Abstract

According to strict morphological, biochemical, cytogenetic and molecular criteria including the Philadelphia (Ph) chromosome and bcr/abl fusion gene and protein, chronic myeloid leukemia is a malignant disease with an obligate transition into acute leukemia, whereas essential thrombocytopenia (ET), polycythemia vera (PV) and agnogenic myeloid metaplasia (AMM) feature by a benign proliferation of the 3 hematopoietic cell lines. Increase and clustering of abnormal enlarged megakaryocytes together with no or various degrees of increased erythropoiesis and/or granulopoiesis in bone marrow biopsy specimens appears to be a pathognomonic clue for the diagnosis of prefibrotic MPD when WHO bone marrow features are applied. On top of established WHO bone marrow features in combination with JAK2V617F mutation screening, and laboratory features including endogenous erythroid colony (EEC) formation and serum erythropoietin (EPO) levels we propose updated European clinical, molecular and pathological (ECMP) criteria for the differential diagnosis of true ET, PV and chronic idiopathic myelofibrosis (CIMF) or AMM. The ECMP criteria reduced the platelet count of 600 x10^9/l to the upper limit of normal (>400 x10^9/l) as inclusion criterion for the diagnosis of thrombocytopenia in various MPDs. When WHO and ECMP criteria are applied, PVSG-defined ET includes three distinct entities: true ET, early PV mimicking ET, and thrombocytopenia associated with early CIMF-0 mimicking ET. Compared to characteristic bone marrow features pathognomonic for MPD, spontaneous EEC and low serum EPO levels are not sensitive enough as isolated markers for the diagnosis and differential diagnosis of prefibrotic ET, PV and CIMF-0. Bone marrow histology assessment remains the gold standard criterion for the diagnosis and staging of the MPDs. PVSG-defined ET and the presence of the JAK2V617F mutation, EEC, PRV-1 overexpression and low serum EPO levels is consistent with early PV (“forme fruste” PV) when ECMP criteria are applied. The combination of JAK2V617F mutation and increased hematocrit (>0.051 males and >0.48 females) is consistent with the diagnosis PV (specificity 100%, sensitivity 95%) without the need of red cell mass measurement. About half of the ET and CIMF patients are JAK2V617F positive (sensitivity 50%). The degree of JAK2V617F positivity of granulocytes is related to disease stage: heterozygous in true ET and early PV and mixed hetero/homozygous to homozygous in overt and advanced PV and CIMF-1 to 3. The proposed ECMP criteria for the differential diagnosis of ET, PV, CIMF in patients with JAK2V617F positive and JAK2 wild type MPD should be evaluated in prospective management studies in search for the most relevant prognostic factors of therapeutic significance.

Key words: myeloproliferative disorders, essential thrombocytopenia, polycythemia vera, chronic idiopathic myelofibrosis, erythropoietin, endogenous erythroid colony assay, JAK2 V617F mutation, bone marrow pathology

Introduction

In the 19th century chronic myeloid leukemia (CML) and polycythemia vera (PV) have been described as primary distinct disease entities1,2,3. In 1960 Nowell and Hungerford described the presence of a minute chromosome in leukemic cells of patients with chronic myeloid leukemia (CML). This minute chromosome was called Philadelphia chromosome or Ph after the city of discovery4. Using banding techniques Janet Rowley (1973) discovered that the Ph originated from a translocation between the long arms of chromosomes 9 and 22, 9(9;22)(q34;q11)5. Two groups working together in scientific friendship discovered that a hybrid gene is generated by the translocation consisting of the BCR gene on chromosome 22 and the ABL oncogene originating from chromosome 96. This results in a BCR/ABL fusion gene with high tyrosine kinase activity and CML-transformation capacity7,8. Ninety-five percent of all CML patients are Ph+; 90% are Ph+/BCR/ABL+, 5% are Ph+/BCR/ABL-, and 5% are Ph-/BCR/ABL-, the latter group usually diagnosed as atypical CML, juvenile CML, chronic neutrophilic leukemia or chronic myelomonocytic leukemia7. According to strict morphological, biochemical, cytogenetic and molecular criteria including the Ph+ chromosome and bcr/abl fusion gene and protein, CML is a malignant disease with an obligate transition into acute leukemia, whereas essential thrombocytopenia (ET), PV and agnogenic...
The concept of PV as a trilinear MPD

Wasserman extended in 1954 the 1950 concept of Dameshek on PV as a trilinear MPD and distinguished at least five subsequent stages in the natural history of PV.

Table 1. Polycythemia Vera Study Group (PVSG) criteria for the diagnosis of essential thrombocythemia (ET), polycythemia vera (PV) and modified criteria for the diagnosis of chronic idiopathic myelofibrosis (CIMF)

<table>
<thead>
<tr>
<th>PVSG criteria for the diagnosis of ET/17,18</th>
<th>Platelet count &gt; 600 x 10^9/L (ECMF &gt; 400 x10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- No known cause of Reactive Thrombocytosis</td>
<td></td>
</tr>
<tr>
<td>- Normal hemoglobin and red cell mass to exclude overt PV</td>
<td></td>
</tr>
<tr>
<td>- Sizable iron bone marrow to exclude PV</td>
<td></td>
</tr>
<tr>
<td>- No tear drop erythrocytes and no leuko-erythroblastosis</td>
<td></td>
</tr>
<tr>
<td>- No features of MDS in bone marrow smear and biopsy</td>
<td></td>
</tr>
<tr>
<td>- Absence of Ph1 chromosome (brc/abl) to exclude CML</td>
<td></td>
</tr>
<tr>
<td>- Myelofibrosis grade 1 up to grade 2 is allowed in the absence of collagen fibrosis of bone marrow</td>
<td></td>
</tr>
</tbody>
</table>

CIMF-1 defined ET with leuko-erythroblastosis in the peripheral blood (early clinical stage CIMP-0 and CIMP-1, table 3) when ECMAP criteria are applied.

