludes any firm conclusions. When HU is used in ET patients that have previously received alkylating agents (58), or in combination with ^{32}P (52), an increased risk of AML and other cancers has clearly been demonstrated. Some patients will experience other significant side effects from HU, including gastrointestinal disturbance, skin pigmentation, mucocutaneous and leg ulcers. The latter can be seen in up to 10% of patients, and do not heal until HU is discontinued. Several case reports of skin cancers in HU-treated patients also exist. In the later stages, the so called “spent phase”, anemia may be an unwelcome side effect of HU.

Conflicting data has been reported with regards to the effects of HU therapy and $JAK2_{V617F}$ allele burden. While some investigators found no effects (225), Ricksten et al reported a decrease in 9 PV pts from 25 % positive neutrophils before therapy to 10 % (median) after 12 months (226), and

similar data has also recently been presented by Girondon et al (227) and Besses et al (228). In these trials no PV patient became $JAK2_{V617F}$ negative, whereas JAK2 negativity could be seen in ET.

HU is not recommended during pregnancy (see page 35).

Recommendations:
Hydoxyurea is recommended as one first-line cytoreductive therapy in PV.
Grade A recommendation, evidence level Ib.

The concern for leukaemia transformation with long term HU treatment and the recent favourable results of IFN treatment with a reduction in JAK2 allele burden makes IFN the drug of choice in PV, especially in younger patients.The use of HU should therefore be limited in patients below 60 years.
Grade C recommendation, evidence level IV.

Interferon-α
Interferon-α (IFN) suppresses the proliferation of haematopoietic progenitors both pluripotent and lineage-committed. IFN was first shown to be effective in correcting thrombocythemia in ET and PV, and later shown to control the excess red cell mass in PV. Lengfelder et al. (59) have summarized treatment results with IFN in 279 PV patients. Besides correction of thrombocythemia in around 90% of patients, a reduction of splenomegaly was seen in 77%. Control of pruritus was achieved in 81% of patients, and in 82% the frequency of phlebotomies was reduced. One study (60) also documented a reduced rate of venous thrombosis on treatment but not the rate of arterial thrombosis compared to the rates on prior treatments. Treatment is usually continuous but occasionally can be stopped for prolonged periods of time. No randomized trials of IFN in PV have been reported to date.

Clear disadvantages associated with conventional IFN therapy are the need for frequent subcutaneous injections and a relatively high rate of side effects, leading to discontinuation of therapy in 21% of PV on metaanalysis (59), and in up to 41% in individual studies. Therefore, investigators have studied pegylated interferon α-2b (PEG-IFN) compatible with once-weekly dosing, and have documented that PEG-IFN is effective in reducing platelet counts in thrombocythemic MPD patients with a toxicity similar to conventional unmodified IFN (61,62). In the recent Nordic 24-month phase II feasibility study (62) of pegylated interferon α-2b (PegIntron®, PEG-IFN) treatment in 21 PV and 21 ET, 29/42 patients (69%) achieved a complete platelet response (CR). No thromboembolic or hemorrhagic complications were observed, compared to 12 thrombotic events in 42 patients (29%) in the 24 months preceding inclusion, highlighting the importance of controlling thrombocythemia in MPD patients with previous thrombosis. Phlebotomy requirements were reduced in the majority of PV patients. Side effects were the cause of therapy failure in 16/23 patients. Nineteen patients completed the planned 2-year treatment in CR, and only 8/19 patients reported any side effects at 2 years.

IFN has not been implicated as a possible leukemogenic agent. It is the only therapy in PV where studies have shown a modulation of fundamental abnormal biological processes. Reports include reversal of chromosome abnormalities (63,64), restoration of polyclonal hematopoiesis in individuals with previously monoclonal hematopoiesis and the suppression of erythropoietin-independent erythroid colony growth (65), and normalization of PRV-1 expression (62,66). Jones et

al (67) recently reported that the median percentage of mutated $JAK2_{V617F}$ alleles was not different between PV patients treated with phlebotomy alone, hydroxyurea, anagrelide or imatinib. The authors found a significantly lower V617F level in 7 IFN treated patients compared to the other patient groups, and hypothesized that IFN therapy had reduced V617F levels. Samuelsson et al (68) demonstrated that PEG-IFN conferred a 1.2-3.6-fold reduction in the percentage of $JAK2_{V617F}$ positive cells in 5/8 patients. Kiladjian et al (69) has reported that therapy with pegylated Interferon α-2a (Pegasys®) was very well tolerated. Sequential samples for % $JAK2_{V617F}$ monitoring, available in 29 pts, showed a decrease in 26 pts (229). Median % $JAK2_{V617F}$ decreased from 45 % before interferon α -a to 22,5 %, 17,5 %, 5 %, and 3 % after 12, 18, 24 and 36 months respectively. Molecular CR was obtained in 7 pts lasting from 6+ to 18 + months. Molecular CR persisted after IFN discontinuation in 5 pts. Similar results have been presented by Quintas-Cardema et al. (230). Larsen et al (231,232) has also shown in 7 pts that long-term treatment with interferon α-2b is able to induce complete molecular remissions with normalization of the bone marrow morphology, which may even be sustained after discontinuation of α-2b for up to 20 months. Finally, Kiladjian et al have reported that IFN -α2b therapy can eradicate the $JAK2_{V617F}$+ clone even in bone marrow erythroid progenitors (233).

Recommendation: IFN-α is theoretically superior for treating PV as it is effective in controlling proliferation of all cell lineages and there is no risk of leukemogenesis. Molecular remissions can be acheived with IFN. It is most likely to be tolerated in patients below 60 years for whom it is recommended. Pegylated forms of IFN seem equally effective as conventional IFN.
Grade B recommendation, evidence level III.

Since the median time to clinical response to interferon is long, patients with platelet values >1500 or with a vascular complication (demanding prompt lowering of platelet values) should receive hydroxyurea as initial therapy, and later on be switched to interferon.

Anagrelide
Anagrelide is described in more detail in the chapter on ET. It has been shown to control the platelet count in MPD patients including PV. The most common side effects are palpitation, headache and diarrhea. Due to its positive inotropic effect, anagrelide is not recommended in patients with heart failure or coronary heart disease. It was initially claimed that side effects of anagrelide were mild, with only 16% of patients discontinuing treatment because of side effects at 5 years (70). In a non-randomised study in 60 MPD patients of long-term tolerance over a two year time period, Birgegard et al (71) found a drop-out rate of 50% mainly caused by side effects. The remaining 30 patients had a continued platelet response with few or no side effects. Other studies (72,73) have corroborated this finding with similar discontinuation rates. Additionally, anagrelide was discontinued in 148/405 patients (37%) in the PT-1 trial. Since anagrelide is megakaryocyte specific it is only effective in controlling the platelet count, and probably does not control progression of PV, for example an increasing splenomegaly.

Recommendation: Anagrelide may be used to control thrombocytosis in PV patients that can not tolerate or do not respond to IFN or HU, and when HU is considered a less suitable alternative due to a concern for an increased leukemic risk.
Grade C recommendation, evidence level IV.

Combination therapy
In clinical practice where it is sometimes difficult to reach the desired treatment goal due to side effects of single agents, combination therapy with HU and anagrelide, IFN and anagrelide, or HU and IFN may have advantages since side effects can be reduced with retained or improved clinical efficacy. However, no studies of such combinations have yet been published.

Busulfan
The EORTC conducted a trial comparing ^{32}P to *intermittent* busulfan (74). Phlebotomy was added in each arm to maintain the Hct between 0.42 and 0.47. Overall survival at 10 years was significantly better (p=0.02) in the busulfan group, 70%, compared to 55% for ^{32}P. The major reason for the difference was an increase in vascular deaths in the ^{32}P arm, 25/140, compared to 8/145 in busulfan treated patients. There were no differences between the arms for other complications such as leukaemia or non-haematological malignancy, 15/140 (^{32}P) and 14/145 (busulfan), respectively.

Recommendation:
Low dose ***intermittent*** busulfan is more efficacious in controlling PV than ^{32}P.
Grade A recommendation, evidence level Ib.
Since busulfan is an alkylating agent it should be reserved for patients 75 years or older, or for patients not tolerating HU, IFN or anagrelide.
Grade B recommendation, evidence level III.

Radioactive phosphorus (^{32}P)
^{32}P can control PV, intermittent treatment is required and follow-up can therefore be limited. It is valuable in older patients if compliance with continuous oral therapy is a problem.

The PVSG-01 study randomized patients to phelebotomy alone, ^{32}P or chlorambucil given daily on alternate months. Median survival was significantly longer in the phlebotomy arm, 12.6 years, compared to 10.9 years for ^{32}P-treated patients and 9.1 years in those allocated to chlorambucil (75). This difference was due to a higher risk of leukemia which at 10 years was 9.6% for ^{32}P and 13.5% for chlorambucil, compared to 1.5% in the phlebotomy arm. In addition, non-haematological malignancies were more common for ^{32}P treated patients, 3.5 times, and chlorambucil treated patients, 2.5 times. There was a large degree of cross-over between arms, with 91% of patients randomised to phlebotomy having changed to alternative treatments by ten years in the French subgroup of patients in the trial (36).

In a recent study, Finazzi et al (57) followed 1638 patients for a median of 2.5 years and found that older age was the main independent risk factor for progression of PV to MDS or AML (hazard ratio 4.3, p=0.0294). Also, exposure to ^{32}P, busulfan and pipobroman (hazard ratio 5.46, p=0.0023) had an independent role in producing an excess risk of progression, compared to patients treated with phlebotomy only or interferon. Cumulative dose of ^{32}P has not been shown to predict for leukemic evolution (76).

Recommendation: ^{32}P is effective in PV, but less so than *intermittent* busulfan. Since it increases the leukaemic transformation rate its use should be limited to patients older than 75 years.
Grade A recommendation, evidence level Ib.

Non-recommended therapeutic options in PV

Imatinib

Results of imatinib therapy has been reported in some 60 patients with PV. In most patients, the need for phlebotomy is reduced but platelet responses have been unpredictable. Dosages of 800 mg may be more effective than 400 mg.

Recommendation: Due to the limited effects and very high cost of imatinib this agent can not be recommended in PV at the present time.

Pipobroman

The results of the randomized trial between hydroxyurea and pipobroman have been described above. A non-randomized trial of pipobroman therapy in 179 previously untreated PV patients found a high risk of solid tumors and AML/MDS, the actuarial risk of the latter being 24% at 18 years. In patients younger than 60 years at diagnosis, AML/MDS represented 51% of causes of death (53).

Recommendation: Since pipobroman is not available in the Nordic countries no recommendation can be made.

Chlorambucil

The alkylating agent chlorambucil was associated with a higher risk of acute leukaemia in the randomized trial PVSG-01.

Recommendation: Chlorambucil can not be recommended in the treatment of PV

Grade A recommendation, evidence level Ib.

Summarized recommendations for the management of PV

- Phlebotomy to maintain the Hct to < 0.45 (grade B recommendation)
- Aspirin 75 - 100 mg/day unless it is contraindicated (grade A)
- Cytoreduction should be given to;
  - All patients > 60 years of age with any degree of platelet elevation in order to prevent thrombosis (grade C)
  - All patients with a previous thrombotic event with any degree of platelet elevation in order to prevent thrombosis (grade C)
  - All patients with platelets > 1500 x 10^9/L in order to prevent bleeding (grade B)
- Cytoreduction can also be considered in patients:
  - with a leukocyte count > 15 x 10^9/L in order to prevent thrombosis (grade C)*
  - < 60 years with platelets > 600 x 10^9/L in order to obtain molecular remission (grade C)*
  - with poor tolerance of phlebotomy (grade C)
  - with symptomatic or progressive splenomegaly (grade C)
  - with other evidence of disease progression e.g. weight loss, night sweats (grade C)

* not applicable for anagrelide

Choice of cytoreductive therapy, if indicated

(Grade C recommendation, evidence level IV)

<60 years: 1st line interferon-α, 2nd line hydroxyurea, 3rd line anagrelide*

60-75 years: 1st line hydroxyurea, 2nd line interferoαn-, 3rd line anagrelide*

>70 years: 1st line hydroxyurea, 2nd line consider combination therapy (HU+ana*, HU+IFNα-) 3rd line *intermittent* busulfan, 4th line ^{32}P

* anagrelide only if the indication for therapy is thrombocytosis

Investigation of thrombocythemia

ET is a chronic myeloproliferative disorder that primarily involves the megakaryocytic lineage, and is characterized by sustained thrombocytosis in the blood and increased numbers of large, mature megakaryocytes in the bone marrow. The $JAK2_{V617F}$ mutation is found in approximately 50 -55% of the patients. In a small subset of JAK2 $_{V617F}$ negative patients, mutations of the thrombopoietin receptor MPL (mostly MPLW515L) have been identified. Like JAK2 $_{V617F}$, this is a gain-of -function mutation.

The traditional diagnostic criteria of the PSVG were based mainly on a sustained increase in platelet count > 600 x 10^9/L and the exclusion of reactive thrombocytosis and other myeloproliferative or myelodysplastic disorders.
The new WHO guidelines published in 2007 (200) reduce the diagnostis criteria to four (Table 3). The platelet threshold is lowered to 450x10^9/L, as the earlier use of a threshold level of 600 x 10^9/L compromises the detection of early-phase disease, since the 95th percentile for normal platelet count, adjusted for gender and race, is below 400 x 10^9/L. The demonstration of $JAK2_{V617F}$ mutation that is present in 50-55% of ET patients, or the demonstration of other clonal marker, is included. But since the importance of the $JAK2_{V617F}$ marker is limited by its lower frequency in ET, a bone marrow biopsy is still required (as in the former 2001 WHO criteria (77)) to help with the differential diagnosis between $JAK2_{V617F}$ -negative ET and reactive thrombocytosis and to differentiate ET from other chronic myeloid neoplasms, including cellular phase/prefibrotic PMF and myelodysplastic syndromes (MDS). Of special consideration is the differentiation between ET and PMF grade 0 (CPMF0), which shows a different morphology of the megakaryocytes (smaller, hypolobulated and clustered). There is not a general consensus about this issue, and some hematologists/pathologists still rely on the PSVG criteria.

A separate criterion requires the absence of WHO criteria that would otherwise classify a patient as having PV, PMF, chronic myelogenous leukemia (CML), or MDS. In rare cases CML has a clinical phenotype similar to PV or ET. In cases with $JAK2_{V617F}$ mutation negativity where bone marrow biopsy cannot exclude the possibility of CML, bcr/abl or Philadelphia chromosome analysis is important (10). Criterion 4 underlines the need to exclude reactive thrombocytosis either through the demonstration of $JAK2_{V617F}$ or MPL mutations, or, in mutation-negative patients, by clinical assessment.

Table 3. Revised WHO criteria for essential thrombocytemia (ET)

1. Sustained platelet count > 450 x 10^9/L*
2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes; no significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis
3. Not meeting WHO criteria for PV,† PMF,‡ CML,§ MDS,¶ or other myeloid neoplasm
4. Demonstration of $JAK2_{V617F}$ or other clonal marker, or in the absence of a clonal marker, no evidence for reactive thrombocytosis

Diagnosis requires meeting all 4 criteria.
* During the work-up period.
† Requires the failure of iron replacement therapy to increase hemoglobin level to the PV range in the presence of decreased serum ferritin. Exclusion of PV is based on hemoglobin and hematocrit levels, and red cell mass measurement is not required.
‡ Requires the absence of relevant reticulin fibrosis, collagen fibrosis, peripheral blood leukoerythroblastosis, or markedly hypercellular marrow for age accompanied by megakaryocyte morphology that is typical for PMF_ small to large with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous or irregularly folded nuclei and dense clustering.
§ Requires the absence of *BCR-ABL*.
¶ Requires absence of dyserythropoiesis and dysgranulopoiesis.
_ Causes of reactive thrombocytosis include iron deficiency, splenectomy, surgery, infection, inflammation, connective tissue disease, metastatic cancer, and lymphoproliferative disorders. However, the presence of a condition associated with reactive thrombocytosis does not exclude the possibility of ET if the first three criteria are met.

Practical steps in the diagnosis of essential thrombocytemia (ET)

The lowered platelet treshold of 450 x 10^9/L should allow the detection of early-phase disease. The 95th percentile for normal platelet count, adjusted for gender and race, is below 400 x10^9/L. If patients, however, should experience ET- related symptoms or complications with platelet counts lower than this diagnostic threshold, the diagnosis should be suspected, especially when this is combined with an ET- related feature like erythromelalgia, early or atypical venous or arterial thrombosis, splenomegaly, or unexplained sustained leukocytosis (78).

The first step is to rule out the possibility of reactive thrombocytosis (table 4), which may be caused by inflammatory disorders, chronic infections or non-myeloid malignancy. Iron deficiency can cause thrombocytosis exceeding $1500x10^9/1$, and is important to discover. Once reactive thrombocytosis is ruled out, the second step should be $JAK2_{V617F}$ mutation screening and bone marrow biopsy. Exclusion of bcr/abl (FISH or pcr) or Ph-chromosome by cytogenetic investigation is in our opinion necessary only if JAK2 is negative and the bone marrow biopsy cannot exclude CML (see diagnostic algoritm below), but the WHO criteria require the absence of bcr-abl.

A serum or plasma-EPO concentration should be measured, since a subnormal value gives an indication of MPD diagnosis, and in the case of an established ET diagnosis a subnormal EPO has prognostic significance.