<table>
<thead>
<tr>
<th>PV criteria for the diagnosis of PV/14,15</th>
<th>A. Major criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1. Increased red cell mass: male &gt;35 ml/kg, female &gt;32 ml/kg</td>
<td></td>
</tr>
<tr>
<td>A2. Normal arterial oxygen (O2) saturation &gt;92%</td>
<td></td>
</tr>
<tr>
<td>A3. Spleiotomy or palpation</td>
<td></td>
</tr>
</tbody>
</table>

B. Minor criteria

- B1. Thrombocytosis, platelet count >400 x 10^9/L
- B2. Leukocytosis >12 x10^9/L (no fever or infection)
- B3. Increased leukocyte alkaline phosphatase score (LAP) (no fever or infection)
- B4. Increased platelet count (>200 x10^9/L)

Diagnosis PV:

A1 + A2 + A3 or A1 + A2 + any two from category B establishes the diagnosis of PV.

Increased red cell mass is a crude criterion and overlooks the early thrombocytemic and erythroid stages of PV.

Table 2. WHO bone marrow features and European clinical, molecular and pathological (ECMP) criteria for the diagnosis of essential thrombocythemia (true ET/15,16,17)

<table>
<thead>
<tr>
<th>Clinical and molecular criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1. Sustained platelet count above the upper limit of normal: &gt;600 x10^9/L</td>
</tr>
<tr>
<td>C2. Presence of large or giant platelets in a peripheral blood smear</td>
</tr>
<tr>
<td>C3. Presence of MPD17 or JAK2V617F mutation</td>
</tr>
<tr>
<td>C4. Normal values for hemoglobin, hematocrit, white blood cell differential count</td>
</tr>
<tr>
<td>C5. Presence of the Philadelphia chromosome or any other cytogenetic fusion-gene abnormality</td>
</tr>
</tbody>
</table>

According to WHO/ECMP criteria C1 + P1 and P2 establish the diagnosis of true ET. A typical ET bone marrow histological picture (figure 2, 3 and 4A) excludes PV, CIMF, CML, MDS, RARS-T and reactive thrombocytosis.17,18

Bone marrow stimulation by an unknown factor or factors, and second, a lack or diminution in the normal inhibitory factor or factors11. This hypothesis of Dameshek has recently confirmed by the discovery of the JAK2V617F mutation demonstrating that the V617F mutation induces a loss of inhibitory activity of the JH2 pseudokinase part on the JH1 kinase part of JAK2, leading to enhanced activity of the normal JH1 kinase activity of JAK2, which makes the mutated hematopoietic stem cells hypersensitive to hematopoietic growth factors TPO, EPO, IGF1, SCF and G-CSF, resulting in trilinear myeloproliferation (Figure 1).13

myeloid metaplasia (AMM) form the Ph-chromosome and brc/abl negative chronic myeloproliferative disorder featured by a benign proliferation of the 3 hematopoietic cell lines (figure 1)13.

In 1950, William Dameshek described polycythemia vera (PV) as a chronic disorder of the bone marrow characterized by excessive production of nucleated red cells, granulocytes and megakaryocytes peripheral blood erythrocytosis, leukocytosis and thrombocytosis (figure 1).13 Some cases however show only a moderate elevation of erythrocytes with an extreme degree of thrombocytosis, while in others the leukocyte counts may be at or close to leukemic levels, with only slight increase in red cells or platelets.13 According to Dameshek all “stops” to blood production in the bone marrow seem to have been pulled out in PV. As to the etiology of PV, Dameshek proposed two highly speculative possibilities: first, the presence of excessive

Figure 1. The concept of Dameshek in 1950 on polycythemia vera (PV) as a trilinear myeloproliferative disorder (MPD) due to one hypothetical stimulus, appeared to be caused by the acquired JAK2V617F mutation discovered by Vainchenker et al in 2005.

The unifying concept of Dameshek in 1951 on the chronic myeloproliferative disorders (CMPDs) essential thrombocythemia (ET), polycythemia vera (PV), agnogenic myeloid metaplasia (AMM) has been broken up by the PVSG into Ph-positive thrombocythemia and CML complicated by myelofibrosis (MF) and the Ph-negative MPDs ET, PV and MF either positive or negative for the acquired JAK2V617F mutation.
Table 3. WHO bone marrow features and European clinical, molecular and pathological (ECMP) criteria for diagnosis and staging chronic idiopathic myelofibrosis CIMF17,23

<table>
<thead>
<tr>
<th>Clinical and molecular criteria</th>
<th>Pathological criteria (WHO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 1. Usually associated with or preceded by PVSG-defined ET and no preceding true ET, PV CML, CMML, HES or MDS. Absence of Philadelphia chromosome</td>
<td>P 1. Increased cellularity due to chronic megakaryocytic and granulocytic myeloproliferation (CARMAG) and no or relative reduction of erythroid precursors.</td>
</tr>
<tr>
<td>ECMP staging</td>
<td>P 2. Dense clustering and increase in atypical giant to medium sized megakaryocytes containing bulbous (cloud-like) hypolobulated nuclei and definitive maturation defects (figures 2, 3, 4C).</td>
</tr>
<tr>
<td>Early Clinical Stage</td>
<td>Grading of myelofibrosis (MF)100</td>
</tr>
<tr>
<td>Platelet count &gt;400 x10^9/l, usually pronounced around 1000 x10^9/l</td>
<td>MF 0: prothrombotic CIMF-0: scattered linear reticulin with no intersections (cross-over) corresponding to normal bone marrow</td>
</tr>
<tr>
<td>No leuko-erythroblastosis, no anemia</td>
<td>MF 1: early fibrotic CIMF-1: loose network of reticulin with many intersections, especially in peripheral areas, no collageneration</td>
</tr>
<tr>
<td>No or slight splenomegaly on echogram CIMF-G or CIMF-1</td>
<td>MF 2: fibrotic CIMF-2: diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis.</td>
</tr>
<tr>
<td>Intermediate Clinical Stage</td>
<td>MF 3: classic CIMF-3: diffuse and dense increase in reticulin with extensive intersections course bundle of collagen often associated with significant osteosclerosis</td>
</tr>
<tr>
<td>Definitive leuko-erythroblastosis</td>
<td>MF &gt;2: endstage hypocellular with extensive osteosclerosis</td>
</tr>
<tr>
<td>Anemia grade 1: Hb &lt;12 l/g &gt;10 g/dl, or 1.75e6, 25 mole/mlormal/l</td>
<td>According to WHO/ECMP criteria, C 1 and P1 plus P2 establish CIMF – any other peripheral blood criterion and grading of secondary myelofibrosis (MF) contribute to ECMP staging of WHO defined CIMF17,23</td>
</tr>
</tbody>
</table>