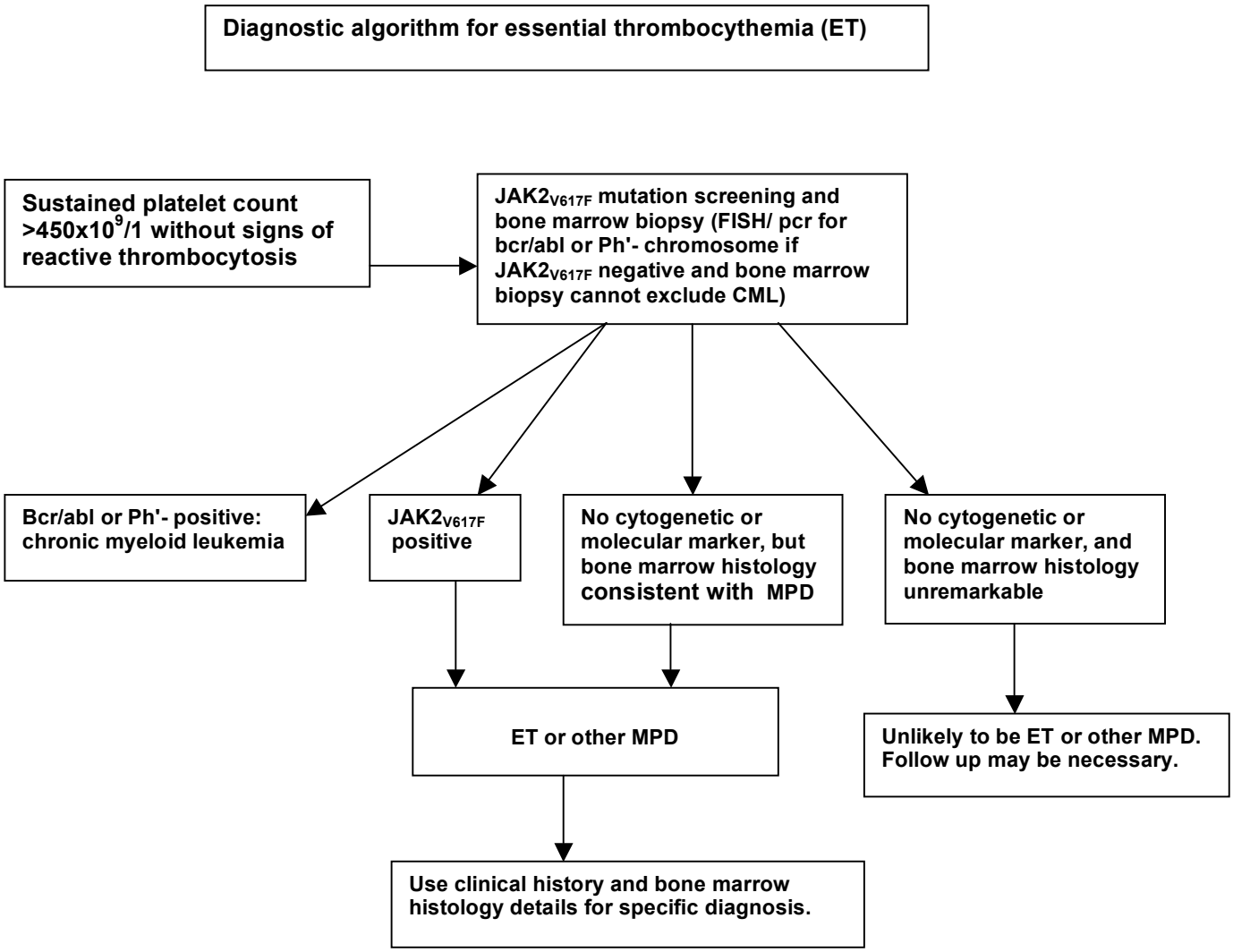
Table 4. Causes of Secondary (Reactive) Thrombocytosis.

Transient processes
Acute blood loss incl. after surgery
Recovery ("rebound") from thrombocytopenia e.g. after alcohol abuse
Acute infection or inflammation
Response to exercise

Sustained processes
Iron deficiency
Hemolytic anemia
Asplenia (functional or after splenectomy)
Cancer
Chronic inflammatory or infectious diseases
- Connective-tissue disorders e.g. rheumatoid arthritis
- Temporal arteritis
- Inflammatory bowel disease
- Tuberculosis
- Chronic pneumonitis

Drug reactions
- Vincristine
- All*trans* -retinoic acid
- Cytokines
- Growth factors

Modified after Schafer AI. Thrombocytosis. N Engl J Med 2004;350:1211-9 (79).



Essential thrombocythemia (ET)

Introduction

The annual incidence of ET was found to be 2.3/10^5 in a large study from Gothenburg 1983-99 (29). A significantly lower incidence was reported from Denmark, i.e. 0.59/10^5 (40). The incidence increases with age and is twice as high in women compared to men; the median age at diagnosis is about 70 years (29,80). Rozman et al (81) and Passamoni et al (82) have reported no significantly increased mortality rate for ET patients. Other studies have found a shorter life expectancy; Fenaux et al (83) found a 73.5% survival 7 years after diagnosis and Jensen et al (40) reported 76% survival at 5 years, both significantly shorter than expected in the normal population. In a recent report from

the Mayo Clinic survival during the first decade of disease was similar to control subjects, while it became significantly worse during the second and third decade after ET diagnosis (84).

Differences in survival and prognosis depend upon which criteria are used to establish the diagnosis of ET. The PVSG criteria have been used in most studies, and bone marrow histo-pathology was used only to differentiate ET from obvious PMF. On the other hand, if WHO criteria are used, in which bone marrow investigation plays a central role, true ET, CPMF-0 and CPMF-1 are differentiated, and these entities have very different prognosis (see chapter on PMF and ref 78).

ET has been considered to be a clonal stem cell disease (85). However, clonality assays have shown that a large proportion of ET patients display polyclonal features when HUMARA technique was applied (86,87). Furthermore, 50-55 % of ET patients carry the $JAK2_{V617F}$ mutation, predominantly in the heterozygous form (i.e. less than 50% $JAK2_{V617F}$ allele burden), while the remaining patients have wild type JAK2 (4,6,88). The measurement of $JAK2_{V617F}$ with specific quantitative methods tends to increase the frequency of patients having a mutation. In a small subset of JAK2 $_{V617F}$ negative patients, mutations of the thrombopoietin receptor MPL (mostly MPLW515K) have been identified. These results indicate that ET is a heterogenous disorder. The findings that 30-50 % of the patients display a subnormal EPO concentration at diagnosis (89), high PRV-1 gene expression (90) and in vitro growth of endogenous erythroid colonies (91) further supports this heterogeneity.

Complications and risk factors

Major thrombotic events are reported to be more frequent compared with the normal population. A wide variation in frequencies is found, 9-84%, mostly depending on different types of institution (1). From Gothenburg a study comprising 70 ET patients described that 19 patients (27%) had experienced major thrombotic events close prior to diagnosis and thereafter (90). Cortelazzo et al (92) compared the overall risk of thrombotic episodes in 100 patients with ET and 200 patients with MGUS; and found the frequencies to be 6.6%/patient-year and 1.2%/patient-year, respectively. Passamonti et al retrospectively evaluated 605 ET patients and found a 10 year risk of thrombosis of 14 % (234).
Several risk factors for developing thrombotic complications have been identified (level 1b); age above 60 years, previous history of thrombosis and long duration of thrombocytosis are major risk factors (92,93,234). A low risk cohort of ET patients, i.e. below 60 years, no earlier vascular events and platelets below $1500x10^9$/L, was prospectively compared with a control group; and was found to have no significant increased incidence of thrombosis (level IIa) (94). Tefferi et al retrospectively reported an analysis of 99 young ET patients with platelets > 1000 x 10^9/L and without risk factors for thrombosis or hemorrhage. These patients showed similar rates of thrombohemorrhagic complications whether (n=75, median age 45) or not (n=24, median age 31) prophylactic cytoreductive treatment was given (235). Median follow-up was shorter in those not receiving therapy, 174 months, compared to those not treated (88 months).
Three other cohort studies have dealt with conventional cardiovascular risk factors and it can be summarized that ET patients with presence of arterial hypertension, hypercholesterolemia, male gender or smoking experienced more thromboembolic events (level III) (95,96).

Circulating platelet-leukocyte aggregates have been found more frequent in MPD patients with thromboembolic events, and is suggested as an explanation to the increased risk of thrombosis in these patients (40,41). Leukocyte count ≥15 x 10^9/L at diagnosis of ET was found to be an

independent risk factor for thrombosis and survival (level II) (84). In a recent published retrospective study by Carobbio et al (217) 439 ET patients were evaluated with respect to leukocyte counts and risk of thrombosis. Leukocyte count above the median level of 8.7 x 10^9/L was found to be an independent risk factor for thrombosis. The authors also interpreted their data that treatment with HU, which decreased the leukocyte levels, had a significant antithrombotic efficacy. A study by Hsiao et al (207) supports the hypothesis of the role of leukocytosis in thrombogenesis in ET patients; the patients who had thrombotic complications had significantly higher leukocyte levels compared with the patients without this complication. These finding all support the opinion that leukocytosis should be taken into account when ET patients are evaluated for risk of thrombotic complications and in the decision of introduction of myelosuppressive treatment. However, the level of leukocytosis when this risk appears has not been determined and more studies confirming that cytoreductive treatment reduces the complication risk have to be done before firm treatment recommendations based on leukocyte counts can be made.

Other risk factors have been reported in smaller retrospective studies (level III); clonal hematopoiesis has been suggested to be a risk factor for thrombosis (87,97). ET patients with subnormal plasma EPO concentration at diagnosis and high PRV-1 gene expression were shown to experience more major macrovascular events compared to patients with normal plasma EPO concentration and PRV-1 gene expression (89,90).

Many different studies have assessed the influence of the JAK2V617F mutational status or JAK2V617F allele burden on the risk of thrombosis in ET, but the results have been conflicting. The PT-1 study, comprising 809 patients, showed that carriers of the JAK2V617F mutation suffered significantly more venous thromboses, but not arterial, compared to patients with the wild type JAK2 (98). Another cohort of 179 ET patients and 77 PV patients showed significantly more thrombosis in ET patients with the $JAK2_{V617F}$ mutation compared with wild type patients. Thus, the $JAK2_{V617F}$ positive ET patients did not differ significantly, in this respect, from PV patients (99). Similar results have been reported by Hsiao et al (207). From the Mayo clinic a first report did not show significant differences between patients with or without $JAK2_{V617F}$ mutation (84), but in a recent study of 176 ET patients the risk for venous thrombosis was significantly greater in patients with $JAK2_{V617F}$ mutation compared with patients with wild type JAK2 (208). Furthermore, a study by Antonioli et al (209) show that increased $JAK2_{V617F}$ allele burden is correlated with a higher frequency of arterial thrombosis. Carobbio and colleagues (236,237) measured risk factors for thrombosis in a cohort of 657 patients with ET, and found that *JAK2V617F* allele burden did not confer an increased risk of thrombosis in a multivariate model. Instead, they noted that *JAK2V617F* allele burden correlated with total white blood cell count, and that leukocytosis with blood cell count greater than 9.4 x 10^9/L was predictive of thrombotic risk. Similar findings have been observed in PV (45). These data suggest that JAK2V617F-mediated effects on thrombosis may, at least partly, be mediated by an increase in neutrophil count and/or increased neutrophil activation.
This is consistent with other studies showing leukocytosis as a risk factor.

Other risk factors have been reported in smaller retrospective studies (level III); clonal hematopoiesis has been suggested to be a risk factor for thrombosis (87,97). ET patients with subnormal plasma EPO concentration at diagnosis and high PRV-1 gene expression were shown to experience more major macrovascular events compared to patients with normal plasma EPO concentration and PRV-1 gene expression (89,90).

Microvascular events seem to be less frequent compared with macrovascular complications. Also here large differences are reported, at least partly due to different definitions. In the Gothenburg study 5 out of 70 patients (7%) experienced aggressive microvascular events, i.e. transient ischemic attacks and/or erythromelalgia (16). The frequency of microvessel disease increase progressively with higher $JAK2_{V617F}$ allele burden, according to a recent study (207). Low dose aspirin has proven efficacy in 80-90% of ET patients with microvascular events (level II) (40,100).

Hemorrhagic complications are reported to be less frequent compared to thrombotic events, 4-69% (1). In the above mentioned Gothenburg study only 4 out of 70 ET patients (6%) suffered from major bleedings (90). After diagnosis the incidence in another study was 0.33%/patient-year (92). High platelet counts, i.e. > 1000 x 10^9/L, was found to be associated with mucosal and gastro-intestinal bleedings (level III) As a possible explanation it has been proposed that an inverse relationship between platelet count and von Willebrand factor is present (101).

Transformation from ET to PV/MF/AML. Reliable frequencies cannot be given as the majority of studies addressing this issue are retrospective and the patients have been treated with different therapies and the follow-up has been of varying length. It can clearly be stated that ET patients with the $JAK2_{V617F}$ mutation have a significantly increased transformation rate to PV and MF compared with patients with the wild type JAK2 (level II) (98). In a study by Cervantes et al (102) the transformation rate into MF was reported to be 8% after 10 years of ET diagnosis (level III). In a mutual population based study of MPD patients in Göteborg and Dijon, France 271 patients with ET were followed for a median time of 15 years. 21 of these developed AML, giving an incidence of 0,37 cases/year. The mean time from diagnosis to AML transformation was 76 months and the median survival time after AML diagnosis was only 4 months (238). The Italian study by Passamonti at al showed a 10 year risk of MF transformation of 3.9%, with anemia at diagnosis as a risk factor. The 10 year risk for AML development was 2.6%, with age > 60 years of age at ET diagnosis as risk factor (234). The ongoing discussion concerning the role of bone marrow biopsy, and the possibility of misinterpreted early stages of idiopathic MF in the group of ET diagnosed with conventional criteria, gives a considerable uncertainty to such figures.

Regarding the transformation rate into AML the vagueness is even greater, since retrospective studies always include different treatment modalities. The report from the Mayo Clinic shows a cumulative probability for AML transformation of 1.4% at 10 years and 8.1% at 20 years. In this study, the risk of transformation to AML or any other myeloid disorder did not appear to be increased with exposure to HU treatment (84).

Clinical management of ET

The main task in the management of ET patients is to prevent the major complications and to decide which treatment options to use in different situations. It should be noted that the majority of studies describing effects of therapy in ET have included patients diagnosed using the old PVSG criteria.

Aspirin prophylaxis

The ECLAP study was designed to establish the value of low-dose ASA (100 mg) in PV patients in a large randomized setting (35). There are no indications that the platelet function differ in the closely related disorders ET and PV. Aspirin is well documented to prevent arterial thrombosis in the general population (104). Aspirin is effective in preventing microvascular symptoms in ET (40,100). Several retrospective studies suggest a benefit for the use of ASA prophylaxis in the prevention of thrombosis, especially in combination with cytoreductive therapy (40,104,105). However, some caution has to be taken in ET patients with very high platelet counts (101) and, of course, in patients with a history of major hemorrhage.

Recommendation:
Aspirin 75-100 mg daily to all ET patients except patients with high platelet levels (i.e. > 1500 x 10^9/L) where cytoreductive therapy should be used initially. Also, aspirin should not be used in patients with a history of major bleedings or other contraindications. The combination of anagrelide and aspirin should be used with some caution.
Grade B recommendation, level IIb.

Platelet lowering therapy
Few prospective randomized studies have been performed in ET. An Italian group randomized 114 high-risk patients (age >60 years or prior thrombosis) to hydroxyurea (HU) treatment or no cytoreductive therapy. The goal in the treatment arm was to lower the platelet count to below 600 x 10^9/L. There was no difference in aspirin or ticlopedine use in the two groups. The patients on HU experienced significantly fewer thrombotic events compared with the patients in the control group, 4% and 24% respectively (39).

Several retrospective studies have indicated age and previous history of vascular events as risk factors (92,93). The incidence of thrombotic events in a low-risk ET population (age <60 years, no history of vascular events and platelet count <1500x10^9/L) did not differ significantly from control subjects in one prospective study (94). Other retrospective reports have confirmed a low rate of thrombotic events in similar low-risk patients (106,107).

Cardiovascular risk factors in the normal population are, naturally, also associated with thrombotic complications in ET patients (93,95,96).

Leukocytosis at ET diagnosis has been shown to be a risk factor for thromboembolic events and survival in several studies (84, 217,236,237).

Clonal hematopoiesis has been suggested to be a risk factor for thromboembolic events (87,97), however, the methods to determine clonality are not widely spread and are costly. Factor V Leiden mutation, subnormal EPO concentration at diagnosis and high PRV-1 gene expression (89,90,94) are all shown to be associated with increased risk for vascular events, but these studies are all retrospective and comprise fairly small cohorts of ET patients.

Several studies with ET patients with $JAK2_{V617F}$ mutation displayed more thrombotic events and higher PV transformation risk compared to patients with the wild type JAK2 (98, 205,206).

Summarized recommendations for platelet lowering therapy in ET:

ET patients with age > 60 years and/or patients with a history of previous thromboembolic or hemorrhagic event should be treated with cytoreductive therapy.
Grade A recommendation, level Ib.

ET patients with a platelet count > 1500 x 10^9/L should be treated with cytoreductive therapy in order to avoid bleeding.
Grade B recommendation, level III.

The goal of cytoreductive therapy should be platelets below 400 x 10^9/L.
Grade B recommendation, level III.

ET patients < 60 years and a platelet count < 1500 x 10^9/L and without other risk factors should not be treated with cytoreductive therapy.
Grade B recommendation, level IIa.

ET patients with microvascular disturbances, cardiovascular risk factors (i.e. arterial hypertension, hypercholesterolemia and/or smoking) and/or leukocytosis at diagnosis could be considered for cytoreductive therapy.
Grade B recommendation, level III.

ET patients with proven clonal hematopoiesis, subnormal EPO concentration at diagnosis and/or $JAK2_{V617F}$ mutation could be considered for cytoreductive therapy.
Grade C recommendation, level IV.

Choice of cytoreductive treatment in ET

Hydroxyurea (HU)
HU is a non-alkylating, non-specific myelosuppressive drug, acting by blockage of ribonucleside reductase. Few randomized trials have been preformed. Cortelazzo et al (39) reported a prospective trial including 114 ET patients randomized to treatment with HU or control; all patients treated with HU got a respons in platelet counts. All treated patients achieved platelet levels below 600x10^9/L with a starting dose of 15mg/kg in a median time of 30 days (range16-60). As mentioned above the HU- treated patients also experienced significantly less thrombotic complications compared to the control subjects.

The British PT-1 study included 809 ET patients with high risk for vascular complications (54). The patients received low-dose aspirin and were randomized to either HU or Anagrelide (Ana), the

median follow-up time was 39 months. The primary end-points in the study were arterial thrombosis, venous thrombosis, serious hemorrhage, or death of vascular complications. Both treatment modalities proved to be effective in lowering platelet counts. Patients receiving Ana, were more likely to withdraw from the treatment due to side effects and adverse events ($p<0.001$). The patients randomized to HU + aspirin, as compared with patients randomized to Ana + aspirin, had a decreased rate of arterial thrombosis ($p=0.004$) and serious hemorrhage ($p=0.008$), but an increased rate of venous thromboembolism ($p=0.006$). Furthermore, the patients with HU + aspirin had significantly lower risk for transformation to MF ($p=0.01$). The main side effects for the patients treated with HU were; nonthrombotic cardiovascular events, gastroentrologic events and hematologic events (lower frequency than patients in the Ana group); and dermatologic events (higher frequency compared with the Ana group). Post-hoc $JAK2_{V617F}$ mutational analysis of the PT-1 trial showed that the increased risk of arterial thrombosis associated with anagrelide therapy was seen in $JAK2_{V617F}$ -positive but not in the $JAK2_{V617F}$ -negative ET patients (98). Although these results were obtained retrospectively, these data are consistent with recent data suggesting that either $JAK2_{V617F}$ -positive disease or a high $JAK2_{V617F}$ allele burden predicts increased sensitivity to hydroxyurea in PV/PMF (239). Thus, the benefits of hydroxyurea over anagrelide may be specific to $JAK2_{V617F}$ -positive ET. A preliminary report of a smaller study has not been able to confirm the difference in vascular complications, instead showing non-inferiority of anagrelide compared to HU (210).