Stage 1). Pure erythrocythemia is featured by increased hemoglobin, hematocrit and red cell mass with normal leukocytes, thrombocytes and spleen size, which is labelled as idiopathic erythrocytosis.

Stage 2). The polycythemic stage of PV is featured by thrombocytosis, erythrocytosis and no or slight myeloid metaplasia, leukocytosis and/or splenomegaly.

Stage 3). Myeloid metaplasia in PV patients presents with no or different grades of reticulin and collagen fibrosis in the bone marrow and progressive splenomegaly during long-term follow in about one third of the cases.

Stage 4). The polycythemic stage with various degrees myelofibrosis and splenomegaly following PV may elapse 5 to 25 years before a period of normal red cell values so-called spontaneous remission of PV occurs. This stage must be considered as the beginning of spent phase PV and may last a few to several years. At this point the spleen is frequently large and very firm to palpation, the liver is enlarged to a moderately degree in most patients, thrombocytopenia is frequent and may be pronounced with bizarre and giant platelets, and white cells are usually increased with granulocytic leukocytosis (leukocytosis) accompanied by a small percentage of immature forms.

Stage 5). Post-PV myeloid metaplasia shows various degrees of leuko-erythroblastosis of the peripheral blood and may progress to extreme myelofibrosis with a dry tap on aspiration and massive splenomegaly. At this end-stage histopathology of bone marrow biopsy shows a similar picture and...
can not been differentiated from agnogenic myeloid metaplasia with no previous history of PV.

**Three phenotypes of MPD: ET, PV and CIMF**

The criteria the Polycythemia Vera Study Group (PVSG) originate from the early 1970s to classify the chronic myeloproliferative disorders (MPD) as 3 disease entities ET, PV and idiopathic myelofibrosis (IMF) and separate these 3 MPDs from Philadelphia chromosome-positive chronic myeloid leukaemia (CML) (figure 1, table 1)\(^1,14-18\). The criteria of Ph-chromosome-negative myeloproliferative disorders according to the 1990 Hannover bone marrow classification\(^19\), the Rotterdam clinical and pathological criteria between 1997 and 2000\(^21\) and the World Health Organisation (WHO) in 2001\(^21\) are an attempt to integrate bone marrow morphological criteria alongside PVSG clinical criteria for essential thrombocytopenia (ET), polycythaemia vera (PV) and chronic idiopathic myelofibrosis (CIMF). However, early stages of PV and ET are not recognized by the PVSG and 2001 WHO classifications and many patients will be diagnosed as unclassifiable MPD, indicating the need to modify the diagnostic inclusion criteria for early and overt stages of MPD. In the present study we propose a new set of European clinical, molecular and pathological (ECMP) criteria in a joint effort by clinicians and pathologists to integrate PVSG criteria, WHO bone marrow features and the use of new laboratory and molecular markers for diagnostic differentiation of each of the early and overt MPD phenotypes more accurately as a sound basis on which proper treatment guidelines are to be recommended.

**Table 5.** ET according to PVSG17,18, WHO21 and ECMP72,73 criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>ECMP</th>
<th>PVSG</th>
<th>WHO21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>&lt; 0.001</td>
<td>20-30</td>
<td>25-30</td>
</tr>
<tr>
<td>Serum EPO</td>
<td>Normal</td>
<td>Normal</td>
<td>Decreased</td>
</tr>
<tr>
<td>Platelets</td>
<td>&gt;400 ECP</td>
<td>&gt;400 WHO</td>
<td>&gt;400 ECP</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Bone marrow: ET picture</td>
<td>ET picture</td>
<td>PV picture</td>
<td>CIMF picture</td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td>Normal large / giant and mature</td>
<td>Abnormal</td>
<td></td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>JAK2 V617F</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>EEC</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>PV-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clonality</td>
<td>polyclonal</td>
<td>monoclonal</td>
<td>Monoclonal</td>
</tr>
</tbody>
</table>