There is a concern for the risk for transformation to AML and secondary malignancies with long-term treatment with HU. No randomized long-term studies in patients with ET have been performed. Several cohort studies with long follow-up find a higher incidence for transformation in HU-treated patients compared with untreated patients (106,107). However, the HU-treated patients were not comparable to patients without requirement for myelosuppressive treatment as they can be suspected to have had a more aggressive disease. In the long-term follow up of 322 ET patients with different treatments the risk of transformation to AML, MF or any other myeloid disorder did not appear to be increased with exposure to HU treatment (84). The large Italian study comprising 605 ET patients also found no correlation between HU and AML development, the only factor in multivariate analysis to be significant was age > 60 years at diagnosis (234). It can clearly be stated that patients treated with HU followed by alkylating agents suffer from more secondary malignancies (58). Furthermore, patients treated with HU followed by radiophosphorous suffer an increased transformation risk (108). Other late occurring side effects of HU are described in the PV chapter.

Two studies have shown a rapid reduction of the $JAK2_{V617F}$ allele burden in PV and ET patients after initiation of therapy with HU, thus, significant decrease of mutated cells were seen within 4 months of treatment (226,227), whereas another found limited effects (225). The clinical importance of the possible effect on $JAK2_{V617F}$ mutated cells has not been studied.

HU should not be used when pregnancy is planned or during pregnancy.
Starting dose recommended is 500-1000 mg daily. The dose range usually used is 500-1500 mg daily.

Recommendations
Hydroxyurea is recommended as a first-line myelosuppressive therapy in ET
Grade A recommendation, evidence level Ib.

As there is still some anxiety about the possibility of leukemia transformation the use of HU should be limited in patients below 60 years of age.
Grade C recommendation, evidence level IV.

Interferon-α
Interferon-α (IFN) suppresses growth of multipotent hematopoietic progenitor cells. No leukemogenic risk has been reported. No randomized trials have been published. In the Italian guidelines for treatment of ET (1) 15 studies have been summarized, including 292 patients. The weekly dose IFN varied from 6 MU to 70 MU. 85% of treated patients responded, while 15% were resistant. Complete normalization of platelet counts was achieved in 54% of the patients. If splenomegaly was present, 66% of IFN treated patients had a reduction and 17% had complete normalization of spleen size. Positive effects on clinical symptoms were correlated to platelet count normalization. Side effects (mainly flu-like syndrome) were common in the beginning of treatment, but decreased with time; 16.5% of patients stopped IFN medication due to side effects. Other side effects of IFN are described in the PV chapter.

Pegylated IFN given weekly has been shown to have equal efficacy as IFN given trice weekly (109,62). The Swedish pegylated IFN study is described in more detail in the PV section. A decrease in $JAK2_{V617F}$ allele burden has been reported during pegylated IFN-α–2a therapy in 8/15 ET pts, with 2 patients achieving molecular CR (230). Starting dose of pegylated IFN-α-2b (PegIntron) is 0.5µg/kg/week, for pegylated IFN-α–2a (Pegasys) a flat dose of 90µg/week is used. If conventional IFN is used the recommended starting dose is 3MU 3 times a week. The maintenance dose range usually used is 1 MU - 5 MU subcutaneous injection three times weekly.

If there is a requirement for myelosuppressive treatment during pregnancy IFN is recommended (110).

Recommendation
IFN treatment is well documented and safe in ET. IFN can be used in younger patients where long-term use of HU is of concern and in patients who do not tolerate HU. If cytoreductive therapy is indicated IFN is the treatment of choice when pregnancy is planned and during pregnancy.
Grade B recommendation, evidence level III.

Anagrelide
Anagrelide acts on the post-mitotic phase of megakaryocyte development, showing a selective effect on megakaryocytes in vitro. Several non-randomized studies have shown good efficacy in ET with normalization of platelet counts in about 70 %. Side effects are rather common, and the drop-out rate is 30-50 % within one year. The most common side effects are palpitations (> 50%), headache (> 50%) and loose stools/diarrhea (15-20 %). On the other hand, when well tolerated, the long term results are good (71,111). A slight lowering of hemoblobin levels is often seen soon after initiation of anagrelide therapy. The most commonly accepted explanation for this finding is vasodilation. However, in the only published long term study of anagrelide use in ET, 24 % of

treated patients experienced a decrease of more than 30g/L hemoglobin during long-term treatment (112).

The PT-1 study (37) is described in more detail above (HU in ET). 405 ET patients were randomized to the angrelide + aspirin arm. Only 19 patients were withdrawn from the study due to lack of platelet control. 148/405 patients were withdrawn mostly because of side effects and adverse events. The most frequent side effects were: nonthrombotic cardiovascular events in 88 patients (palpitations most frequent), gastrointestinal events in 59 patients (diarrhea/loose stools most frequent), hematologic events in 35 patients (anemia most frequent) and dermatologic events in 29 patients (rash most frequent). The incidence of major gastrointestinal bleeding was also significantly higher in the anagrelide + aspirin arm, therefore, the combination of anagrelide and aspirin should be used with some caution. In the PT-1 study there was a significant difference between the two therapy arms with respect to hematologic transformation; 16/405 in the anagrelide + aspirin arm developed MF, compared with 5/404 in the HU + aspirin arm (p=0.01). There is no theoretical evidence to suggest that anagrelide is responsible for these transformations, rather lack of preventing such spontaneous transformation. Further, 4 patients in the angrelide + aspirin arm developed AML compared with 6 patients in the HU + aspirin arm.

Anagrelide should not be used when pregnancy is planned or during pregnancy, since there is no data available concerning effects on the fetus.

The recommended starting dose is 0.5 mg every 12 hours. The degree of side effects can be decreased by increasing the dose with no more than 0.5mg/day/week. The dose range usually used is 1-3 mg daily.

Recommendation
Anagrelide can be used in younger patients where long-term use of HU is of concern and in patients who do not tolerate HU. Patients should have normal cardiac function.
Grade B recommendation, evidence level III.

Busulfan
Busulfan (BU) is an alkylating agent. No randomized tials in ET patients are performed. Non-randomized studies have reported good and long-lasting responses on platelet counts. Vascular disturbances were well controlled in the patients with good platelet response (105,112). There is a concern of increased AML transformation rate with use of alkylating agents, Finazzi et al (58) reported a high transformation rate for ET patients treated with HU and previously treated with BU.

BU is mostly given as intermittent treatment 4-6 mg daily until response (normally 2-6 weeks).

Recommendation
Intermittent BU treatment can be used in ***elderly*** ET patients. Since busulfan is an alkylating agent it should be reserved for patients 75 years or older, or for patients where HU, IFN or anagrelide are not suitable.
Grade B recommendation, evidence level IIb.

Radioactive phosphorus

Radioactive phosphorus (P^{32}) has been frequently used since the 1970-ies. Two randomized trials have been conducted, both versus melfalan. In the PVSG 10 study the response rate was 67%. Long-term follow-up showed that 5/7 ET patients previously treated with HU developed AML. A Swedish non-randomized study reported 10% AML transformation after P^{32} treatment (113).). In a large retrospective population based study from Göteborg the survival of patients treated with P^{32} was not significantly different compared with patients treated with HU, combinations of myelosuppressive agents or patients without cytoreductive therapy (unpublished data)

Recommendation

P^{32} treatment can be used in *elderly* ET patients where HU, IFN or anagrelide are not suitable.

Grade A recommendation, evidence level Ib.

Summarized recommendations for the management of ET

- Aspirin 75-100 mg daily to all ET patients except when platelets are > 1500 x 10^9/L, in patients with bleeding symptoms or in patients with other contraindications to aspirin. (Grade B recommendation)

- Platelet lowering therapy should be given to;
  - all patients over 60 years of age
  - all patients with earlier thromboembolic complications
  (Grade A recommendation)
  - all patients with platelets > 1500 x 10^9/L
  (Grade B recommendation)

- The goal of platelet lowering therapy should be platelets < 400 x 10^9/L
(Grade B recommendation)

- Platelet lowering therapy could be considered in patients with:
  - microvascular disturbances, leukocytosis, and/or cardiovascular risk factors (i.e. arterial hypertension, hypercholesterolemia and/or smoking) (Grade B recommendation)
  - proven clonal hematopoiesis, subnormal EPO concentration, $JAK2_{V617F}$ mutation and/or factor V Leiden mutation (Grade C recommendation)

Choice of cytoreductive therapy in ET (grade C recommendation, evidence level IV)

- Hydroxyurea is the best documented therapy in ET
- However, due to the concern of possible increased risk of leukemia transformation with long-term use it is not recommended as 1st line therapy in younger patients

- < 60 years (where platelet lowering is the indication for treatment):
1st line interferon-α or anagrelide, 2nd line hydroxyurea
- < 60 years (where leukocyte lowering or constitutional symptoms are the indications for treatment):

1st line interferon- α, 2nd line hydroxyurea

- 60 - 75 years:
  1st line hydroxyurea, 2nd line interferon-α or anagrelide
- > 75 years:
  1st line hydroxyurea, 2nd line consider combination therapy (HU-Ana, HU-IFN), 3rd line busulfan, 4th line radiophosphorus

Management of complications in PV and ET

Acute thrombotic events and secondary prophylaxis

Acute thrombotic events should be managed according to current guidelines, individual risk factors should be examined and control of the Hct and platelet count optimized. In emergency situations such as acute cerebrovascular complications or severe digital ischemia acute platelet apheresis or hemodilution/erythropheresis can be used in order to achieve a rapid reduction in blood counts (114). Since the effect is brief, cytoreductive therapy with hydroxyurea must be started as soon as possible.

In a retrospective study (115) 235 PV and 259 ET patients were followed after an arterial (n=341) or venous (n=160) thrombotic event. With a median follow-up of 5.3 years 166 patients (34%) suffered a new event, giving an incidence of 7,6 % patients-years. Age > 60 predicted for a new event, a previous arterial thrombosis predicted for a new arterial event, previous venous thrombosis predicted a new venous event. Increased leukocyte count at time of first thrombosis was a risk factor for recurrence in patients < 60 years. Cytoreductive therapy reduced the incidence of rethrombosis in the entire cohort by 50% due to a marked reduction of arterial events. Significant prevention of rethrombosis was independently achieved in patients with previous venous thrombosis by both oral anticoagulants and antiplatelet drugs, in those with acute coronary syndrome by cytoreduction, and in those with cerebrovascular disease by antiplatelet drugs. Since no prospective trials exist, it remains unclear whether it is better to give a short course of warfarin or to continue with long term therapy for secondary prevention of venous thromboembolism. The role of the ADP-receptor antagonist clopidogrel in MPD patients with arterial thrombosis is currently unclear.

Myeloproliferative diseases (MPDs) represent the commonest cause of splanchnic vein thrombosis (SVT), including Budd-Chiari syndrome (BCS) and portal vein thrombosis (PVT). The largest published series of 241 SVT patients (104 BCS, 137 PVT) showed that $JAK2_{V617F}$ was found in 45% of BCS and 34% of PVT, while JAK2 exon 12 and MPL515 mutations were not detected (222). $JAK2_{V617F}$ analysis was more effective than S-EPO, EEC:s or bone marrow biopsy as a screening test for MPD. Liver dysfunction at the time of diagnosis was more severe in MPD pts. However, underlying MPD had no impact on overall survival, probably due to the fact that they had shorter time to liver decompressive therapy (angioplasty, surgical or radiologic shunting) or transplantation. Thus, the prognostic influence of greater severity at presentation with BCS in MPD patients was offset by earlier relief of hepatic venous outflow block. If confirmed in other studies, this finding would justify including MPD among the arguments for early interventions to relieve the outflow block. No evidence based guidelines can be given regarding long-term therapy after SVT, most clinicians tend to favour continued warfarin therapy if possible. Normalization of any abnormal blood counts is also important.

Bleeding in PV and ET

As mentioned previously, the most important cause of bleeding in ET and PV is acquired von Willebrand´s disease associated with high platelet counts. 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 (116-118). In view of reports of thrombotic complications among non-hematological patients at high risk for thrombosis treated with desmopressin, this agent is not recommended (114). The same is true for von Willebrand factor-containing plasma products (100).

Pruritus
Pruritus, typically aquagenic, can be a severe clinical problem in PV. Antihistamines may be of benefit (119). Several studies describe improvements with treatment with IFN (59,120). Tefferi & Fonseca (121) reported 10 patients with PV who were treated with selective serotonin re-uptake inhibitors for other reasons and had great improvement of pruritus. Benefit has been shown with phototherapy using psoralen and ultraviolet A light (122).

Elective surgical interventions
It is generally recommended to use cytoreductive agents in order to normalise blood counts before elective surgery, but this recommendation is not based on solid data from well-controlled studies. However, a recent study of surgical interventions in 105 PV and 150 ET patients showed a high risk of thrombosis despite good hematological control (123). Thus, despite a median platelet count of 477 and a hematocrit of 0.43 as well as prophylactic heparin in 56%, aspirin in 74% and warfarin in 6%, an incidence of thrombosis of 8% was seen. It has also been shown that perioperative complications after splenectomy have decreased after prompt use of cytoreductive agents to counteract postsplenectomy thrombocytosis (124). These findings imply that it is of benefit to control elevated counts before invasive surgery.

Transformation to AML
The results after conventional AML induction chemotherapy are dismal in patients developing AML after PV, ET or PMF, with a very short median survival. Results are not significantly better than palliative therapy. If possible it is recommended that patients undergo allogeneic stem cell transplantation after induction chemotherapy.

Thepot et al (240) has recently presented data on azacytidine therapy in 17 pts with MDS or AML post MPD not eligible for transplantation. The median number of cycles administered was 4 (range 1–9). The overall response rate (including CR, PR, CRi) was 10/17 (59 %). 9/10 responders were still alive after 2 to 16 months (median 5.5).

Pregnancy in PV and ET

There is only limited information in the medical literature about the management of PV in pregnancy. Of the 20 pregnancies reported there were 12 live births but 3/12 suffered early neonatal death. A recent series of pregnancies (125) reviewed a further 16 pregnancies in 8 women and documented significantly greater chance of live birth with aggressive management. The risks of pregnancy in PV are probably similar to those for patients with ET where about 300 pregnancies are reported in the literature. 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

growth retardation, intrauterine death and stillbirth (8%) (126-128). Major maternal complications occur in approximately 8% of ET patients. In a study by Passamonti et al ET patients with JAK2 mutation showed increased risk of pregnancy complications (211).

An overview of the literature does not enable confident management guidelines to be drawn up, but guidelines summarized below have recently been published (128). We recommend that pregnant MPD patients are followed in haematological centers that have experience in handling this situation in close collaboration with an obstetrical department. Therapeutic strategies for PV and ET in pregnancy are influenced by the patients' disease status and prior obstetric history. If any of the following factors are present then the pregnancy is likely to be at high risk of complication to the mother and/or fetus:

- previous venous or arterial thrombosis in mother
- previous haemorrhage attributed to PV/ET
- previous pregnancy complication that may have been caused by PV/ET
- significant ante- or postpartum hemorrhage
- severe preeclampsia
- platelet count rising to $>1{,}000x10^9/l$

Therapeutic options include antithrombotic treatment, phlebotomy in PV and cytoreductive agents, although the expected natural fall of the platelet count and Hct during pregnancy may anyway obviate or reduce the need for the latter. Spontaneous remissions of ET had also been described during pregnancy (129,130). The target Hct for a non-pregnant female has yet to be determined but in pregancy the Hct should be maintained within the normal range appropriate for gestation. There is currently no evidence for maintaining Hct lower than this in pregnancy.

Platelet-reducing therapy is important in pregnancy if platelets are high, in order to avoid loss of the fetus and other complications. Where cytoreduction is deemed necessary, IFN is the drug of choice. There are no reports of teratogenic effects in animals or adverse effects in the admittedly small numbers of pregnancies exposed to this drug. One still-birth and one malformed infant, and teratogenicity in animals, has been reported with HU. Hence hydroxyurea is probably contraindicated at the time of conception, which also also applies to male patients, and during pregnancy. Anagrelide is not recommended because of insufficient documentation of its use in pregnancy. Thus hydroxyurea or anagrelide should be gradually withdrawn 3-6 months prior to conception and may be substituted with IFN if necessary.

Low dose aspirin seems advantageous during pregnancy in ET (128). We recommend that in the absence of clear contraindications all patients should be on aspirin 75mg throughout the pregnancy. About two weeks before delivery is expected, aspirin is substituted by low molecular weight heparin which is given until 6 weeks after delivery (grade C recommendation, evidence level IV).

Low molecular weight heparin (LMWH) has been used anecdotally in women with PV or ET and previous thrombosis and/or fetal morbidity. If the mother or fetus is at high risk for complication (see previous page), the use of LMWH is indicated during the whole pregnancy. The first six weeks post-partum is a high risk period for venous thrombosis. Blood counts may rise rapidly, thus on-going haematological monitoring is important. In the puerperium we recommend thrombosis prophylaxis for 6 weeks with LMWH for all women with MPD. Breast feeding is safe with heparin,

but contra-indicated with the cytoreductive agents. The doses of LMHW that have been reported are dalteparin 5000 U or enoxaparin 40mg daily**.**

Primary Myelofibrosis

Introduction

Primary myelofibrosis (PMF), agnogenic myeloid metaplasia or myelofibrosis with myeloid metaplasia (MMM) is characterized by progressive accumulation of connective tissue and endothelial proliferation in the bone marrow being accompanied by extramedullary haematopoiesis with enlargement of the spleen and liver (131-140). The disease is rare with an estimated incidence in the Western countries of 0.4-0.7 new cases per 100 000 person/year (80). It is a disease of mainly elderly people with a median age at presentation of about 65 years, although about 25% of patients are aged 55 years old or less (137). The median survival of PMF patients ranges from 3.5-5 years (135,141) with a very wide range, since some patients die after 1 or 2 years from diagnosis and others survive even for decades. This variability is partly age-related but also reflects that PMF is a very heterogeneous disorder in terms of presentation and evolution. Accordingly, the clinical spectrum of the disease at diagnosis ranges from asymptomatic patients who may not need treatment even for several years to those patients with severe constitutional symptoms together with anemia and symptoms related to the enlarged spleen from the very onset of the disease or shortly after. The clinical phenotype – asymtomatic versus severe constitutional symptoms – is related to the degree of myeloproliferation and myeloid metaplasia, which together with the development of transfusion-dependent anaemia often determine when to initiate treatment in individual patients. Although the clinical course is chronic in the large majority of patients a subgroup is characterised by a rapidly lethal course with death within a few months from diagnosis (=acute myelofibrosis) (132,133).

Although described many years ago (77,142,143), it has only in recent years been increasingly recognized that the classical clinical phenotype of PMF as described above is preceded by a prefibrotic stage, which clinically may mimick ET. This early stage of PMF is now widely accepted as a distinct clinico-pathological disease entity implying that a large number of patients previously categorized as ET in the future will be correctly diagnosed as *early prefibrotic idiopathic myelofibrosis* (77,142,143). Indeed this novel concept of PMF may also profoundly change the clinical phenotype, course and prognosis of ET (77,102,144). A stepwise evolution of the disease process in PMF from an early ET-like phenotype, featured clinically by the hyperproliferation of megakaryocytes – thrombosis and bleeding - to classical PMF, in which the consequences of bone marrow failure and myeloid metaplasia – leucoerythroblastic anemia, tear-drop poikilocytosis, bone marrow fibrosis and huge splenomegaly - are the dominating clinical features is in fact very similar to the evolution of PV. Thus, PV may progress to myelofibrosis with myeloid metaplasia (MMM), also called the ”spent phase” of the disease, which evolves in approximately 10-15% of the patients after an average of 10 years from diagnosis (144). This stage of the disease is clinically and histopathologically indistinguishable from PMF and should be treated accordingly. Before this end stage of PV patients may remain in a transitional stage of the disease (”transitional myeloproliferative disorder”) for several years being featured by pancytosis, huge spenomegaly and bone marrrow fibrosis (145). This dynamic evolution of PMF shares some similarity with the different disease phases in CML, although the evolution of PMF is much slower.

As also outlined above it seems logic that a continuum exist from “early” myelofibrosis with no or minimal bone marrow fibrosis to the advanced stage of myelofibrosis with myeloid metaplasia. However, the concept of prefibrotic myelofibrosis as a separate disease entity easily to be distinguished from ET by distinct histopathological features (77,142,143) has recently been challenged by the findings of no differences between patients with "prefibrotic myelofibrosis" or "true ET", neither clinically, biochemically or in regard to JAK2 status, thromhemorragic complications, survival, or myelofibrotic transformation (213). A most recent study comparing the prognostic role of bone marrow grading according to the European consensus report (214) with other prognostic scoring systems (135,141,215) has confirmed that bone marrow fibrosis is a significant marker of disease progression and associated with different survival and also clearly distinguishing overall survival in the different risk groups of patients with PMF (216).

Pathogenesis

Both fibrogenesis and angiogenesis are considered to develop consequent to the intramedullary release of various growth factors from rapidly proliferating, large and dysplastic megakaryocytes which are always located in clusters near to sinusoids (146-149). These growth factors include among others platelet-derived growth (PDGF), basic fibroblast growth factor (bFGF), transforming growth factor beta (TGF-beta) and vascular endothelial growth factor (VEGF) , which are mitogenic for fibroblast and endothelial proliferation , giving rise to the major histopathological hallmarks of the disease – myelofibrosis, osteosclerosis and angiogenesis in the bone marrow and spleen. Among these GFs TGFbeta1 may have a particular role, since this GF has the capacity to trigger all three stroma changes in PMF - fibrosis, osteosclerosis and angiogenesis (150).

In 2005 a major breakthrough in the understanding of the pathogenesis of PMF and allied diseases occurred by the identification of the JAK2 mutation in a large proportion of patients with CMPDs, including about half those with PMF (4-7,151-154). In regard to PMF, JAK2-positivity has been reported to be associated with higher leukocyte counts, a history of thrombosis or pruritus and a less frequent need of blood transfusions (151,153). Concerning the impact of the JAK2 mutation upon survival and leukemic transformation in PMF the results have been divergent (151-154,241-244), The largest study by Barosi et al clearly demonstrated the JAK2 mutation to be an independent predictor of evolution towards large splenomegaly, need of splenectomy and leukemic transformation (243), whereas another study by Tefferi et al found significantly shortened overall and leukemia-free survival for those myelofibrosis patients with a JAK2 mutational load in the lower quartile as compared to those in the upper quartile allele burden group; They concluded that a low V617F allele burden in PMF might indicate the presence of an overriding V617F-negative clone being associated with a more aggressive disease phenotype (244). In patients with post-polycythemia vera and post-essential thrombocythemia myelofibrosis $JAK2_{V617F}$ mutational status and allele burden have been shown to have little influence on clinical phenotype and prognosis (245).

In 2006 and later other mutations in the thrombopoietin receptor (MPL-W515L,MPL-W515K) were described being present in about 5-10 % of the patients (212,246-252**).** These mutations occur at the level of the multipotent hematopoietic stem cell (250). Only the MPL W515 mutation triggers spontaneous MPL activation. The other MPL mutations may synergize with $JAK2_{V617F}$ or other not yet characterized molecular events (252). In patients with PMF the occurrence of the MPLW515L/K mutation is more frequently seen in females and patients harbouring the mutation are older with more severe anemia and accordingly more likely to require blood transfusions (212).

Despite the divergent results on the correlation beteween clinical correlates and the prognostic impact of the JAK2-mutation in myelofibrosis patients, a new concept of these diseases as a biological continuum from ET over PV to MMM has emerged. Thus, in most studies the JAK2-mutational load has been shown to reflect the "tumor burden" as assessed by a rising leukocyte count and/or increasing splenomegaly during disease progression towards myelofibrotic and leukemic transformation (243). All these aspects have been reviewed (246,253)

Diagnosis

As outlined previously PMF/MMM is a very heterogeneous disease in clinical presentation and evolution. Although the diversity of clinical presentations and the various patterns of evolution is partly explained by the occurrence of transitions within the chronic myeloproliferative disorders as typified by the well-described transitional stages between PV and postpolycythemic myelofibrosis (144,145) the broad clinical spectrum of PMF/MMM as reported in the literature is certainly also partly explained by different criteria used for diagnosis of the disease.

The first formulation of diagnostic criteria in PMF/MMM were advanced by the Polycythaemia Vera Study Group (PVSG) to be used cooperatively in studies of pathogenesis and evolution of the disease. These criteria included the following: 1) a leukoerythroblastic blood picture, 2) splenomegaly, 3) bone marrow fibrosis involving more than one third of the sectional area of a bone marrow biopsy and 4) the absence of well-established diagnostic criteria for other CMPs (ie, no increased red blood cell mass and no Philadelphia chromosome) and exclusion of systemic disorders accompanied by bone marrow fibrosis (155).

Although the diagnostic criteria in most reported larger series of PMF/MMM have included bone marrow fibrosis together with leucoerythroblastic anaemia, teardrop polikilocytosis and variable splenomegaly (131,132,136,137,141) some series already in the 1980-ies included patients with no or minimal bone marrow fibrosis in the early phase of the disease ("early myelofibrosis"), the designation "primary myelofibrosis-osteomyelosclerosis " being used only for those patients with features of classical chronic primary myelofibrosis (142). Accordingly, already about 20 years ago the concept of "early prefibrotic myelofibrosis" was born, but only in recent years the stepwise evolution of the disease process has been widely accepted in terms of the Cologne criteria for diagnosis of PMF/MMM (World Health Organization classification of the disease). Using the Cologne criteria of PMF/MMM in the diagnostic classification will significantly change the future clinical spectrum of PMF as well as ET, taking into account that this spectrum includes a prodromal stage of PMF/MMM which in most previous studies has been classified as ET (77,143).

Besides the Cologne project on definition of diagnostic criteria in PMF/MMM the Italian Cooperative Group on Myeloproliferative Disorders developed a definition using the literature-derived evidence on sensitivity and specificity of a core set of diagnostic criteria and the consensus methodology (138,139). Since then these diagnostic criteria have been widely used in clinical studies. A major problem with this set of criteria is that they inevitably include PV-patients with huge spleens, but still only a slightly lowered or normal Hb-concentration consequent to an expanded plasma volume (haemodilution) and an increased red cell mass. Accordingly, these patients are categorized as primary MMM although they fulfil the golden standard criterium for diagnosis of PV – an increased red cell mass. Other diagnostic criteria were also proposed which took into account the presence or absence of the JAK2 mutation (154). In regard to diagnostic criteria for PMF, we recommend the recently published revised WHO criteria (201), which are also

based upon the Cologne-criteria, implying the acceptance of a prefibrotic cellular phase of the disease as outlined above.

Revised WHO criteria for primary myelofibrosis

Major criteria
1. Presence of megakaryocyte proliferation and atypia,* usually accompanied by either reticulin and/or collagen fibrosis, or, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (ie, prefibrotic cellular-phase disease) 2. Not meeting WHO criteria for PV, CML, MDS, or other myeloid neoplasm** 3. Demonstration of JAK2617V>F or other clonal marker (eg, MPL515W>L/K), or in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic diseases***

Minor criteria
1. Leukoerythroblastosis
2. Increase in serum lactate dehydrogenase level****
3. Anemia****|
4. Palpable splenomegaly****

Diagnosis requires meeting all 3 major criteria and 2 minor criteria.

* Small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei and dense clustering.

**Requires the failure of iron replacement therapy to increase hemoglobin level to the polycythemia vera range in the presence of decreased serum ferritin. Exclusion of polycythemia vera is based on hemoglobin and hematocrit levels. Red cell mass measurement is not required. Requires the absence of bcr-abl. Requires the absence of dyserythropoiesis and dysgranulopoiesis.

***Secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies. It should be noted that patients with conditions associated with reactive myelofibrosis are not immune to primary myelofibrosis and the diagnosis should be considered in such cases if other criteria are met.

****Degree of abnormality could be borderline or marked.

Investigations

Stage 1

Full blood count

According to the older literature the large majority of patients presents with anemia, low Hb-concentrations (< 100 g/L) in particular being recorded in those with very large spleens (131). In most patients the anaemia is normocytic normochromic but an elevated MCV may be seen owing to B12- or folate deficiency or reticulocytosis consequent to Coombs positive hemolysis. A microcytic anaemia secondary to iron deficiency is not uncommon owing to bleeding from the gastroinsteinal tract.

Neutrophilia is found in most patients at the time of diagnosis unless the patient is diagnosed in the advanced "burnt out" phase of the disease with pancytopenia and huge splenomegaly. Immature red (erytroblasts) and white cells (myelocytes, metamyelocytes, promyelocytes and occasionally blast cells) are classically seen in peripheral blood ("leucoerythroblastic blood picture"). In classical PMF the platelet count is normal or decreased at the time of diagnosis being mainly explained by platelet pooling and sequestration in the enlarged spleen (133). In some patients immune-mediated platelet destruction may also account for low platelets.

With the recognition of an initial prefibrotic stage of PMF (early prefibrotic PMF) (77,142,143) a proportion of the patients have another clinical phenotype being featured by the hypercellular phase of the disease (normal or near normal Hb-concentration, neutrophil leucocytosis, and thrombocytosis) and less by myeloid metaplasia (no or only modest enlargement of the spleen). These patients have been/are being misclassified as ET or an undifferentiated MPD.

Iron status

Asessment of iron status at the time of diagnosis by available analyses (plasma iron, plasma transferrin, plasma ferritin, or plasma soluble transferrin receptor) and during the course of the disease - when indicated by clinical suspicion – is of particular importance for the early diagnosis of bleeding from the gastrointestinal tract, which is likely to develop in those patients with massive splenomegaly due to portal hypertension (hypertensive gastropathy, ruptured esophageal varices, gastric or duodenal ulcer) as a consequence of a hyperdynamic portal circulation. In some patients another contributing factor for the bleeding tendency may be decreased platelet function. Furthermore, investigation of iron status is indicated before and during treatment with erythropoietin. Replacement by iron may occasionally also disclose those patients with "subclinical" PV, who have bled from eg. the GI-tract.

Liver and renal function

These tests are recommended in all patients to obtain baseline values at diagnosis with reference to potential later development of abnormal tests during the course of the disease secondary to extramedullar haematopoiesis in the liver or decreased renal function due to hyperuricaemia or more rarely hypercalcemia.

CRP

C-reactive protein should be performed when assessing plasma ferritin levels to exclude elevated ferritin values to reflect current inflammation. In addition, CRP is an important measure when

assessing if current inflammation or infection contribute to hypermetabolic symptoms (low-grade fever, weight loss, sweats, fatigue).

Bone marrow biopsy
In all patients suspected of a diagnosis of PMF a bone marrow biopsy is mandatory and a sine qua non to identify the characteristic cellular and stroma features of the disease. These include a vast amount of large morphologically atypical megakaryocytes with hypolobulated and hyperchromatic nuclei, dense clustering and the surrounding fibroblast-and endothelial proliferation, the latter being absent or minimal in the prefibrotic stage of the disease (77,143).

JAK2 mutation analysis
As noted previously the $JAK2_{V617F}$ -mutation is positive in about half of PMF-patients, which may be associated with a poorer prognosis (151). However, this issue is controversial and further studies are required to delineate if JAK2-positive MF-patients have a clinical phenotype and a prognosis which differs from those who are JAK2-negative (152). Assessement of JAK2-mutation burden is recommended in all patients at the time of diagnosis and during follow-up in JAK-2 positive patients to follow the "tumor burden" at the molecular level, eg. during IFN-α treatment of JAK2-positive patients in the hypercellular phase of the disease. In addition, quantitative $JAK2_{V617F}$ analysis is recommended in the setting of bone marrow transplantation to monitor residual disease.

Cytogenetic analysis / FISH-analysis/ pcr bcr-abl
The role of cytogenetics as a prognostic marker in PMF has recently been underscored (254-256). Indeed, karyotype complements the International Prognostic Scoring System (256).
In a series of 109 patients cytogenetic findings were categorized as normal vs abnormal or favourable (normal or with sole abnormalities of 13q- or 20q-) vs unfavourable (all other abnormalities). Cytogenetic analysis was abnormal in 33 % of the patients and 21 % displayed an unfavourable karyotype. At a median follow-up of 35 months an unfavourable karyotype predicted shortened survival (256). In young patients a cytogenetic analysis is also indicated as a prognostic parameter to be included in the decision-making for a bone marrow transplant (155-159).
If the aspiration yields no material for cytogenetic analysis (dry tap) a FISH-analysis on peripheral blood (Ph-chromosome) or a pcr for the bcr-abl should be performed to exclude CML, which initially may present with only elevated platelet counts or only moderately elevated leucocyte and platelet counts.

Stage 2 Investigations

Plasma/serum erythropoietin
In patients with enlargement of the spleen but still normal or only slightly lowered Hb-concentration an analysis of plasma/serum erytropoietin and red cell mass estimation should be performed to categorize these patients correctly as PV. In addition, measurement of Epo is important as a screening test for a favourable outcome of treatment with erythropoietin. Patients with a value <125 U/L respond more readily to treatment (160,161). However, Epo levels above 125 U/L are also compatible with a response to therapy. Accordingly, plasma Epo is recommended in all patients considered candidates for Epo-treatment.

CD34+ count

The number of circulating CD34+ cells are always increased in patients with classical PMF/MMM, a cut-off levels of 15/ul discriminating patients with PV from those patients with incipient bone marrow fibrosis and myeloid metaplasia (162-164). Monitoring of the CD34+ has been proposed as a means in assessing disease activity, highly elevated and increasing levels being interpreted as imminent leukemic transformation (162). Others have not been able to confirm these findings (164). The CD34+ count is recommended as an additional potentially useful marker of disease activity in PMF-patients.

X-ray of the skeleton
This investigation has previously been recommended in all patients at the time of diagnosis to obtain information on the degree of osteomyelosclerosis, which may be of prognostic significance (133). However, this is nowadys seldom performed due to the desciption of more robust prognostic factors.

Complications and associated diseases

Complications of PMF are very common contributing significantly to morbidity and mortality. Most commonly are infectious (20-60 %) cardiovascular (20-50 %), thromboembolic (10-40 %) or haemorrhagic (30 %) complications . Transformation to acute leukaemia is seen in about 20-30 % of the patients. Second malignancies are seen in about 10 %. Various autoimmune diseases may be present at debute or arise during the course of the disease (131-140).

Recommendations for assessing risks of complications in PMF.
A firm risk assessment for an individual complication is not possible in patients with PMF, although some studies have indicated that high circulating CD34+ counts and a high JAK2 allele burden is associated with progressive splenomegaly and leukemic transformation. Thus, in patients below the age of 65 years of age a progression in the Lille score or elevation of CD34+ cells in peripheral blood to > 300 x 10^6/L should be considered for the possibility of SCT (see page 42 KOLLA sid nr). However, as outlined in a previous section both the CD34 count and the JAK2 allele burden are controversial parameters as predictors of disease progression.

A highly discriminative prognostic system has been reported based upon 1054 patients consecutively diagnosed with PMF at seven centers (257). Overall median survival was 69 months (95 % CI: 61-76). Multivariate analysis of parameters obtained at disease diagnosis identified age > 65 years, presence of constitutional symptoms, hemoglobin level < 100 g/L, leukocyte count > 25 x 10^9/L and circulating blast cells > 1% as predictors of shortened survival. Based on the presence of 0 (low risk), 1 (intermediate risk-1), 2 (intermediate risk-2) or > 3 (high risk) of these variables, four risk groups with no overlapping in their survival curves were delineated. Respective median survivals were 135, 95, 48 and 27 months ($p < 0.0001$). Compared to prior prognostic models, the new risk stratification system displays higher predictive accuracy, replicability and discriminating power (257). In 409 patients with assessable metaphases, cytogenetic abnormalities (observed in 30% of cases) were associated with shorter survival and contributed to prognosis, but only in patients in the intermediate-risk groups. No prognostic influence for either JAK2 V617F mutational status (n= 345) or blood CD34+ cell count (n=150) was noted. Comparisons of the Lille scoring system and the new prognostic scoring system is given below.

Lille scoring system for predicting survival in PMF

No of adverse prognostic factors*	Risk group	Median survival (months)
0	Low	93
1	Intermediate	26
2	High	13

* i.e hemoglobin < 100 g/L and WBC count < 4 or > 30 x 10^9/L

New Prognostic Scoring System (International Working Group for Myelofibrosis Research and Treatment)

No of adverse prognostic factors*	Risk group	Median survival (months)
0	Low-risk	135
1	Intermediate risk-1	95
2	Intermediate risk-2	48
>/=3	High-risk	27

* > 65 years; presence of constitutional symptoms; hemoglobin < 100 g/L; WBC count > 25 x 10^9/L and circulating blast cells >/= 1 %.

Conventional Treatment Modalities

Cytoreductive Therapy

The treatment of PMF/MMM aims at reducing the hypermetabolic symptoms associated with clonal myeloproliferation/myeloaccumulation and myeloid metaplasia (131-133,135,137). Conventionally patients with the hyperproliferative form of PMF have been treated with Myleran (busulphan, BU) (165), Alkeran (Melphalan) (166) or hydroxyurea (HU) (167-169). Busulphan and melphalan are leukemogenic and HU may be leukemogenic - at least when being used sequentially in this patient group (170). In younger patients conventional or pegylated alpha-interferon may be useful alternatives (171-175), although in some patients treatment with α-IFN was associated with significant side effects and accordingly not recommended in recent reports on α-2-IFN treatment of PMF (174,175). However, significant reductions in the JAK2-allelle burden in PV during long-term

treatment with alpha-interferon in concert with normalisation of the bone marrow in a subset of patients might certainly indicate that alpha-interferon may also have a role in younger patients in the hypercellular phase of PMF, post-polycythaemic and postthrombocythemic myelofibrosis. Early treatment with alpha-interferon may postpone or even inhibit the ultimate development of the burnt-out phase of the disease with huge debilitating splenomegaly and bone marrow failure due to progressive accumulation of connective tissue in the bone marrow (172,258,259). Patients with progressive splenomegaly and increasing leuco- and thrombocytosis following splenectomy have been successfully treated with 2-chlorodeoxyadenosine (176).

There are no data in the literature concerning therapy of thrombocytosis in PMF in order to minimize the risk of thrombosis. We therefore recommend that clinicians follow the guidelines regarding platelet reduction given in ET, since the disease processes are similar.

Cytotoxic treatment is associated with the development or deterioration of anaemia in most patients. Besides treatment with iron, folic acid and vitamin B12, the anemia may be corrected by treatment with recombinant human erythropoetin, danazol, glucocorticoids or thalidomide (134,140).