**Table 6.** Staging of PV patients according to WHO/ECMP criteria: therapeutic implications\(^21,72,73\)

<table>
<thead>
<tr>
<th>PV, ECP stage</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Diagnosis</td>
<td>Early PV</td>
<td>polycythemic PV</td>
<td>Classic PV</td>
<td>Advanced PV</td>
<td>Post-PV myelofibrosis</td>
<td>Spent phase PV</td>
</tr>
<tr>
<td>LAP-score and/or PRV-1</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
</tr>
<tr>
<td>Red cell mass</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N-</td>
</tr>
<tr>
<td>Serum EPO</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Leukocytes x109/l</td>
<td>&lt;12</td>
<td>&lt;12</td>
<td>N &gt;12</td>
<td>&gt;15</td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Platelets x109/l</td>
<td>&gt;400</td>
<td>&gt;400</td>
<td>&gt;400</td>
<td>&gt;1000</td>
<td>variable</td>
<td>variable</td>
</tr>
<tr>
<td>Peripheral blood red cells</td>
<td>Hemoglobin g/dl (mmol/l)</td>
<td>&lt;16 (10)</td>
<td>&gt;16 (10)</td>
<td>&gt;16 (10)</td>
<td>variable</td>
<td>N-</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>&lt;0.51</td>
<td>&gt;0.51</td>
<td>&gt;0.51</td>
<td>&gt;0.51</td>
<td>variable</td>
<td>N-</td>
</tr>
<tr>
<td>Erythrocytes x1012/l</td>
<td>&lt;6</td>
<td>&gt;6</td>
<td>&gt;6</td>
<td>&gt;6</td>
<td>variable</td>
<td>N-</td>
</tr>
<tr>
<td>WHO bone marrow</td>
<td>Early PV</td>
<td>Early PV</td>
<td>Trilinear PV</td>
<td>Trilinear PV</td>
<td>Trilinear /MF</td>
<td>MF</td>
</tr>
<tr>
<td>Bone marrow cellularity (%)</td>
<td>MF 0</td>
<td>MF 0</td>
<td>MF 5</td>
<td>MF 1/2</td>
<td>MF 3</td>
<td></td>
</tr>
<tr>
<td>Spleenomegaly</td>
<td>slight</td>
<td>no</td>
<td>Slight/moderate</td>
<td>moderate</td>
<td>large</td>
<td>large</td>
</tr>
<tr>
<td>Spleen size, echogram cm</td>
<td>12-15</td>
<td>12</td>
<td>12-15</td>
<td>12-20</td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Specific MPD markers</td>
<td>EEC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Molecular JAK2V617F</td>
<td>++</td>
<td>+/++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>BFU-e</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Therapeutic implications</td>
<td>First line treatment</td>
<td>Aspirin</td>
<td>Phlebotomy</td>
<td>Aspirin</td>
<td>INF/HU</td>
<td>Supportive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Splenectomy</td>
<td></td>
</tr>
</tbody>
</table>
Limitations of PVSG and 2001 WHO criteria for the diagnoses of ET, PV and CIMF

The Polycythemia Vera Study Group (PVSG) reduced since 1986 the platelet count of 1000 to 600 x10^9/l as the arbitrary minimum for the diagnosis of ET (table 1)17,18. Lengfelder et al showed that this minimum of 600x10^9/l platelets according to the PVSG excluded early stage ET at platelets between normal and 600 x10^9/l in 29% of 143 ET cases when bone marrow biopsy was included in the investigations employed to diagnose ET22. These data confirmed the need to modify the PVSG criteria by lowering the platelet counts to 400 x10^9/l as the upper limit of normal for the clinical diagnosis of ET (table 2)20,23,24.

The 2001 WHO bone marrow criteria separated true ET (table 2) from thrombocythemia associated with prefibrotic and early fibrotic stages of CIMF-0 and CIMF-1 (table 3)21,24,25. Early stage CIMF-0 and CIMF-1 manifest typical clinical and laboratory features of ET with no features of leuko-erythroblastosis and therefore are diagnosed as ET by the PVSG criteria (table 1)24. In retrospect, 97% of PVSG-defined ET patients according to Lengfelder et al showed bone marrow features with typical increase and/or clustering of enlarged megakaryocytes with abnormal morphology diagnostic for MPD22. This was associated with normal cellularity in 52% consistent with true ET, with increased erythropoiesis in 17% consistent with early PV, and with increased cellularity due to pronounced granulopoiesis in 45% consistent with prefibrotic CIMF22.

The 2001 WHO criteria combined a typical ET histological bone marrow picture with platelet count in excess of 600 x10^9/l for the diagnosis of ET21, thereby excluding early stage ET22-24. Only one-third of PVSG-defined ET is diagnosed as true ET when the 2001 WHO clinical and bone marrow criteria are applied25. Unclassifiable early stage CMPD according to the 2001 WHO criteria include 3 types of early stage MPD: 1) initial ET with a typical ET bone marrow but platelet count below 600 x10^9/l; 2) initial PV with a typical PV bone marrow, platelet count less than 600 x10^9/l, low serum erythropoietin (EPO), normal red cell mass and hematocrit less than 0.51; and 3) initial masked MPD with splenomegaly and normal or slightly increased platelet count and hematocrit21.

According to Wasserman, PV frequently presents with initial erythrocytosis (idiopathic erythrocytosis), and distinguished at least five subsequent stages in the natural history of PV23. The PVSG used 3 major and 4 minor clinical criteria as inclusion criteria for the diagnosis of PV in the PVSG-01 study (Table 1)16,17. Increased red cell mass is a crude inclusion criterion for the diagnosis of PV (Louis Wasserman personal communication ASH 1995) and corresponds to high hematocrit values between 0.48 and 0.76, increased platelet count above 400 x10^9/L in two-third and palpable spleen in two-third of about 400 PV patients in the PVSG-
One third of PV patients have normal platelet count and spleen at time of presentation. Pre-treatment bone marrow biopsy specimens in 103 PV patients of the PVSG-01 study with increased red cell mass showed increased cellularity from 50 to 80% in two-thirds consistent with early stage PV and from 80 to 100% in one-third consistent with overt stage PV at the bone marrow level.