Choice of cytoreductive Therapy

Hydroxyurea
The efficacy and safety of HU in the treatment of PMF has been reported in several studies (167-169). Whereas HU lowers elevated leucocyte and platelets counts within days several months may elapse before regression of an enlarged spleen is recorded on clinical examination. In some patients bone marrow fibrosis may regress during treatment with BU (165) and HU (168), although not reproduced in most recent larger studies (177).

Recommendations:
Hydroxyurea is recommended as a first-line cytoreductive therapy in PMF patients not eligible for transplantation.
Grade B recommendation, evidence level III.
Owing to the growing concern about the leukemogenecity of HU alternative agents should be preferred in younger patients, i.e α-interferon (PegIntron - pegylated interferon α-2b, Pegasys - pegylated interferon α-2a).
Grade C recommendation, evidence level IV.

Busulphan (BU)
This agent has been previously used extensively in the treatment of PMF (46). It gradually within weeks to months lowers leucocyte-and platelet counts. Symptoms related to the enlarged spleen may also vanish in concert with a steady but slow decrease in spleen size. 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%) (170). Combinational therapy with BU and danazol has been reported to be well-tolerated with alleviation of hypermetabolic symptoms and rise in Hb-concentration in selected patients (178).
Grade B recommendation, evidence level III.

Interferon-α (IFN)
Theoretically, IFN might be a useful agent for the treatment of PMF by inhibiting clonal myeloproliferation – suppression of early progenitors and in particular the megakaryocyte cell lineage – and also endothelial proliferation. Several studies have been conducted showing that IFN may be efficacious in PMF, in particular patients in the hyperproliferative stage of the disease (171-175). However, owing to its side effects elderly patients do not tolerate the drug very well. IFN is not leukemogenic. Regression of bone marrow fibrosis is not the rule.
As previously described the JAK2-mutation is found in about half those patients with PMF. The findings of a steady decrease in the level of the JAK2-mutation in a large number of PV patients during treatment with pegylated interferon α-2a supports the contention that IFN-treatment may influence the disease at the molecular level, at least in half of the patients (JAK2-positive).
Recommendations : IFN-α is recommended as the drug of choice for patients who might be candidates for transplantation with PMF, post-polycythemia and post-thrombocythemia myelofibrosis in the hyperproliferative phase of the disease.
Grade B recommendation, evidence level III.

2-Chlorodeoxyadenosine (2-CdA)
This agent 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 leucocytosis and thrombocytosis following splenectomy. It is administered at 0.05-0.1 mg/kg for 7 days monthly for up to five treatment cycles (176).
Recommendations : 2-CdA is recommended in symptomatic patients refractory to other conventionally used cytoreductive therapy.
Grade B recommendation, evidence level III.

Anagrelide
Anagrelide 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 (179-181). Although interfering with the proliferation of megakaryocytes and therefore theoretically with the production of cytokines responsible for the development of myelofibrosis and osteosclerosis this agent does not inhibit progression of myelofibrosis or the production of GFs in PMF (180) or essential thrombocythaemia (54,181).
Recommendation: Anagrelide is recommended in PMF-patients with symptomatic thrombocythaemia and intolerance to other conventional cytoreductive drugs
Grade B recommendation, evidence level III.

Radiotherapy

Spleen and lung irradiation
Several reports have documented that radiation of the spleen may benefit symptomatic patients with huge spleens (182). 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 6-8 months.
Radiation of the spleen prior to splenectomy is associated with an increased risk of postoperative bleeding. Radiation of the lungs (whole-lung external beam radiotherapy in a single fraction of

100 cGy) may induce marked clinical improvement and decrease in pulmonary artery systolic pressure in patients with pulmonary hypertension due to myeloid metaplasia (183).
Recommendations: Spleen irradiation should be considered in elderly patients with symptomatic splenic enlargement, refractory to conventional cytoreductive therapy, and not candidates for splenectomy. Lung irradiation may be used to alleviate symptoms of pulmonary hypertension consequent to myeloid metaplasia in the lungs.
Grade B recommendations, evidence level III.

Radiation therapy to other sites
Symptomatic extramedullary haematopoiesis – 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) (184-186).
Recommendations: Low-dose radiation therapy alleviates symptomatic foci of myeloid metaplasia.
Grade B recommendations, evidence level III.

Splenectomy

In addition to mechanical discomfort 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 anaemia and thrombocytopenia consequent to pooling and sequestration of red blood cells and platelets. All these features of hypersplenism is alleviated by splenectomy with symptomatic improvement in most patients and a rise in Hb-concentration in about half of the patients. Accordingly, main indications for splenectomy in PMF include – in addition to pronounced mechanical discomfort - episodes of upper gastrointestinal bleeding secondary to portal hypertension (varices), transfusion-dependent anaemia and low platelet counts . Since the procedure is associated with significant morbidity (infection, thrombosis and bleeding - about 25-30%) and mortality (7-10%) (187,188) conditioning and timing of the patient and surgeon are of utmost importance. There is no evidence in the literature to support the contention, that splenectomy is followed by an increased risk of leukemic transformation (188). Splenectomy prior to SCT in patients with huge spleens is a matter of debate.

Recommendations: Splenectomy should be considered in patients with huge splenomegaly associated with repeated upper gastrointestinal bleeding episodes due to portal hypertension and/or cytopenias secondary to haemodilution, splenic pooling and sequestration of blood cells.
Grade B recommendations, evidence level III.

Stem cell transplantation (SCT)

Allo- SCT is the only curative therapy for patients with PMF. In two larger series reporting the outcome of HLA-identical SCT the 3- and 5-year probability of survival was 58% and 47% +/- 8% , respectively (155,156). In general, karyotypic abnormalities, ostemyelosclerosis and high-risk score (Lille Score) at the time of transplantation is associated with a poor outcome. A trend for increased treatment related mortality has been reported when TBI has been used in the conditioning regimen. The transplant related mortality is about 25-30%. Long term survival has been poor for patients over 45 years and was only 14 % in a large study.

Induction of remission after donor lymphocyte infusion has been demonstrated in PMF as evidence of a graft versus myelofibrosis (GvMF) (189, 190, 190a) and implying reduced-intensity allo-SCT to have a possible role in PMF. Since then several reports have shown very encouraging results of non-myeloablative allo-SCT with a low mortality and high 1 year survival rates of 54 % and 90 % and an overall and disease-free survival at 3 years of 84 % (157-159). A study from Sweden has confirmed these findings showing a much better outcome after reduced-intentensity allo-SCT than after concentional allo-SCT (159). Several studies including the Swedish study have shown a good tolerabality and good long-term survival for patients up to 65 years of age.
Recommendations: Allo-SCT with myeloablative or reduced intensity conditiong is indicated in young (< 40 years of age) high risk patients with PMF. Reduced intensity transplantation should be considered for patients with biological age of 40-65 years with high risk features at diagnosis or later during the course of the disease (elevation of Lille score and/or increase in peripheral blood CD34+ cells > 300 x 10^6/L.

Grade B recommendations, evidence level III.

Treatment of Anemia in Primary Myelofibrosis

As outlined previously the anaemia in PMF is multifactorial and deficiency of iron, vitamin B12 and folate should always be excluded before adding other therapies. The hemoglobin level nessecitating therapy should be determined in the individual patient. As a general guideline, treatment of anemia should be considered at Hb levels < 110g/L in symptomatic patients and in patients with a reduced functional capacity.

Androgens

Androgens stimulate bone marrow function and have been an important treatment option for the anaemia, which has been shown to improve in about 40% of the patients, in particular in those patients with only moderate splenomegaly and normal cytogenetics (191). Danazol – a semisynthetic androgen - has much fewer side effects than conventional androgens (nandrolone, fluoxymesterolone, oxymethalone) and accordingly has virtually substituted these agents. Danazol has proved to be very effective with responses in about 40% of the patients. In general, the treatment is well tolerated with only moderate toxicity and most frequently a slight increase in liver enzymes (191). However, androgenic side effects can be seen in female patients. Danazol is administered at a dose of 200mg x 3/day. Monitoring of liver function is recommended regularily (once/monthly). Most patients respond within the first 2-3 months but a subgroup of patients have a late response occurring about 6-8(-9) months after starting therapy (191). A synergistic effect between human recombinant erythropoietin and danazol has been recorded (160). However, at the present time danazol is only available with special license in Finland, Norway and Sweden (from Sanofi/Aventis or affiliated companies).

Recommendations: Danazol is , if available, recommended as one first-line therapy in the treatment of anaemia in PMF .
Grade B recommendations, evidence level III.

Glucocorticoids
Treatment with glucocorticoids is indicated in those patients with Coombs positive immune haemolysis, but may also be effective in patients without overt haemolytic activity.

Recommendations: Prednisolone is recommended as the drug of choice in the treatment of Coombs positive anaemia in PMF. Prednisolone may also be useful in occasional patients without biochemical evidence of immune haemolysis.
Grade B recommendations, evidence level III.

Erythropoietin
In recent years several studies have shown that human recombinant erythropoietin effectively increases the Hb-concentration in about 40-50 % of the patients. The required dose is about 10.000 U three times per week. Darbepoietin-alpha administered once a week is equally effective – the recommended dose is in the range 150-300ug/week. A goal of EPO therapy of hemoglobin around 120 g/L has been advocated by the FDA and ASH/ASCO when EPO is used in patients with other hematological malignancies in order to minimize the risk of thrombosis. It seems reasonable to suggest this also in PMF. A plasma erythropoietin below 125 U/l has been clearly associated with a higher probability of response (160-161). However, higher S-EPO levels do not preclude patients from responding. In the first reports on Epo-treatment of PMF enlargement of the spleen was recorded in some patients. However, this has not been observed in subsequent studies. A single study has found that a few non-responsive patients may be Epo-responsive when adding thalidomide, possibly being explained by the immunomodulating effects of thalidomide therapy (192). A similar syngergistic effect has been seen in a single patient treated with erythropoietin and danazol (160).

Recommendations : Erythropoietin is recommended in the treatment of anaemia in PMF in patients not responding to danazol, or in patients in whom danazol therapy is considered inappropriate/not available.
Grade B recommendations, evidence level III.

Thalidomide and thalidomide analogues
As outlined above some patients with PMF may benefit from treatment with thalidomide as evidenced by an increase in the Hb-concentration and a decrease in spleen size (193,194). However, in a few studies no benefit of thalidomide was recorded and treatment was associated with significant side effects and a high drop out rate (195,196). Low-dose thalidomide (50mg/day) in combination with prednisolone appears to be highly effective with an increase in the Hb-concentration in about 60% of the patients (197). However, there is still a need to minimize non-haematologic toxicity associated with thalidomide therapy. In this context the novel thalidomide analoges seem very promising. CC-5013 (Lenalidomide; Revlimid) is an immunomodulatory analog of thalidomide that is substantially more potent than the parent drug in terms of both anti-angiogenic and anti-TNF-alpha activity. The compound has also significantly fewer non-haematologic toxicities and has shown very promising results in both multiple myeloma, the

myelodysplastic syndrome as well as in PMF (198, 260-263). A study included three consecutive patients with del(5q)-associated PMF or post-PV MF (all three patients JAK2V617F-positive, 263). One of these patients had been previously reported (262). A very impressive effect of lenalidomide therapy was recorded and screening for del(5)(q) was recommended in all patients with PMF or post-PV MF. If found, lenalidomide therapy should be offered and continued indefinitely, if tolerated (263).
Recommendations: Low-dose thalidomide (50mg/day) + prednisolone (1mg/kg for 2 weeks and afterwards tapering to the lowest dose maintaining an adequate Hb-concentration) is recommended for patients not responding to danazol monotherapy or erythropoietin. In the rare patient harbouring del(5)(q) lenalidomide should be considered.

Grade B recommendations, evidence level III.

JAK2 inhibitors
No clinical recommendations can be given at this time, since therapy with JAK2 inhibitors is a strictly experimental treatment modality at the moment. They are not available outside of clinical trials. Preliminary data with different JAK2 inhibitors presented at major meetings suggest that these agents have profound effects on splenomegaly and constitual symptoms, but have very limited or no effects on blood counts.

Evidence levels and recommendations grades

Where possible and appropriate, recommendation grade (A, B and C) and evidence level (I-IV) are given (for definitions see Table 1). Grade A does not imply that a treatment is more recommendable than a grade B, but implies that the given recommendation regarding the use of a specific treatment is based on at least one randomised trial.

Table 1.
A) Levels of evidence

Level	Type of evidence
Ia	Evidence obtained from meta-analysis of randomised trials
Ib	Evidence obtained from at least one randomised controlled trial
IIa	Evidence obtained from at least one well-designed controlled study without randomisation
IIb	Evidence obtained from at least one other type of well-designed quasi-experimental study
III	Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case control studies
IV	Evidence obtained from expert committee reports and/or clinical experiences of respected authorities

B) Grades of recommendation

Grade	Evidence level	Recommendation
A	Ia, Ib	Required: At least one randomised controlled trial as part of the body of literature of overall good quality and consistency addressing specific recommendation
B	IIa, IIb, III	Required: Availability of well-conducted clinical studies but no randomised clinical trials on the topic of recommendation
C	IV	Required: Evidence obtained from expert committee reports or opinions and /or clinical experiences of respected authorities. Indicates absence of directly applicable studies of good quality

References 2007 version

1. Barbui T, Barosi G, Grossi A, Gugliotta L, Liberato LN, Marchetti M, et al. Practice guidelines for the therapy of essential thrombocythemia. A statement from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation. Haematologica 2004;89:215-32.

2. McMullin MF, Bareford D, Campbell P, Green AR, Harrison C, Hunt B et al. Guidelines for the diagnosis, investigation and management of polycythaemia/erythrocytosis. Br J Haem 2005; 130: 174-195.

3. Vardiman, J.W., Harris, N.L. & Brunning, R.D. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002, 100, 2292-2302.

4. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365:1054-61

5. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, Garcon L, Raslova H, Berger R, Bennaceur-Griscelli A, Villeval JL, Constantinescu SN, Casadevall N & Vainschenker W. A unique clonal JAK2 mutation leading to constitutive signaling causes polycythaemia vera. Nature 2005; 434, 1144-1148.

6. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352:1779-90.

7. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntley BJ, Boggon TJ, Wlodarska I, Clark JJ, Moore S, Adelsperger J, Koo S, Lee JC, Gabriel S, Mercher T, D'Andrea A, Frohling S, Dohner K, Marynen P, Vandenberghe P, Mesa RA, Tefferi A, Griffin JD, Eck MJ, Sellers WR, Meyerson M, Golub TR, Lee SJ & Gilliland DG. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocytosis, and myeloid metaplasia with myelofibrosis. Cancer Cell 2005; 7: 387-397.

8. Steensma DP.The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both "atypical" myeloproliferative disorders and myelodysplastic syndromes. Blood, Aug 2005; 106: 1207 - 1209.

9. Jones AW. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood, 2005; 106: 2162 – 2168.

10. Campbell P and Green T. Management of Polycythemia Vera and Essential Thrombocythemia, Hematology, ASH, 2005:203-208.

11. Tefferi A and Gilliland G. The JAK2 V617F Tyrosine Kinase Mutation. Status Report and Immediate Implications for Disease Classification and Diagnosis. Mayo Clin Proc. 2005; 80: 947-958.

12. Barbui T and Finazzi G. Indications for cytoreductive therapy in polycythemia vera and essential thrombocythemia. Hematology, 2003: 202-209.

13. Nordin G. A multicentre study of reference intervals for haemoglobin, basic blood cell counts and erythrocyte indices in the adult population of the Nordic countries. Scan J Clin Lab Invest 2004, 64: 385-398.

14. Pearson, T.C. Apparent polycythaemia. Blood Reviews 1991; 5: 205–213.

15. Pearson et al, Interpretation of measured red cell mass and plasma volume in adults: Expert Panel on Radionuclides of the International Council for Standardization in Haematology. Br J Haematol 1995;89:748-56.

16. Sirhan S, Fairbanks VF, Tefferi A. Red cell mass and plasma volume measurements in polycythemia: evaluation of performance and practical utility. Cancer. May 11, 2005

17. Andreasson B, Löfvenberg E, Westin J. Management of patients with polycythaemia vera: results of a survey arnong Swedish haematologists. Eur J Haematol. 2005;74:489-495.

18. Pierre et al. Polycythemia vera. In ”Tumours of Haematopoietic and Lymphoid Tissues”. WHO Classification of Tumours. IARC Press. 2001.

19. Berlin NI. Diagnosis and classification of the polycythemias. Semin Hematol. 1975;12:339-351.

20. Pearson & Messinezy, The diagnostic criteria of polycythaemia rubra vera. Leuk Lymphoma. 1996;22 Suppl 1:87-93.

21. Whitehead et al. The effects of cigarette smoking and alcohol consumption on blood haemoglobin, erythrocytes and leucocytes: a dose related study on male subjects. Clin Lab Haematol. 1995;17:131-8.

22. Messinezy M. Serum erythropoietin values in erythrocytoses and in primary thrombocythaemia. Br J Haematol, 2002, 117, 47–53.

23. Mossuz P. Diagnostic value of serum erythropoietin level in patients with absolute erythrocytosis. Haematologica 2004; 89:1194-1198.

24. Birgegård G and Wide L. Serum erythropoietin in the diagnosis of polycythaemia and after phlebotomy treatment. Br J Haematol 1992;81:603-606.

25. Messinezy et al. Spleen sizing by ultrasound in polycythaemia and thrombocythaemia: comparison with SPECT. Br J Haematol 1997; 98, 103–107

26. Smith, J.R. and Landaw, S.A. Smokers’ polycythemia. N Engl J Med. 1978;298:6-10.

27. Moore-Gillon, J.C., Treacher, D.F., Gaminara, E.J., Pearson, T.C. & Cameron, I.R. Intermittent hypoxia in patients with unexplained polycythaemia. British Medical Journal, 1986; 293: 588–590.

28. Johns MW. Daytime sleepiness, snoring, and obstructive sleep apnea. The Epworth Sleepiness Scale. Chest. 1993;103:30-6.

29. Johansson P, Kutti J, Andreasson B, Safai-Kutti S, Vilen L, Wedel H. Trends in the incidence of chronic Philadelphia cromosme negative myeloproliferative disorders in the city of Göteborg, Sweden, during 1983-99. J Int Med 2004; 256: 161-165.

30. Chievitz E & Thiede T. Complications and causes of death in polycythaemia vera. Acta Medica Scandinavica, 1962; 172: 513-523.

31. Marchioli R, Finazzi G, Landolfi R, Kutti J, Gisslinger H, Patrono C, Marilus R, Villegas A, Tognoni G, Barbui T. Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. J Clin Oncol 2005; 23: 1-9.

32. Pearson TC & Wetherley-Mein G. Vascular occlusive episodes and venous haematocrit in primary proliferative polycythaemia. Lancet 1978; 2: 1219-1222.

33. Thomas DJ, du Boulay GH, Marshall J, Pearson TC, Ross Russell RW, Symon L, Wetherley-Mein G, Zilkha E. Cerebral blood-flow in polycythaemia. Lancet 1977; 2: 161-163.

34. Berk PD, Wassermann LR, Fruchtman SM, Goldberg JT. Treatment of polycythaemia vera, a summary of clinical trends conducted by the polycythaemia vera subgroup. In: Treatment of Polycythaemia Vera, a Summary of Clinical Trends Conducted by the Polycythaemia Vera Study Group. 1995 pp.166-194. L. P. Wasserman and P. D. Berk. Philadelphia: W.B. Saunders.

35. Landolfi R, Marchioli R, Kutti J, Gisslinger H, Tognoni G, Patrono,C, Barbui T & European Collaboration on Low-Dose Aspirin in Polycythemia Vera Investigators. Efficacy and safety of low-dose aspirin in polycythemia vera. N Engl J Med 2004; 350: 114-124.

36. Najean Y, Dresch C, Rain JD. The very-long-term course of polycythaemia: a complement to the previously published data of the Polycythaemia Vera Study Group. Br J Haematol 1994; 86: 233-235.

37. Berk PD, Goldberg JD, Donovan PB, Fruchtman SM, Berlin NI, Wasserman LR. Therapeutic recommendations in polycythemia vera based on Polycythemia Vera Study Group protocols. Semin Hematol 1986; 23: 132-143.

38. Spivak JL. Polycythemia vera: myths, mechanisms and management. Blood 2002; 104: 4272-4290.

39. Cortelazzo S, Finazzi G, Ruggeri M, Vestri O, Galli M, Rodeghiero F, Barbui T. Hydroxyurea in the treatment of patients with essential thrombocythemia at high risk of thrombosis: a prospective randomized trial. N Engl J Med 1995, 332, 1132-1136.

40. Jensen MK, de Nully Brown P, Lund BV, Nielsen OJ, Hasselbalch HC. Increased circulating platelet-leukocyte aggregates in myeloproliferative disorders is correlated to previous thrombosis, platelet activation and platelet count. Eur J Haematol 2001; 66:143-151.

41. Falanga A, Marchetti M, Vignoli A, Balducci D, Barbui T. Leukocyte-platelet interaction in patients with essential thrombocythemia and polycythemia vera. Exp Hematol 2005; 33: 523-530.

42. Budde U, Schaefer G, Mueller N, Egli H, Dent J, Ruggeri Z, Zimmerman T. Acquired von Willebrand´s disease in the myeloproliferative syndromes. Blood 1984; 64: 981-985.

43. Tartaglia AP, Goldberg JD, Berk P, Wasserman LR. Adverse effects of antiaggregating platelet therapy in the treatment of polcythemia vera. Semin Hematol 1986; 23: 172-176.

44. Najean Y & Rain JD. Treatment of polycythemia vera: the use of hydroxyurea and pipobroman in 292 patients under the age of 65 years. Blood 1997; 90: 3370-3377.

45. Landolfi R, di Gennaro L, Barbui T, de Stefano V, Finazzi G, Marfisi RM, Finazzi G, Tognoni G, Marchioli R. Leukocytosis as a major thrombotic<risk factor in patients with polycythemia vera. Blood 2007; 109:2446-2452.

46. Vanucci AM, Antonioli E, Guglielmini P, Longo G, Pancrazzi A, Ponziani V, Bogani C, Ferrini PR, Rambaldi A, Guerini V, Bosi A, Barbui T. Prospective identification of high-risk polycythemia vera patients based on JAK2(V617F) allele burden. Leukemia 2007; 21: 1952-1959.

47. Ruggeri M, Gisslinger H, Tosetto A, Rintelen C, Mannhalter C, Pabinger I, Heis N, Castaman G, Missiaglia E, Lechner K, Rodeghiero F. Factor V Leiden mutation carriership and venous thromboembolism in polycythemia vera and essential thrombocythemia. Am J Hematol 2002; 71, 1-6.

48. Gisslinger H, Mullner M, Pabinger I, Heis-Vahidi-Fard N, Gisslinger B, Brichta A, Bachleitner T, Mannhalter C. Mutation of the prothrombin gene and thrombotic events in patients with polycythemia vera or essential thrombocythemia: a cohort study. Haematologica 2005; 90: 408-410.

49. Donovan PB, Kaplan ME, Goldberg .D, Tatarsky I, Najean Y, Silberstein EB, Knospe WH, Laszlo J, Mack K, Berk PD. Treatment of polycythemia vera with hydroxyurea. Am J Hematol 1984; 17, 329-334.

50. Kaplan ME, Mack K, Goldberg JD, Donovan PB, Berk PD, Wasserman LR. Long-term management of polycythemia vera with hydroxyurea: a progress report. Semin Hematol 1986 23, 167-171.

51. Fruchtman SM, Mack K, Kaplan ME, Peterson P, Berk PD, Wasserman LR. From efficacy to safety: a Polycythemia Vera Study group report on hydroxyurea in patients with polycythemia vera. Semin Hematol 1997; 34, 17-23.

52. Najean Y & Rain,JD. Treatment of polycythemia vera: use of 32P alone or in combination with maintenance therapy using hydroxyurea in 461 patients greater than 65 years of age. The French Polycythemia Study Group. Blood 1997; 89, 2319-2327.

53. Kiladjian JJ, Rain JD, Bernard JF, Briere J, Chomienne C, Fenaux P. Long-term incidence of hematological evolution in three French prospective studies of hydroxyurea and pipobroman in polycythemia evar and essential thrombocythemia. Semin Thromb Hemost 2006; 32: 417-21.

54. Harrison CN, Campbell PJ, Buck G, Wheatley K, East CL, Bareford D, Wilkins BS, van der Walt JD, Reilly JT, Grigg AP, Revell P, Woodcock BE, Green AR, United Kingdom Medical Research Council Primary Thrombocythemia 1 Study. Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. N Engl J Med 2005; 353: 33-45.

55. Tatarsky I & Sharon R. Management of polycythemia vera with hydroxyurea. Semin Hematol 1997; 34, 24-28.

56. Weinfeld A, Swolin B, Westin J. Acute leukaemia after hydroxyurea therapy in polycythaemia vera and allied disorders: prospective study of efficacy and leukaemogenicity with therapeutic implications. European Journal of Haematology 1994; 52, 134-139.

57. Finazzi G, Caruso V, Marchioli R, Capnist G, Chisesi T, Finelli C, Gugliotta L, Landolfi R, Kutti J, Gisslinger H, Marilus R, Patrono C, Pogliani EM, Randi ML, Villegas A, Tognoni G, Barbui T, ECLAP Investigators. Acute leukaemia in polycythemia vera: an analysis of 1638 patients enrolled in a prospective observational study. Blood 2005; 105: 2664-2670.

58. Finazzi G, Ruggeri M, Rodeghiero F, Barbui T. Second malignancies in patients with essential thrombocythemia treated with busulphan and hydroxyurea: long-term follow-up of a randomized clinical trial. Br J Haematol 2000, 110, 577-583.

59. Lengfelder E, Berger U, Hehlman R. Interferon-α in the treatment of polycythemia vera. Ann Hematol 2000, 79, 103-9.

60. Heis N, Rintelen C, Gisslinger B, Knobl P, Lechner K, Gisslinger H. The effect of interferon alpha on myeloproliferation and vascular complications in polycythemia vera. Eur J Haematol 1999; 62, 27-31.

61**.** Langer C, Lengfelder E, Thiele J, Kvasnicka HM, Pahl HL, Beneke H, Schauer S, Gisslinger H, Griesshammer M. Pegylated interferon for the treatment of high risk essential thrombocythemia: results of a phase II study. Haematologica 2005; 90: 1333-1338.

62. Samuelsson J, Hasselbalch H, Bruserud O, Temerinac S, Brandberg Y, M Merup, Linder O, Bjorkholm M, Pahl H, & Birgegard G. A phase II trial of pegylated interferon α-2b therapy in polycythemia vera and essential thrombocythemia. Feasibility, clinical and biological effects and impact on quality of life. Cancer 2006, 106: 2397-405.

63. Hino M, Futami E, Okuno S, Miki T, Nishizawa Y, Morii H. Possible selective effect of interferon α-2b on a malignant clone in a case of polycythemia vera. Ann Hematol 1993; 66: 161-62.

64. Messora C, Bensi L, Vechi A, Giacobbi F, Temperani P, Berini M, Emilio G, Sacci S. Cytogenetic conversion in a case of polycythaemia vera treated with interferon-alpha. Br J Haematol 1994; 86: 402-04.

65. Liu E, Jelinek J, Pastore YD, Guan Y, Prchal JF, Prchal JT. Discrimination of polycythemias and thrombocytoses by novel, simple, accurate clonality assays and comparison with PRV-1 expression and BFU-e response to erythropoietin. Blood 2003; 101: 3294-3301.
66. Fruehauf S, Topaly J, Villalobos M, Veldwijk MR, Laufs S, Ho AD. Quantitative real-time polymerase chain reaction shows that treatment with interferon reduces the initially upregulated PRV-1 expression in polycythemia vera patients. Haematologica 2003; 88: 349-350.

67. Jones AV, Silver RT, Waghorn K, Curtis C, Kreil S, Zoi K, Hochhaus A, Oscier D, Metzgeroth G, Lengfelder E, Reiter A, Chase AJ, Cross NC. Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. Blood. 2006; 107:3339-3341.

68. Samuelsson J, Mutschler M, Birgegård G, Gram-Hansen P, Björkholm M & Pahl HL. Limited effects on JAK2 mutational status after pegylated interferon α-2b therapy in polycythemia vera and essential thrombocythemia. Haematologica 2006, 91:1281-1282.

69. Kiladjian JJ, Cassinat B, Turlore P, Cambier N, Roussel M, Belluci S, Menot ML, Massonnet G, Dutel JL, Ghomari K, Rousselot P, Grange MJ, Chait Y, Vainchenker W, Parquet N, Abdelkader-Aljassem L, Bernard JF, Rain JD, Chevret S, Chomienne C, Fenaux P. High molecular response rate of polycythemia vera patients treated with interferon alpha-2a. Blood 2006; 108: 2037-2040.

70. Anagrelide Study Group. Anagrelide, a therapy for thrombocythemic states: experience in 577 patients. Am J Med 1992; 92: 69-76.

71. Birgegård G, Björkholm M, Kutti J, Lärfars G, Löfvenberg E, Markevärn B, Merup M, Palmblad J, Mauritzson N, Westin J, Samuelsson J. Adverse effects and benefits of two years of anagrelide treatment for thrombocythemia in chronic myeloproliferative disorders. Hematologica 2004, 89: 520-27.

72. Penninga E, Jensen BA, Hansen PB. Anagrelide treatment in 52 patients with chronic myeloproliferative diseases. Clin Lab Haem 2004; 26: 335-340.

73. Fruchtman SM, Petitt RM, Gilbert HS, Fiddler G, Lyne A. Anagrelide: analysis of long-term efficacy, safety and leukemogenic potential in myeloproliferative disorders. Leuk Res 2005; 29: 481-491.

74. Cooperative Group, European Organization for Research on Treatment of Cancer (E.O.R.T.C.). Treatment of polycythaemia vera by radiophosphorus or busulphan: a randomized trial. "Leukemia and Hematosarcoma". Br J Cancer 1981; 44, 75-80.

75. Berk PD, Goldberg JD, Silverstein MN, Weinfeld,A, Donovan P., Ellis JT,
Landaw SA, Laszlo J, Najean Y, Pisciotta AV, Wasserman LR. Increased incidence of acute leukemia in polycythemia vera associated with chlorambucil therapy. N Eng J Med 1981; 304, 441-447.

76. Najean Y, Rain JD, Dresch C, Goguel A, Lejeune F, Echard M, Grange MJ. Risk of leukemia, carcinoma, and myelofibrosis in 32P- or chemotherapy-treated patients with polycythemia vera: a prospective analysis of 682 cases. Leuk Lymphoma 1996; 22 (suppl 2): 111-119.

77. Thiele J, Kvasnicka HM. A critical reappraisal of the WHO classification of the chronic myeloproliferative disorders. Leukemia & Lymphoma 2006; 47: 381-396.

78. Lengfelder E. Should a platelet limit of 600x 10*9/l be used as a diagnostic criterion in essential thrombocythemia? An analysis of the natural course including early stages. Br J Haem 1998, 100;15-23.

79. Schafer AI. Thrombocytosis. N Engl J Med 2004; 350: 1211-9

80. Mesa RA, Silverstein MN, Jacobsen SJ, Wollan PC, Tefferi A. Population-based incidence and survival figures in essential thrombocythemia and agnogenic myeloid metaplasia: an Olmsted County Study, 1976-1995. Am J Hematol 1999;61:10-5.

81. Rozman C, Giralt M, Feliu E, Rubio D, Cortes MT. Life expectancy of patients with chronic nonleukemic myeloproliferative disorders. Cancer 1991;67:2658-63.

82. Passamonti F, Rumi E, Pungolino E, Malabarba L, Bertazzoni P, Valentini M, et al. Life expectancy and prognostic factors for survival in patients with polycythemia vera and essential thrombocythemia. Am J Med 2004;117:755-61.

83. Fenaux P, Simon M, Caulier MT, Lai JL, Goudemand J, Bauters F. Clinical course of essential thrombocythemia in 147 cases. Cancer 1990; 66: 549-556.

84. Wolanskyj A, Schwager S, McClure R, Larson D, Tefferi A. Essential Thrombocythemia beyond the first decade: Life expectancy, Long-term complication rates, and Prognostic factors. Mayo Clin Proc. 2006; 81: 159-166.

85. Fialkow PJ, Faguet GB, Jacobson RJ, Vaidya K, Murphy S. Evidence that essential thrombocythemia is a clonal disorder with origin in a multipotent stem cell. Blood 1981;58:916-9.

86. Yan L, Elkassar N, Gardin C, Briere J. Clonality assays and megakaryocyte culture techniques in essential thrombocythemia. In: Leuk Lymphoma 1996; 22: 31-40.

87. Harrison CN, Gale RE, Machin SJ, Linch DC. A large proportion of patients with a diagnosis of essential thrombocythemia do not have a clonal disorder and may be at lower risk of thrombotic complications. Blood 1999; 93: 417-424.

88. Goerttler PS, Steimle C, Marz E, Johansson PL, Andreasson B, Griesshammer M, et al. The Jak2V617F mutation, PRV-1 overexpression, and EEC formation define a similar cohort of MPD patients. Blood 2005;106:2862-4.

89. Andreasson B, Lindstedt G, Kutti J. Plasma erythropoietin in essential thrombocythaemia: at diagnosis and in response to myelosuppressive treatment. Leuk Lymphoma 2000;38:113-20.

90. Johansson P, Ricksten A, Wennstrom L, Palmqvist L, Kutti J, Andreasson B. Increased risk for vascular complications in PRV-1 positive patients with essential thrombocythaemia. Br J Haematol 2003;123:513-6.

91. Juvonen E, Ikkala E, Oksanen K, Ruutu T. Megakaryocyte and erythroid colony formation in essential thrombocythaemia and reactive thrombocytosis: diagnostic value and correlation to complications. Br J Haematol 1993;83(2):192-7.

92. Cortelazzo S, Viero P, Finazzi G, D'Emilio A, Rodeghiero F, Barbui T. Incidence and risk factors for thrombotic complications in a historical cohort of 100 patients with essential thrombocythemia. J Clin Oncol. 1990 Mar;8(3):556-62.

93. Besses C, Cervantes F, Pereira A, Florensa L, Sole F, Hernandez-Boluda JC, Woessner S, Sans-Sabrafen J, Rozman C, Montserrat E.Major vascular complications in essential thrombocythemia: a study of the predictive factors in a series of 148 patients. Leukemia. 1999 Feb;13(2):150-4.

94. Ruggeri M, Finazzi G, Tosetto A, Riva S, Rodeghiero F, Barbui T. No treatment for low-risk thrombocythaemia: results from a prospective study. Br J Haematol 1998;103(3):772-7.

95. Watson KV, Key N. Vascular complications of essential thrombocythaemia: a link to cardiovascular risk factors. Br J Haematol. 1993 Feb;83(2):198-203.

96. Jantunen R, Juvonen E, Ikkala E, Oksanen K, Anttila P, Ruutu T. The predictive value of vascular risk factors and gender for the development of thrombotic complications in essential thrombocythemia. Ann Hematol. 2001 Feb;80(2):74-8.

97. Shih LY, Lin TL, Dunn P, Wu JH, Tseng CP, Lai CL, Wang PN, Kuo MC. Clonality analysis using X-chromosome inactivation patterns by HUMARA-PCR assay in female controls and patients with idiopathic thrombocytosis in Taiwan. Exp Hematol. 2001 Feb;29(2):202-8.

98. Campbell PJ, Scott LM, Buck G, Wheatley K, East CL, Marsden JT, Duffy A, Boyd EM, Bench AJ, Scott MA, Vassiliou GS, Milligan DW, Smith SR, Erber WN, Bareford D, Wilkins BS, Reilly JT, Harrison CN, Green AR; United Kingdom Myeloproliferative Disorders Study Group; Medical Research Council Adult Leukaemia Working Party; Australasian Leukaemia and Lymphoma Group. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. Lancet. 2005 Dec 3;366(9501):1945-53.

99. Finazzi G, Rambaldi A, Guerini V, Carobbo A, Barbui T. Risk of thrombosis in patients with essential thrombocythemia and polycytemia vera according to JAK2 V617F mutation status. Haematol 2007; 92 (01): 135.

100. van Genderen PJ, Leenknegt H, Michiels JJ. The paradox of bleeding and thrombosis in thrombocythemia: is von Willebrand factor the link? Semin Thromb Hemost. 1997;23(4):385-9.

101. van Genderen PJ, Michiels JJ. Erythromelalgic, thrombotic and haemorrhagic manifestations of thrombocythaemia.Presse Med. 1994 Jan 22;23(2):73-7.

102. Cervantes F, Alvarez-Larran A, Talarn C, Gomez M, Montserrat E.Myelofibrosis with myeloid metaplasia following essential thrombocythaemia: actuarial probability, presenting characteristics and evolution in a series of 195 patients. Br J Haematol. 2002 Sep;118(3):786-90.

103. Patrono C.Aspirin as an antiplatelet drug. N Engl J Med. 1994 May 5;330(18):1287-94.

104. Regev A, Stark P, Blickstein D, Lahav M.Thrombotic complications in essential thrombocythemia with relatively low platelet counts. Am J Hematol. 1997 Nov;56(3):168-72.

105. Michiels JJ.Normal life expectancy and thrombosis-free survival in aspirin treated essential thrombocythemia. Clin Appl Thromb Hemost. 1999 Jan;5(1):30-6.

106. Tefferi A, Fonseca R, Pereira DL, Hoagland HC.A long-term retrospective study of young women with essential thrombocythemia. Mayo Clin Proc. 2001 Jan;76(1):22-8.

107. Randi ML, Rossi C, Fabris F, Menapace L, Girolami A. Aspirin seems as effective as myelosuppressive agents in the prevention of rethrombosis in essential thrombocythemia. Clin Appl Thromb Hemost. 1999 Apr;5(2):131-5.

108. Murphy S.Therapeutic dilemmas: balancing the risks of bleeding, thrombosis, and leukemic transformation in myeloproliferative disorders (MPD). Thromb Haemost. 1997 Jul;78(1):622-6.