Evidence accumulates that the very early pre-fibrotic stage of chronic myeloproliferative disease is featured by a platelet count in the upper limit of normal (around 400 x10^9/L), the presence of enlarged platelets in a peripheral blood smear in the complete absence of any underlying disorder for reactive thrombocytosis. ET according to the PVSG criteria comprises 3 types of prefibrotic MPDs including true ET, thrombocytopenia associated with early polycythemia vera (PV), and thrombocytopenia associated with prefibrotic chronic idiopathic myelofibrosis (CIMF-0) when the recently defined WHO bone marrow features and the European clinical molecular and pathological (ECMP) criteria are applied (figure 2, table 5). We produced good evidence that characteristic PV bone marrow histology features (irrespective of red cell mass measurements) are seen in four different stages of newly diagnosed MPD patients (figures 3, tables 4 and 6): First, early PV mimicking ET with a hematocrit in the upper limit of normal but increased platelet count without or with slight splenomegaly (stage 0 PV, table 6); Second, erythrocytosis with increased red cell mass, high hematocrit, low serum erythropoietin (EPO), but normal platelet count and spleen size (so-called idiopathic erythrocytosis = stage 1 PV, table 6); Third, classic PV with increased red cell mass, high hematocrit and one or more PVSG B criteria PV (overt stage 2 and 3 PV, table 6); Fourth, unclassifiable MPD or masked PV with pronounced splenomegaly, normal hemoglobin and hematocrit, normal or slightly elevated platelet count. Thiele et al has nicely worked out this concept and confirmed that characteristic PV bone marrow histopathological features are seen in classic PV and in early PV mimicking ET (tables 4 and 6). The 2001 WHO criteria com-
cells (HSCs) acquires the Jak2V617F mutation and homozygosity is the second genetic step in which both alleles of HSCs carry the Jak2V617F mutation due to mitotic recombination occurring at times that HSCs are dividing. Progression of heterozygous ET from 1 to 100% is associated with progression of ET to early stage PV, very likely with mild splenomegaly and no tendency to develop myelofibrosis. Homozygosity for the Jak2V617F mutation is associated with a more rapid onset (still rather slow), and with pronounced trilineal myeloproliferation of megakaryocytes, erythropoiesis and granulopoiesis leading to pancytosis of the bone marrow. The degree of homozygosity for the Jak2V617F mutation in PV may range from 1% to 100% during long-term follow-up. If less than 25% of the HSCs are homozygous for the Jak2V617F mutation the result of JAK2 measurement in granulocytes using PCR technique will be less than 50%. If more than 25% of the HSCs carry the Jak2V617F mutation the result of JAK2 measurement will be more than 50%. If during follow-up all HSCs carry the Jak2V617F mutation the maximum positivity of the Jak2V617F mutation using PCR technique will be 100%. Consequently, PV patients may progress from low percentage to high percentage positivity for the homozygous Jak2V617F mutation, which is associated with progressive splenomegaly and post-PV myelofibrosis during long-term follow-up. About 95% of PV patients are positive for the Jak2V617F mutation either homozygous or heterozygous or combined. Among the 5% of PV negatives Jak2V617F, the majority do carry an exon and/or JAK2V617F in granulocytes in congenital (primary) polycythemia (CP), congenital erythrocytosis (CE) idiopathic erythrocytosis (IE) or acquired (secondary) erythrocytosis (SE) without the need for red cell mass measurement. PV patients with RCM may have normal hemoglobin and hematocrit because of associated iron deficiency and/or significant splenomegaly. 

Bone marrow histopathology has a sensitivity and specificity of near to 100% (gold standard) to differentiate a typical trilineal hypercellular bone marrow with small and enlarged (pleomorphic) megakaryocytes in early and overt PV (table 4) from the presence of isolated increased erythrocytosis and normal megakaryocytes in congenital (primary) polycythemia (CP), congenital erythrocytosis (CE) idiopathic erythrocytosis (IE) or acquired (secondary) erythrocytosis (SE) without the need for red cell mass measurement. In none of the PV patients is the information from RCM measurement found to be of additional diagnostic value, because all PV patients with increased red cell mass not only show a typical PV bone marrow histology picture but also the presence of one or more specific markers endogenous erythroid colony (EEC), low serum EPO, increased platelet count, and/or splenomegaly for the diagnosis of PV.

Spontaneous EEC and low serum EPO levels are specific confirmative criteria for the diagnosis of PV, but have insufficient diagnostic sensitivity as isolated parameters to differentiate between PV, CP, CE, SE, ET and normal controls. About 50% of PVSG defined ET patients show not
only spontaneous EEC but also increased PRV-1 expression42-46 together with low serum EPO levels42,46,47 indicating that EEC/PRV-1-positive ET comprises a biological subgroup of ET patients reflecting early PV (“forme fruste” PV) that is at risk for progression to overt PV (tables 5 and 6). Considering the finding of clustered enlarged or giant megakaryocytes according to ECMP criteria as diagnostic for MPD (ET, PV or CIMF) in 46 MPD patients with splanchic thrombosis, the sensitivity of increased red cell mass for the diagnosis of MPD was 63%, of low serum EPO level 52%, of EEC 72%, and of splenomegaly 74%, indicating the superiority of bone marrow histopathology to detect masked, early and overt stages of MPD46. In patients with splanchic vein thrombosis (Budd-Chiari syndrome or portal vein thrombosis) without signs of overt MPD, the laboratory markers EEC, PRV-1 expression and low serum EPO are insensitive but the combination of JAK2V617F mutation and bone marrow histology assessment is highly sensitive and specific to diagnose early ET and PV 48-52.

The role of JAK2V617F mutation in the pathogenesis and classification of trilinear MPD

The discovery of the JAK2 V617F mutation in 2004 by William Vainchenker and his team in France (JAK2 V617F France) was immediately appreciated as a real evolutionary event, and rapidly confirmed in 2005 by several investigators44,53. JAK2 plays an essential role in hematopoiesis by mediating signals from several hematopoietic cytokines including EPO, TPO IL-3 G-CSF, and GMSCF42,44-55. The JAK2 V617F mutation makes the mutated hematopoietic progenitor cells hypersensitive to these cytokines, thereby leading to growth advantage of the mutated above the non-mutated normal trilinear hematopoietic cells in the bone marrow (figure 4)24,53. The JAK2V617F mutation is detectable in CD34+ hematopoietic bone marrow cells, erythroblasts, in cells of spontaneous EEC, blood platelets and granulocytes53-55. Applying allele-specific polymerase chain reaction (PCR) analysis in PVSG-defined MPD patients, a high frequency of the JAK2V617F mutation of 95% (92-97%) in PV, and a lower frequency of 53% (49-57%) in ET and 52% (44-55%) in idiopathic myelofibrosis (IMF) are described44,56. Only 3 to 4% of ET, 24 to 27% of PV and 6 to 18% of IMF patients are homozygous for the JAK2V617F mutation24,56. Two hypotheses have been proposed by Vainchenker’s group of French investigators Delhommeau, James, Pisani, Villeval and Casadevall to explain why three different phenotypes of MPD are caused by the same JAK2V617F mutation: the “dosage” hypothesis and the “additional events” hypothesis (figure 4)43-55. According to the dosage hypothesis (based on animal studies and different mutation states of JAK2V617F in MPD patients), the level and duration of JAK2V617F directly contribute to the phenotypic diversity of trilinear MPDs. According to this model (based on animal studies and different mutation states of JAK2V617F in MPD patients), the level and duration of JAK2V617F directly contribute to the phenotypic diversity of trilinear MPDs (figure 4)53,55. The hypothesis to explain that the level of kinase activity regulates the disease phenotype of MPD is based on different densities of thrombopoietin (TPO) and EPO receptors (TPOR and EPOR) on hematopoietic progenitor cells and on difference of response of TPOR and EPOR to various levels of JAK2V617F activity55. TPOR or MPL is expressed at high levels in megakaryocytic cells where it controls TPO physiologic levels. It is possible that activation of a few TPO receptors by low levels of JAK2V617F (heterozygous) is sufficient to send a signal to megakaryocytic cells. A slight increase in numbers of mutated megakaryocytes and platelets (about 200 x10^9/l mutated platelets) might be enough to produce platelet-mediated microvascular circulation disturbances44,53. Conversely, EPOR is expressed at low levels on hematopoietic progenitor cells and therefore high levels of JAK2V617F may be required to activate EPOR and generate PV-like phenotype44,53. Sustained high levels of JAK2V617F during long-term follow-up subsequently may lead to a high level activation of EPOR and granulocyte colony stimulating factor receptor (G-CSF)R leading to extramedullary hematopoiesis (splenomegaly) and cytokine mediated secondary myelofibrosis53,55. The degree of JAK2V617F positivity (progression from heterozygous to homozygous in figure 4) is strongly cor-
related with (polycythemia rubra vera-1 gene) PRV-1 over expression in granulocytes, with the ability to form spontaneous EEC and with progressive post-PV myelofibrosis15,55,57. Scott et al recently showed that BFU-e colonies are already homoyzogenous for the JAK2V617F mutation in PV patients with a heterozygous pattern of JAK2V617F in their peripheral blood granulocytes58. In contrast, the BFU-E colonies from heterozygous patients with ET did not contain a subpopulation of JAK2V617F homozygous cells58. In a recent study, JAK2V617F was detected in 75% of ET (n=60) and in 97% of PV patients (n=62)59. Allelic ratios exceeding 50% JAK2V617F indicating the presence of granulocytes homoyzogenous for JAK2V617F were found in 70% of PV at diagnosis but never in ET59. Passamonti et al produced good evidence that transition from heterozygosity to homozygosity for the JAK2V617F mutation represents a very important step in the progression from classic PV to post-PV myelofibrosis59.

JAK2V617F may be be dependent not only on the amount of heterozygous and homoyzogenous mutant protein, but also on the various pathway regulating JAK2 activity including MPL, JAK2, STAT-3 signalling pathway53. This has led to the recent discovery of the MPLW515L and MPLW515K mutations as the underlying etiology in some ET and CIMF patients60,61. Pikman et al identified a somatic activating mutation in the transmembrane domain of MPL, the thrombopoietin receptor (TPOR), in 4 of 45 (9%) of JAK2 wild type myelofibrosis patients60. In a mouse model transplant assay, expression of the MPLW515L, but not wild type MPL, resulted in a fully penetrant MPD characterized by marked thrombocytosis and leukocytosis with no evidence of polycythemia. Thrombocytopenia with leukocytosis was caused by dual proliferation of megakaryopoiesis and granulopoiesis (myelopoiesis) with increased numbers and clustering of atypical dysmorphic megakaryocytes in the bone marrow, myelofibrosis and marked splenomegaly due to extramedullary hemopoiesis consistent with true CIMF56. Pardanani et al screened 1182 PVSG-defined MPD patients (318 ET, 242 PV, and 290 IMF) and 64 controls for MPL515 mutations regardless of JAK2V617F mutation49. MPL mutations either MPLW515L (n=17) or MPLW515K (n=5) was detected in 20 patients (de novo IMF in 12 = 4%, ET in 4 = 1%) and post-ET myelofibrosis in 1, but not in PV and controls61. Six cases carried the MPLW515L and JAK2V617F alleles indicating that these alleles have functional complementation in IMF. These experimental and clinical observations indicate that MPL515 mutation related myelofibrosis may represent a distinct entity of JAK2 wild type IMF distinct from JAK2V617F trilinear MPD. According to the “additional events” hypothesis, alternative and/or additional molecular abnormalities modify, or precede a homoyzogenous state deferred to by the JAK2V617F mutation alone, combinations carrying the JAK2V617F and MPL515 mutations49,61 or other combinations of still unknown mutations (figure 4)67. Mechanisms other than mitotic recombination such as duplication of the mutated allele is observed in a proportion of PV and CIMF patients displaying a gain of 9, mostly due to trisomy 953. Therefore, there may be an overlap between “dosage“ and “additional molecular events” hypotheses. Long-term studies are warranted to delineate the chronology and impact of various putative additional molecular abnormalities especially in cases of progressive disease from JAK2V617F positive true ET to PV and subsequent CIMF and in cases with combined JAK2V617F/MPL515 mutations or JAK2-wild-type/MPL515 positive ET and CIMF. Sex appears to be a powerful genetic background modifier in JAK2V617F-positive MPDs as ET is more common in females and PV in males.