109. Sacchi S, Gugliotta L, Papineschi F, Liberati AM, Rupoli S, Delfini C, Ruggeri M, Cavanna L, Bucalossi A, Benedetti E, Ferrandina C, Vinci G, Morselli M, Torelli G. Alfa-interferon in the treatment of essential thrombocythemia: clinical results and evaluation of its biological effects on the hematopoietic neoplastic clone. Italian Cooperative Group on ET. Leukemia. 1998 Mar;12(3):289-94.

110. Griesshammer M, Struve S, Harrison CM. Essential thrombocythemia/polycythemia vera and pregnancy: the need for an observational study in Europe. Semin Thromb Hemost. 2006 Jun;32(4 Pt 2):422-9.

111. Storen EC, Tefferi A. Long-term use of anagrelide in young patients with essential thrombocythemia. Blood. 2001 Feb 15;97(4):863-6.

112. Van de Pette JE, Prochazka AV, Pearson TC, Singh AK, Dickson ER, Wetherley-Mein G. Primary thrombocythaemia treated with busulphan. Br J Haematol. 1986 Feb;62(2):229-37.

113. Brandt L, Anderson H.Survival and risk of leukaemia in polycythaemia vera and essential thrombocythaemia treated with oral radiophosphorus: are safer drugs available? Eur J Haematol. 1995 Jan;54(1):21-6.

114. Elliott MA, Tefferi A. Pathogenesis and management of bleeding in essential thrombocythemia and polycythemia vera. Curr Hematol Rep 2004; 3: 344-351.

115. de Stefano V, Za T, Rossi E, Vannuchi A, Ruggeri M, Elli E, Mio C, Tieghi A et al. Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia; incidence, risk factors, and effects of tretaments. Haematologica 2008; 93: 372-380.

116. Grima KM. Therapeutic apheresis in hematological and oncological diseases. J Clin Apheresis 2000; 15: 28-52.

117. Greist A. The role of blood component removal in essential and reactive thrombocytosis. Ther Apher 2002; 6: 36-44.

118. Adami R. Therapeutic thrombocythapheresis: a review of 132 patients. Int J Artif Organs 1993; 16 (suppl 5): 183-184.

119. Weick JK, Donovan PB, Najean Y, Dresch C, Pisciotta AV, Cooperberg AA, Goldberg JD. The use of cimetidine for the treatment of pruritus in polycythemia vera. Arch Int Med 1982; 142: 241-242.

120. Taylor PC, Dolan G, Ng, JP, Paul B, Collin R, Reilly JT. Efficacy of recombinant interferon-alpha (rIFN-alpha) in polycythaemia vera: a study of 17 patients and an analysis of published data. Br J Haematol 1996; 92: 55-59.

121. Tefferi A. & Fonseca R. Selective serotonin reuptake inhibitors are effective in the treatment of polycythemia vera-associated pruritus. Blood 2002; 99; 2627.

122. Jeanmougin M, Rain JD, Najean Y. Efficacy of photochemotherapy on severe pruritus in polycythemia vera. Ann Hematol 1996; 73: 91-93.

123. de Stefano V, Za T, Rossi E, Vannuchi A, Ruggeri M, Elli E, Mio C, Tieghi A et al. Postsurgery outcomes in patients with polycythemia vera and essential thrombocythemia: retrospective survey.Blood. 2008;111:666-71

124. Mesa RA, Nagorney DS, Schwager S, Allred J, Tefferi A. Palliative goals, patient selection, and perioperative platelet management: outcomes and lessons from 3 decades of splenectomy for myelofibosis with myeloid metaplasia at the Mayo cliniv. Cancer 2006; 107: 361-370.

125. Robinson, S.E., Radia, D., Pocock, M., Duncan, J., Mumford, A., Machin, S., Bewley, S., Hunt, B.J. & Harrison, C. (2004) Polycythaemia and pregnancy: a review of treatment and outcome of 16 pregnancies in 8 women. British Journal of Haematology, 125, 66.

126. Harrison CN. Pregancy and its management in the Philadelphia negative myeloproliferative disorders. Br J Haematol 2005; 129: 293-306.

127. Griesshammer, M., Bergmann, L. & Pearson, T. Fertility, pregnancy and the management of myeloproliferative disorders. Bailliere's Clinical Haematology 1998; 11, 859-874.

128. Griesshammer M, Struve S, Harrison CM. Essential thrombocythemia/polycythemia vera and pregnancy: the need for an observational study in Europe. Semin Thromb Hemost 2006; 32: 422-29.

129. Turhan AG, Humphries RK, Cashman JD, Cuthbert DA, Eaves CJ, Eaves AC . Transient suppression of clonal hemopoiesis associated with pregnancy in a patient with a myeloproliferative

disorder. J Clin Invest 1988;81:1999-2003

130. Samuelsson J & Swolin B. Spontaneous remission during two pregnancies in a patient with essential thrombocythaemia. Leuk Lymphoma. 1997; 25:597-600.

131. Silverstein MN; Gomes MR, Remine WH, Elveback LR. Agnogenic myeloid metaplasia. Natural history and treatment. Arch Intern Med 1967; 120:545-550.

132. Ward HP & Block MH. The natural history of agnogenic myeloid metaplasia (AMM) and a critical evaluation of its relationship with the myeloproliferative syndrome. Medicine (Baltimore) 1971; 50: 357.

133. Hasselbalch HC. Idiopathic Myelofibrosis. A Review. Eur J Haematol 1990; 45:65-72.

134. Reilly JT. Idiopathic myelofibrosis: pathogenesis, natural history and management. Blood Rev 1997; 11(4): 233-42.

135. Cervantes F, Pereira A, Esteve J, Rafel M, Cobo F, Rozman C & Montserrat E. Identification of "short-lived" and "long-lived" patients at presentation of idiopathic myelofibrosis. Br J Haematol 1997; 97: 635-640.

136. Cervantes F, Pereira A, Esteve J, Cobo F, Rozman C, Montserrat E. The changing profile of idiopathic myelofibrosis: a comparison of the presenting features of patients diagnosed in two different decades. Eur J Haematol 1998; 60: 101-105.

137. Cervantes F, Barosi G, Demory JL, Reilly J, Guarnone R, Dupriez B et al. Myelofibrosis with myeloid metaplasia in young individuals: disease characteristics , prognostic factors, and identification of risk groups. Br J Haematol 1998; 102: 684-690.

138. Barosi G. Myelofibrosis with myeloid metaplasia: Diagnostic definition and prognostic classifications for clinical studies and treatment guidelines. J Clin Oncol 1999; 17: 2954-2970.

139. Barosi G, Ambrosetti A, Finelli C, Grossi A, et al. for the ICSG on MMM (Italia Cooperative Study Group on Myelofibrosis with Myeloid Metaplasia. Br J Haematol 1999; 104: 730-737.

140. Tefferi A. Myelofibrosis with myeloid metaplasia N Engl J Med 2000; 342(17):1255-65

141. Dupriez, B., Morel, P., Demory, J.L., Lai, J.L., Simon, M., Plantier, I. & Bauters, F. Prognostic factors in agnogenic myeloid metaplasia: a report on 195 cases with a new scoring system. Blood 1996; , 88: 1013–1018.

142. Thiele J, Simon KG, Fischer R. Follow-up studies with sequential bone marrow biopsies in chronic myeloid leukemia and so-called primary(idiopathic) osteo-myelofibrosis. Path Res Pract 1988; 183: 434-445.

143. Thiele J, Kvasnicka HM, Vardiman J. Bone marrow histopathology in the diagnosis of chronic myeloproliferative disorders: a forgotten pearl. Best Pract Res Clin Haematol 2006; 19(3): 413-437.

144. Najean Y, Arago JP, Rain JD, Dresch C. The „spent“ phase of polycythaemia vera: hypersplenism in the absence of myelofibrosis. Br J Haematol 1984; 56: 163-170.

145. Pettit JE, Lewis SM, Nicholas AW. Transitional myeloproliferative disorder. Br J Haematol 1979; 43:167-184.

146. Castro-Malaspina H. Pathogenesis of myelofibrosis: role of ineffective megakaryopoiesis and megakaryocyte components. In Berk PD, Castro-Malaspina H, Wasseran LR (eds): Myelofibrosis and the Biology of Connective Tissue. New York,NY, Alan R.Liss Inc, 1984: 427-454.

147. Le Bousse-Kerdiles MC,Martyre MC. Dual implication of fibrogenic cytokines in the pathogenesis of fibrosis and myeloproliferation in myeloid metaplasia with myelofibrosis. Ann Hematol 1999 78:437-44.

148. Tefferi A. Pathogenesis of myelofibrosis with myeloid metaplasia. J Clin Oncol 2005; 23(33): 8520-8530.

149. Reilly JT. Idiopathic myelofibrosis: pathogenesis to treatment. Haematological Oncology 2006; 24:56-63.

150. Dong M, Blobe GC. Role of transforming growth factor-beta in haematological malignancies. Blood 2006; 107(12):4589-4596.

151. Campbell PJ, Griesshammer M, Dohner K, Dohner H, Hasselbalch HC et al. The V617F mutation in JAK2 is associated with poorer survival in idiopathic myelofibrosis. Blood 2006 107(5): 2098-2100.

152. Mesa RA, Powell H, Lasho T, Dewald G, McClure R, Tefferi A. JAK2 (V617F) and leukemic transformation in myelofibrosis with myeloid metaplasia. Leuk Res 2006; 30(11):1457-1468.

153. Tefferi A, Lasho TL, Schwager SM, Steensma DP, Mesa RA, Li CY, Wadleigh M, Gilliland DG. The JAK2(V617F) tyrosine kinase mutation in myelofibrosis with myeloid metaplasia:lineage specificity and clinical correlates. Br J Haematol 2005; 131:320-328.

154. Campbell PJ & Green AR. Mechanisms of Disease: The Myeloproliferative Disorders. N Engl J Med 2006; 355(23):2452-2466.

155. Guardiola P, Anderson JE, Bandini G et al. Allogeneic stem cell transplanation for agnogenic myeloid metaplasia: a European Group for Blood and Marrow Transplantation , Societe Francaise de Greffe de Moelle, Gruppo Italiano per il Trapianto del Midollo Osseo, and Fred Hutchinson Cancer Research Center Collaborative Study. Blood 1999; 93: 2831-2838.

156. Deeg HJ, Gooley TA, Flowers ME et al. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. Blood 2003; 102:3912-3918.

157. Hessling J, Kroger N, Werner M et al. Dose-reduced conditioning regimen followed by allogneic stem cell transplantion in patients with myelofibrosis with myeloid metaplasia. Br J Haematol 2002; 119:769-772.

158. Barosi G, Bacigalupo A. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. Current Opinion in Hematology 2006; 13:74-78.

159. Merup M, Lazarevic V, Nahi H, Andreasson B, Malm C, Nilsson L, Brune M, Leblanc K, Kutti J, Birgegard G; the Swedish Group for Myeloproliferative Disorders. Different outcome of allogeneic transplantation in myelofibrosis using conventional or reduced –intensity conditioning regimens. Br J Haematol 2006; 135:367-373.

160. Hasselbalch HC, Clausen NT, Jensen BA. Successful treatment of anemia in idiopathic myelofibrosis with recombinant human erythropoietin. Am J Hematol 2002; 70(2):92-9.

161. Cervantes F. Modern management of myelofibrosis. Br J Haematol 2004; 128:583-592.

162. Barosi G, Viarengo G, Pecci A et al. Diagnostic and clinical relevance of the number of circulating CD34(+) cells in myelofibrosis with myeloid metaplasia. Blood 2001; 98: 3249-3255.

163. Andreasson B, Swolin B, Kutti J. Patients with idiopathic myelofibrosis show increased CD34+ cell concentrations in peripheral blood compared to patients with polycythaemia vera and essential thrombocythaemia. Eur J Haematol 2002; 68:189-193.

164. Arora B, Sirhan S, Hoyer JD, Mesa RA, Tefferi A. Peripheral blood CD34 count in myelofibrosis with myeloid metaplasia: a prospective evaluation of prognostic value in 94 patients. Br J Haematol 2004; 128:42-48.

165. Manoharan A & Pitney WR. Chemotherapy resolves symptoms and reverses bone marrow fibrosis in in myelofibrosis. Scand J Haematol 1984;33:453-459.

166. Petti, MC,Latagliati R,Spadea T, Spadea A, Montefusco E, Aloe Spiriti MA, Avissati G, Breccia M, Pescarmona E & Mandelli F. Melphalan treatment in patients with myeloid metaplasia . Br J Haematol 2002; 116:576-581.

167. Lofvenberg E & Wahlin A. Management of polycythaemia vera, essential thrombocythaemia and myelofibrosis with hydroxyurea. Eur J Haematol 1988; 41: 375-381

168. Lofvenberg E, Wahlin A, Roos G & Ost A, Reversal of myelofibrosis by hydroxyurea. Eur J Haematol 1990; 44:33-38.

169. Manoharan A. Management of myelofibrosis with intermittent hydroxyurea. Br J Haematol 1991; 77: 252-254.

170. Nielsen I, Hasselbalch HC. Acute Leukemia and myelodysplasia in patients with Philadelphia chromosome negative chronic myeloproliferative disorder treated with hydroxyurea alone or with hydroxyurea after busulphan. Am J Hematol 2003; 74(1): 26-31.

171. Gilbert HS. Long term treatment of myeloproliferative disease with interferon alpha-2b: feasibility and efficacy. Cancer 1998; 83:1205-1213.

172. Bachleitner-Hofmann T, Gisslinger H. The role of interferon-alpha in the treatment of idiopathic myelofibrosis. Ann Hematol 1999; 78(12):533-8.

173. Tefferi A, Elliot MA, Yoon SY, Li CY, Mesa RA, Call TG, Dispenzieri A.Clinical and bone marrow effects of interferon alpha therapy in myelofibrosis with myeloid metaplasia. Blood 2001; 97(6):1896.

174. Radin AI, Kim HT, Grant BW, Bennett JM, Kirkwood JM, Stewart JA, Hahn RG, Dutcher JP, Wiernik PH, Oken MM. Phase II study of alpha2 interferon in the treatment of the chronic myeloproliferative disorders. Cancer 2003; 98(1):100-9.

175. Quintas-Cardama A, Kantarjian HM, Giles F, Verstovsek S. Pegylated interferon therapy for patients with Philadelphia chromosome-negative myeloproliferative disorders. Semin Thromb Hemost 2006; 32:409-416.

176. Tefferi A, Silverstein MN, Chin-Yang Li. 2-chlorodeoxyadenosine treatment after splenectomy in patients who have myelofibrosis with myeloid metaplasia.Br J Haematol 1997; 99: 352-357.

177. Thiele J, Kvasnicha HM, Schmitt-Graff A, Hulsemann R, Diehl V. Therapy-related changes of the bone marrow in chronic idiopathic myelofibrosis. Histol Histopathol 2004; 19(1):239-250.

178. Fontana V, Ahn ER, Jimenez J, Dudkiewics P, Jy W, Ahn YS. Danazol in combination with busulphan induces long-term remission in myeloproliferative disorders (MPD). Blood 2004; 104: Abstract 4748.

179. Pettit RM , Silverstein MN & Petrone ME. Anagrelide for control of thrombocythemiia in polycythemia vera and other myeloproliferative states. Seminars in Hematology 1997; 34: 51-54.

180. Yoon SY, Li CY, Mesa RA & Tefferi A. Bone marrow effects of anagrelide therapy in patients with myelofibrosis with myeloid metplasia 1999; 106:682-688.

181. Birgegård G. Anagrelide treatment in myeloproliferative disorders. Sem Thromb Hemost 2006; 32(3): 260-6.

182. Elliott MA, Chen MG, Silverstein MN et al. Splenic irradiation for symptomatic splenomegaly associated with myelofibrosis with myeloid metaplasia . Br J Haematol 1998; 103:505-511.

183. Steensma DP, Hook CC, Stafford SL et al. Low-dose, single fraction , whole-long radiotherapy for pulmonary hypertension associated with myelofibrosis with myeloid metaplasia. Br J Haematol 2002; 118:813-816.

184. Price F & Bell H. Spinal cord compression due to extramedullary hematopoiesis. Successful treatment in a patient with long lasting myelofibrosis. JAMA 1985; 252:2876-2877.

185. Leinweber C, Order SE & Calkins AR. Whole-abdominal irradiation for the management of gastrointestinal and abdominal manifestations of agnogenic myeloid metaplasia. Cancer 1991; 68:1251-1254.

186. Barlett RP, Greipp PR, Tefferi A, Cupps RE, Mullan BP, Trastek VF. Extramedullary hematopoiesis manifesting as a symptomatic pleural effusion. Mayo Clinic Proceedings 1995; 70: 1161-1164.

187. Mesa R, Nagomey DS, Schwager S et al. Palliative goals, patient selection, and perioperative platelet management:outcomes and lessons from 3 decades of splenectomy for myelofibrosis with myeloid metaplasia at the Mayo. Cancer 2006; 107: 361-370.

188. Barosi G, Ambrosetti A, Centra A et al. Splenectomy and risk of blast transformation in myelofibrosis with myeloid metaplasia. Italian Cooperative Study Group on Myelofibrosis with Myeloid Metaplasia. Blood 1998; 91:3630-3636.

189. Byrne JL, Beshti H, Clark D et al. Induction of remission after donor leucocyte infusion for the treatment of relapsed chronic idiopathic myelofibrosis following allogeneic transplantation : evidence for a " graft vs myelofibrosis" effect. Br J Haematol 1999; 108:430-433.

190. Cervantes F, Rovira M, Urbano-Ispizua A, Rozman M, Carreras E, Montserrat E. Complete remission of idiopathic myelofibrosis following donor lymphocyte infusion after failure of allogeneic transplantation: demonstration of a graft-versus-myelofibrosis effect. Bone Marrow Transplant 2000b; 26:697-699.

190a. Siragusa S, Passamonti F, Cervantes F, Tefferi A. Survival in young patients with intermediate-/high-risk myelofibrosis : Estimates derived from databases for non transplant patients. Am J Hematol 2008; Dec 2 (Epub ahead of print).

191. Cervantes F, Alvarez-Larran A, Domingo A, Arella-Rodrigo E, Montserrat E. Efficacy and tolerability of danazol as a treatment for the anaemia of myelofibrosis with myeloid metaplasia: long-term results in 30 patients. Br J Haematol 2005; 129:771-775.

192. Visani G, Mele A, Malagola M, Isidori A, Finelli C, iccaluga PP. Sequential combination of thalidomide and erythropoietin determines transfusion independence and disease control in idiopathic myelofibrosis previously insensitive to both drugs used as single agents. Leukemia 2003; 17(8):1669-70.

193. Marchetti M, Barosi G, Balestri F, Viarengo G, Gentili S, Barulli S, Demory JL, Ilariucci F, Volpe A, Bordessoule D, Grossi A, Le Bousse-Kerdiles MC, Caenazzo A, Pecci A, Falcone A, Broccia G, Bendotti C, Bauduer F, Buccisano F, Dupriez B. Low-dose thalidomide ameliorates cytopenias and splenomegaly in myelofibrosis with myeloid metaplasia : a Phase II trial. J Clin Oncol 2004; 22(3): 424-31.