WHO bone marrow features and ECMP criteria for the diagnosis of ET, PV and CIMF

In 1980s Georgii and Thiele independently defined the pathological features of ET, PV and idiopathic myelofibrosis (IMF) or agnogenic myeloid metaplasia (AMM) as derived from histopathological morphology of bone marrow biopsies19,62. ET is defined by persistent increase of platelets in excess of 400 x109/l without the Ph1+ chromosome together with mononuclear proliferation of mature enlarged megakaryocytes in the bone marrow with normal cellularity, normal erythropoiesis and normal granulopoiesis (figure 2)19,62. PV is defined as a trilinear proliferation of megakaryopoiesis, erythropoiesis and granulopoiesis in which the erythropoiesis was most prominent together with variable degrees of increased platelets, erythrocytes and granulocytes in the peripheral blood in the absence of the Ph1+ chromosome (figure 2)19,62. Georgii regarded myelofibrosis (MF) as a reactive feature secondary to progressive disease5-10 seen in CMGM, PV and CML19,63,64. Therefore, according to Georgii the terms agnogenic myloid metaplasia (AMM) or idiopathic myelofibrosis (IMF) lack accuracy since they are applied to both the prefibrotic hypercellular and advanced fibrotic stages of AMM or IMF. Consequently, Georgii replaced the terms
prefibrotic AMM and IMF by chronic megakaryocytic granulocytic myeloproliferation (CMGM) and proposed in 1990 the Hannover Classification to distinguish 3 distinct primary CMPDs ET, PV and CMGM\textsuperscript{19}. The diagnosis of CMGM according to the Hannover classification\textsuperscript{19} or prefibrotic CIMF-0 according to the Cologne criteria proposed by Thiele\textsuperscript{66} (figure 3) is based on 3 main features. First, the presence of large megakaryocytes with immature cytoplasm and immature cloud-like nuclei not seen in ET and PV. Second, increased granulopoiesis but never disturbed in maturation. Third, erythropoiesis is usually relatively decreased\textsuperscript{19,62,63}. The Hannover classification uses the term CMGM\textsuperscript{19,62,63} and the Cologne classification uses the term prefibrotic chronic IMF (CIMF-0) for the third entity of prefibrotic MPD\textsuperscript{65-71}. The Hannover and Cologne classifications\textsuperscript{19,66} are based on specific bone marrow histology (pathological) features for the three prefibrotic CMPDs true ET, PV and CIMF-0 at the bone marrow level, and have been taken over in 2001 by the WHO\textsuperscript{21}. Michiels and Thiele subsequently proposed the European clinical and pathological (ECP) criteria for the diagnosis of the Ph-negative MPDs to describe the full spectrum of clinical, laboratory, and WHO bone marrow features for true ET, early and overt PV and CIMF-0 (figure 3)\textsuperscript{24,72,73}. In a recent joint effort by clinicians and pathologists, we added the molecular marker and updated the European clinical, molecular and pathological (ECMP) criteria to extend PVSG criteria by including WHO bone marrow features and the use of laboratory and molecular markers for the diagnosis and staging of the three prefibrotic MPDs true ET, PV and CIMF (tables 2, 3 and 4)\textsuperscript{24,72,73}. These ECMP criteria allow a cross talk between clinicians and pathologists to translate PVSG clinical criteria (table 1) by including WHO bone marrow features and new biological and molecular markers (figures 2, and 3, tables 2, 3 and 4) to reach agreement on diagnosis, classification and staging of the MPDs\textsuperscript{72,73}.

For the diagnosis of MPD bone marrow trephine biopsy specimens should be embedded in paraffin or plastic, both with its technical limitations. Paraffin requires decalcification with EDTA (preferable, allows reasonable DNA quality) or acid electrolysis\textsuperscript{28,72,73}. The specimens should have at least 4 evaluable bone marrow spaces with hematopoiesis. Recommended stains include: hematoxylin and eosin (H&E); Giemsa (3 µm sections), periodic acid-Schiff (PAS); Perls for estimation of hemosiderin content; chloro-acetate esterase (Leder) for identification of granulocytic differentiation; silver stain for reticulin; and trichrome-Masson for collagen staining. Immunostains of paraffin embedded specimens should include glycoporphine C for erythropoiesis, myeloperoxidase for granulopoiesis, CD42b, CD61 or FVIII-related antigen for megakaryocytes; CD34 for CD34-positive blasts and CD117 for megakaryoid differentiation. Regarding MPD, clinicians want to receive from their pathologist\textsuperscript{28,72,73} a detailed report according to the Cologne bone marrow evaluation form\textsuperscript{72,73}. According to WHO and ECMP criteria, increased and loose clustering of enlarged mature megakaryocytes with hyperlobulated nuclei in a normocellular bone marrow and platelet count >400 x10\textsuperscript{9}/l represent the hallmark of true ET (table 2, figures 2 and 3). In true ET there is no proliferation or immaturity of granulopoiesis or erythropoiesis. In congenital and acquired erythrocytosis and in reactive thrombocytosis the megakaryocytes are of normal size and morphology and there is no tendency to cluster. A typical histopathological ET picture of the bone marrow excludes RT and distinguishes true ET from early PV, CIMF-0, CIMF-1 (figure 2), and thrombocytosis associated with atypical MPD, MDS, refractory anemia with increased ringed sideroblasts (RARS-T) or Ph\textsuperscript{1}–positive thrombocytosis in CML. The characteristic increase and clustering of small and enlarged pleomorphic megakaryocytes and increased erythropoiesis with increased granulopoiesis and increased cellularity (80-100%) are the diagnostic characteristics of classic PV with increased hematocrit >0.51, low serum EPO and/or JAK2V617F (tables 4 and 6) distinguishing it from congenital and secondary erythrocytosis. A typical histological PV picture with moderately increased bone marrow cellularity (according to age) is seen in patients with early PV mimicking ET or “forme fruste” PV featured by platelet count >400x10\textsuperscript{9}/l and hematocrit <0.51, low serum EPO and/or the presence of the JAK2V617F mutation (tables 4 and 6). A typical histological PV bone marrow picture is also seen in the early erythrocythemic stage 1 PV featured by increased hematocrit >0.51, normal platelet count <400x10\textsuperscript{9}/l, normal spleen, low serum EPO and/or the presence of the JAK2V617F mutation (tables 4 and 6).