194. Strupp C, Germing U, Scherer A, Kundgen A, Modder U, Gatterman N, Haas R. Thalidomide for the treatment of idiopathic myelofibrosis. Eur J Haematol 2004; 72(1): 52-7.

195. Merup M, Kutti J, Birgegard G, Mauritzson N, Bjrokholm M, Markevarn B, Malm C, Westin J, Palmblad J, Samuelsson J. Negligible clinical effects of thalidomide in patients with myelofibrosis with myeloid metaplasia. Med Oncol 2002; 19(2):79-86.

196. Bonn D. Thalidomide: no benefit in myelofibrosis with myeloid metaplasia. Lancet Oncol 2003; 4(2): 70.

197. Mesa RA, Steensma DP, Pardanani A, Li CY, Elliot M, Kaufmann SH, Wiseman G, Gray LA, Schroeder G, Reeder T, Zeldis JB, Tefferi A. A phase 2 trial of combination low-dose thalidomide and prednisolone for the treatment of myelofibrosis with myeloid metaplasia. Blood 2003; 101(7): 2534-41.

198. List A, Kurtin S, Roe DJ, Buresh A, Mahadevan D, Fuchs D et al.Efficacy of lenalidomide in myelodysplastic syndromes. N Engl J Med 2005; 352: 549–557.

New references 2008 update

199. Schmitt-Graeff AH, Teo SS, Olschewski M, Schaub F, Haxelmans S, Kirn A, Reinecke P, Germing U, Skoda RC. JAK2V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis Haematologica. 2008:34-40.

200. Scott LM, Tong W, Levine R, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med. 2007;356:459-468.

201. Tefferi A, Thiele J, Orazi A, Kvasnicka HM, Barbui et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis. Recommendations from an ad hoc international panel. Blood. 2007;110:1092-1097.

202. Teofili L, Giona F, Martini M, Cenci T, Guidi F et al. The revised diagnostic criteria for the Ph-negative myeloproliferative diseases are not appropriate for the diagnostic screening of childhood polycythemia vera and essential thrombocythemia. Blood. 2007;110:3384-3386.

203. Larsen TS, Pallisggard N, Moller MB, Hasselbalch HC. The JAK2 V617F allele burden in essential thrombocythemia, polycythemia vera and primary myelofibrosis – Impact on disease phenotype. Eur J Haematol 2007; 79: 508-15.

204. Gangat N, Strand J, Li CY, Wu W, Pardanani A, Tefferi A. Leucocytosis in polycythaemia vera predicts both inferior survival and leukaemic transformation.Br J Haematol. 2007;138:354-8

205. Tefferi A, Strand JJ, Lasho TL, Knudson RA, Finke CM, Gangat N, Pardanani A, Hanson CA, Ketterling RP. Bone marrow JAK2V617F allele burden and clinical correlates in polycythemia vera. Leukemia. 2007; 21: 2074-5

206. Tefferi A, Lasho TL, Schwager SM, Strand JS, Elliott M, Mesa R, Li CY, Wadleigh M, Lee SJ, Gilliland DG. The clinical phenotype of wild-type, heterozygous, and homozygous JAK2V617F in polycythemia vera. Cancer. 2006;106:631-635.

207. Hsiao HH, Yang MY, Liu YC, Lee CP, Yang WC, Liu TC, Chang CS, Lin SF. The association of JAK2V617F mutation and leukocytosis with thrombotic events in essential thrombocythemia. Exp Hematol. 2007; 35: 1704-7.

208. Kittur J, Knudson RA, Lasho TL, Finke CM, Gagnat N, Wolanskyj AP, Li CY, Wu W, Kettering RP, Pardanani A, Tefferi A. Clinical correlates of JAK2V617F allele burden in essential thrombocythemia. Cancer. 2007; 109: 2279-2284.

209. Antonioli E, Guglielmelli P, Poli G, Bogani C, Pancrazzi A, Longo G, Ponziani V, Tozzi L, Pieri L, Santini V, Bosi A, Vanucchi AM. Influence of $JAK2^{V617F}$ allele burden on phenotype in essential thrombocythemia. Haematol. 2008; 93: 41-48.

210. Gisslinger H, Kralovics R, Gotic M, Holowiecki J, Penka M, Widmann R, Petrides P. Non-inferiority of anagrelide compared to hydroxyurea in newly diagnosed patients with essential thrombocythemia. Blood 2007; 110: 1038a.

211. Passamonti F, Randi ML, Rumi E, Pungolino E, Elena C, Pietra D, et al. Increased risk of pregnancy complications in patients with essential thromocythemia carrying the JAK2(V617F) mutation. Blood. 2007; 110: 485-9.

212. Guglielmelli P, Pancrazzi A, Bergamaschi G, Rosti V, Villani L, Antonioli E, Bosi A, Barosi G, Vannucchi AM; GIMEMA--Italian Registry of Myelofibrosis; MPD Research Consortium . Anaemia characterises patients with myelofibrosis harbouring Mpl mutation. Br J Haematol. 2007; 3:244-7.

213. Wilkins BS, Erber WN, Bareford D, Buck G, Wheatley K, East CL, Paul B, Harrison CN, Green AR, Campbell PJ. Bone marrow pathology in essential thrombocythemia: inter-observer reliablity and utility for identifying disease subtypes. Blood. 2008;111:60-70.

214. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica 2005; 90:1128-1132.

215. Tefferi A, Huang J, Schwager S et al. Validation and comparison of contemporary prognostic models in primary myelofibrosis : analysis base on 334 patients from a single instituon. Cancer 2007; 109:2083-2088.

216. Vener C, Fracchiolla NS, Gianelli U, Calori R, Radaelli F, Iurlo A, Caberlon S, Gerli G, Boiocchi L, Delilers GL. Prognostic implications of the European consensus for grading of bone marrow fibrosis in chronic idiopathic myelofibrosis. Blood 2008; 111; 1862-5.

217. Carobbio A, Finazzi G, Guerini V, Spinelli O, Delaini F, Marchioli R, Borrelli G, Rambaldi A, Barbui T. Leukocytosis is a risk factor for thrombosis in essential thrombocythemia: interaction with treatment, standard risk factors, and Jak2 mutation status. Blood 2007; 109: 2310-13.

New references 2009 update

218. Beer PA, Campbell PJ, Scott LM, Bench AJ, Erber WN, Bareford D, Wilkins BS, Reilly JT, Hasselbalch HC, Bowman R, Wheatley K, Buck G, Harrison CN, Green AR. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. Blood. 2008; 112: 141-9.

219. James C. The JAK2V617F mutation in polycythemia vera and other myeloproliferative disorders: One mutation for three diseases? Hematology Am Soc Hematol Educ Program. 2008; 2008: 69-75.

220. Landgren O, Goldin LR, Kristinsson SY, Helgadottir EA, Samuelsson J, Björkholm M. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24,577 first-degree relatives of 11,039 patients with myeloproliferative neoplasms in Sweden. Blood. 2008; 112: 2199-204.

221. Cassinat B, Laguillier C, Gardin C, de Beco V, Burcheri S, Fenaux P, Chomienne C, Kiladjian JJ; PV-Nord Group. Classification of myeloproliferative disorders in the JAK2 era: is there a role for red cell mass? Leukemia. 2008; 22: 452-3.

222. Kiladjian JJ, Cervantes F, Leebeek FW, Marzac C, Cassinat B, Chevret S, Cazals-Hatem D, Plessier A, Garcia-Pagan JC, Murad SD, Raffa S, Janssen HL, Gardin C, Cereja S, Tonetti C, Giraudier S, Condat B, Casadevall N, Fenaux P, Valla DC. The impact of JAK2 and MPL mutations on diagnosis and prognosis of splanchnic vein thrombosis: a report on 241 cases. Blood 2008; 111: 4922-9.

223. Ruggeri M, Tosetto A, Bolgan A, Rodeghiero F. Efficacy and safety of primary antithrombotic prophylaxis with ticlopidine and with aspirin in patients with essential thrombocythemia and polycythemia vera. Results from a cohort study. Blood 2008; 112: 1743a

224. Kiladjian JJ, Chevret S, Dosquet C, Fenaux P, Chomienne C, Rain JD.Long-Term Outcome in Polycythemia Vera (PV): Final analysis of a randomized trial comparing hydroxyurea to pipobroman. Blood 2008; 112: 1746a.

225. Larsen TS, Pallisgaard N, de Stricker K, Møller MB, Hasselbalch HC. Limited efficacy of hydroxyurea in lowering of the JAK2 V617F allele burden. Hematology. 2009; 14: 11-15.

226. Ricksten A, Palmqvist L, Johansson P, Andreasson B. Rapid decline of JAK2V617F levels during hydroxyurea treatment in patients with polycythemia vera and essential thrombocythemia. Haematologica. 2008; 93: 1260-1.

227. Girodon F, Schaeffer C, Cleyrat C, Mounier M, Lafont I, Santos FD, Vidal A, Maynadié M, Hermouet S. Frequent reduction or absence of detection of the JAK2-mutated clone in JAK2V617F-positive patients within the first years of hydroxyurea therapy. Haematologica. 2008; 93: 1723-7.

228. Besses C, Álvarez-Larrán A, Martínez-Avilés L, Longarón R, Serrano S, Bellosillo B. Major hematological response is the main factor for achieving a major molecular response in JAK2V617F-positive essential thrombocythemia and polycythemia vera patients treated with hydroxyurea. Blood 2008; 112: 660a.

229. Kiladjian JJ, Cassinat B, Chevret S, Turlure P, Cambier N, Roussel M, Bellucci S, Grandchamp B, Chomienne C, Fenaux P. Pegylated interferon-alfa-2a induces complete hematologic and molecular responses with low toxicity in polycythemia vera. Blood 2008; 112: 3065-72.

230. Quintas-Cardama A, Kantarjian HM, Garcia-Manero G, Cortes J, Richie MA, Borthakur G, Verstovsek S. Pegylated interferon-alpha-2A therapy renders high clinical and molecular response rates in patients with essential thrombocythemia and polycythemia vera. Blood 2008; 112: 658a.

231. Larsen TS, Bjerrum OW, Pallisgaard N, Andersen MT, Møller MB, Hasselbalch HC. Sustained major molecular response on interferon alpha-2b in two patients with polycythemia vera. Ann Hematol. 2008 ; 87: 847-50

232. Larsen TS, Boe Møller M, de Stricker K, Nørgaard P, Samuelsson J, Marcher C, Weis Bjerrum O, Andersen MT, Hasselbalch HC. Minimal residual disease and normalization of the bone marrow

after long-term treatment with alpha-interferon2b in polycythemia vera. A report on seven patients in sustained complete hematological remission with major molecular responses. Blood 2008; 112: 1744a.

233. Kiladjian JJ, Dupont S, Cassinat B, Massé A, Vainchenker W, Chomienne C, Delhommeau F, Fenaux P. Can Peg-interferon α-2a eradicate JAK2V617F-positive bone marrow progenitors in polycythemia? Blood 2008; 112: 659a.

234. Passamonti F, Rumi E, Arcaini L et al. Prognostic factors for thrombosis, myelofibrosis, and leukemia in essential thrombocythemia: a study of 605 patients. Haematologica; 2008; 93; 1645-51).

235. Tefferi A, Gangat N, Wolanskyi AP. Management of extreme thrombocytosis in otherwise low-risk essential thrombocythemia: does number matter? Blood 2006; 108: 2493-2494.

236. Carobbio A, Antonioli E, Guglielmelli P, Vannucchi AM, Delaini F, Guerini V, Finazzi G, Rambaldi A, Barbui T. Leukocytosis and risk stratification assessment in essential thrombocythemia. J Clin Oncol. 2008; 26: 2732-6

237. Carobbio A, Finazzi G, Antonioli E, Guglielmelli P, Vannucchi AM, Delaini F, Guerini V, Ruggeri M, Rodeghiero F, Rambaldi A, Barbui T. Thrombocytosis and leukocytosis interaction in vascular complications of essential thrombocythemia. Blood. 2008; 112: 3135-7.

238. Abdulkarim K, Girodon F, Johansson P, Maynadié M, Kutti J, Carli PM, Bovet E, Andréasson B. AML transformation in 56 patients with Ph- MPD in two welld efinied populations. Eur J Haematol. 2009; 82: 106-11.

239. Sirhan S, Lasho TL, Hanson CA, Mesa RA, Pardanani A, Tefferi A.The presence of JAK2V617F in primary myelofibrosis or its allele burden in polycythemia vera predicts chemosensitivity to hydroxyurea. Am J Hematol. 2008; 83: 363-5.

240. Thépot S, Itzykson R, Raffoux E et al. Treatment of progression of myeloproliferative disorders to MDS/AML by azacytidine: A preliminary report on 17 Patients. Blood 2008; 112: 2800a.

241. Rossi D, Deambrogi C, Capello D, Cerri M, Lunghi M, Parvis G, et al. JAK2 V617F mutation in leukemic transformation of Philadelphia-negative chronic myeloproliferative disorders. Br J Haematol 2006; 135: 267–8.

242. Theocharides A, Boissinot M, Girodon F, Garand R, Teo SS, Lippert E, et al. Leukemic blasts in transformed JAK2-V617F positive myeloproliferative disorders are frequently negative for the JAK2-V617F mutation. Blood 2007; 110: 375–9.

243. Barosi G, Bergamaschi G, Marchetti M,Vannucchi AM, Guglielmelli P, Antonioli E, et al. JAK2V617F mutational status predicts progression to large splenomegaly and leukemic transformation in primary myelofibrosis. Blood 2007; 110: 4030–6.

244. Tefferi A, Lasho J, Huang J, Finkel C, Mesa RA, Li CY, Wu W, Hanson CA,Pardani A. Low JAK2V617F allele burden in primary myelofibrosis, compared to either a higher

allele burden or unmutated status, is associated with inferior overall and leukemia-free survival. Leukemia 2008; 1-6.

245. Guglielmelli P, Barosi G, Pieri L, Antonioli E, Bosi A, Vannucchi AM. JAK2V617F mutational status and allele burden have little influence on clinical phenotype and prognosis in patients with post-polycythemia vera and post-essential thrombocythemia myelofibrosis. Haematologica. 2009; 94:144-6.

246. Vannucchi AM, Antonioli E, Guglielmelli P, Pardanani A, Tefferi A. Clinical correlates of JAK2V617F presence or allele burden in myeloproliferative neoplasms: a critical reappraisal Leukemia 2008; 22:1299-307.

247. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, Cuker A, Wernig G, Moore S, Galinsky I, DeAngelo DJ, Clark JJ, Lee SJ, Golub TR, Wadleigh M, Gilliland DG, Levine RL. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006; 3: e270.

248. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, Steensma DP, Elliott MA, Wolanskyj AP, Hogan WJ, McClure RF, Litzow MR, Gilliland DG, Tefferi A. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood 2006; 108:3472-6.

249. Lasho TL, Pardanani A, McClure RF, Mesa RA, Levine RL, Gilliland DG, Tefferi A. Concurrent MPL515 and JAK2V617F mutations in myelofibrosis: chronology of clonal emergence and changes in mutant allele burden over time. . Br J Haematol 2006; 135: 683-7.

250. Pardanani A, Lasho TL, Finke C, Mesa RA, Hogan WJ, Ketterling RP, Gilliland DG, Tefferi A. Extending Jak2V617F and MplW515 mutation analysis to single hematopoietic colonies and B and T lymphocytes. Stem Cells 2007; 25: 2358-62.

251. Chaligné R, James C, Tonetti C, Besancenot R, Le Couédic JP, Fava F, Mazurier F, Godin I, Maloum K, Larbret F, Lécluse Y, Vainchenker W, Giraudier S. Evidence for MPL W515L/K mutations in hematopoietic stem cells in primitive myelofibrosis. Blood 2007; 110: 3735-43.

252. Chaligné R, Tonetti C, Besancenot R, Roy L, Marty C, Mossuz P, Kiladjian JJ,Socié G, Bordessoule D, Le Bousse-Kerdilès MC, Vainchenker W, Giraudier S. New mutations of MPL in primitive myelofibrosis: only the MPL W515 mutations promote a G1/S-phase transition. Leukemia 2008; 22: 1557-66.

253. Kilpivaara O, Levine RL. JAK2 and MPL mutations in myeloproliferative neoplasms: discovery and science. Leukemia 2008; 22: 1813-7.

254. Reilly J. Pathogenetic insight and prognostic information from standard and molecular cytogenetic studies in the BCR-ABL-negative myeloproliferative neoplasms (MPNs). Leukemia 2008; 22: 1818-27.

255. Tam CS, Abruzzo LV, Lin KI, Cortes J, Lynn A, Keating MJ, Thomas DA, Pierce S, Kantarjian H, Verstovsek S. The role of cytogenetic abnormalities as aprognostic marker in primary myelofibrosis: Applicability at the time of diagnosis and later during disease course. Blood 2009; Jan (Epub ahead of print).

256. Hussein K, Huang J, Lasho T, Pardanani A, Mesa RA, Williamson CM, Ketterling RP, Hanson CA, Van Dyke DL, Tefferi A. Karyotype complements the International Prognostic Scoring System for primary myelofibrosis. Eur J Haematol. 2009 Feb 10 [Epub ahead of print]

257. Cervantes F, Dupriez B, Pereira A, Passamonti F, Reilly JT, Morra E, Vannucchi AM, Mesa RA, Demory JL, Barosi G, Rumi E, Tefferi A. A new prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. Blood 2008 Nov 6 [Epub ahead of print]

258. Hasselbalch HC. Myelofibrosis with myeloid metaplasia: the advanced phase of an untreated disseminated hematological cancer. Time to change our therapeutic attitude with early upfront treatment? Leuk Res. 2009; 33 : 11-8.

259. Levy B, Vandris K, Adriano F, Goldman J, Silver RT. Recombinant interferon alpha may retard progression of early primary myelofibrosis by reducing splenomegaly and by changing marrow morphology. Blood 2008; 112: 1758a.

260. List A, Dewald G, Bennett J, Giagounidis A, Raza A, Feldman E et al.Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 2006; 355: 1456–65.

261. Tefferi A, Cortes J, Verstovsek S, Mesa RA, Thomas D, Lasho TL, Hogan WJ, Litzow MR, Allred JB, Jones D, Byrne C, Zeldis JB, Ketterling RP, Mcclure RF, Giles F, Kantarjian HM. Lenalidomide therapy in myelofibrosis with myeloid metaplasia. Blood 2006; 108:1158-64.

262. Mesa RA, Tefferi A, Li CY, Steensma DP. Hematologic and cytogenetic response to lenalidomide monotherapy in acute myeloid leukemia arising from JAK2(V617F) positive, del(5)(q13q33) myelodysplastic syndrome. Leukemia 2006; 20: 2063–2064.

263. Tefferi A, Lasho TL, Mesa RA, Pardanani A, Ketterling RP, Hanson CA. Lenalidomide therapy in del(5)(q31)-associated myelofibrosis: cytogenetic and JAK2V617F molecular remissions Leukemia 2007; 21: 1827–8.