According to Hannover\textsuperscript{19}, Cologne\textsuperscript{66}, WHO\textsuperscript{21} and ECMP criteria\textsuperscript{24,72,73}, CIMF-0 is characterized by hypercellularity of the bone marrow (60-100%) due to increased granulopoiesis, relative decrease of erythropoiesis and the presence of dense clusters of immature megakaryocytes with maturation defects.
centers of excellence (figures 2 and 3)57-73. There are 85 to 90% of the cases, which is only feasible in
 distinguish CIMF-0 from true ET and PV in about
 of experienced hematopathologists are required to
 tological bone marrow preparations in the hands
 of both. The risk of CIMF-0 to transform into early CIMF-1 and subsequent CIMF-2/3 with extramedullary hematopoiesis is clearly dependent on the degree of hypercellularity and on the degree of maturation defects of megakaryopoiesis70,71. High quality histological bone marrow preparations in the hands of experienced hematopathologists are required to distinguish CIMF-0 from true ET and PV in about 85 to 90% of the cases, which is only feasible in centers of excellence (figures 2 and 3)57-73. There are no studies that have examined the concordance between a number of pathologists who have used characteristic histological bone marrow features to assign cases to the prefibrotic stages of true ET, PV and CIMF-0 without knowledge of the clinical findings and biological MPD markers except age. Data on the very long-term natural history of patients with ECMP defined true ET, early PV and CIMF-0 as derived from large scale prospective studies are lacking. We don’t really know whether the early stages of PV patients are at no, low or high risk of progression to post-PV myelofibrosis or whether true ET never progress to CIMF as it is claimed. The bone marrow histology of CIMF-0 with slight dysmegakaryopoiesis can appear to overlap with early PV (hematocrit <0.51) presenting with a trilinear hypercellular bone marrow with relative increased granulopoiesis as compared to erythropoiesis and a similar degree of thrombocytopenia, leukocytosis, increased LAP-score and slight splenomegaly. The histology of CIMF-0 slight dysmorphic megakaryopoiesis may overlap with that of true ET in cases of very mild hyperplasia of granulopoiesis and/or a mixture of mild dysmorphic megakaryocytes and mature enlarged megakaryocytes with hyperploid nuclei. Diagnostic differentiation between true ET, early PV, CIMF-0 at the bone marrow level will be feasible in well equipped pathology laboratories by experienced trained pathologists in the majority of cases, but significant overlap (grey zones) between CIMF-0 with slight dysmegakaryopoiesis versus true ET or early PV due to a rather high interobserver disagreement between hematopathologists in routine daily practice is very likely.

Clinical relevance of ECMP criteria for diagnosis and staging of ET, PV and CIMF

The PVSG-defined ET patients (table 1) comprise three ECMP-defined phenotypes of thrombocythemia at the bone marrow level: true ET, early PV mimicking ET, and CIMF-0 or CIMF-1 without features of leuko-erythroblastosis in the peripheral blood25,70,71. These three ECMP defined ET phenotypes do not differ significantly with regard to peripheral blood features, thrombocytemia related clinical presentation and laboratory findings during long-term follow-up (table 7)70,71. Therefore, patients with true ET and early PV and CIMF-0 mimicking ET are to be treated equally based on clinical risk stratification for thrombotic and bleeding complications irrespective of bone marrow features56. The relevance of recognition of CIMF-0 and CIMF-1 lies in the increased risk of myelofibrotic transformation and decreased prognosis in terms of survival compared to true ET70,71. The prognostic importance of the WHO/ECMP criteria is demonstrated in a large retrospective study of 476 PVSG-defined ET patients (platelet count >600 x10^9/l), who were reclassified according to the WHO bone marrow criteria: true ET in 167, CIMF-0 in 174 and CIMF-1 in 13571. Mean age of true ET patients was 59 years, which is 8 to 10 years younger compared to CIMF-0 (67 years) and CIMF-1 (69 years) patients. The differences in relative 10 years survival rates: 99 ± 7.8% for true ET, 81 ± 11.7% for CIMF-0, and 67 ±17.8% for CIMF-1 patients, are significant due to an increased risk of myelofibrosis and splenomegaly during follow-up. In this retrospective “one-center-study” the majority of CIMF-0 patients have early stage disease without features of leuko-erythroblastosis (table 7), whereas CIMF-1 patients are a mixture of early and intermediated stage disease without and with leuko-erythroblastosis71 (as defined in tables 1 and 3 when applying ECMP criteria). The ECMP criteria for diagnosis and staging of CIMF patients do clearly separate early stage CIMF-0 and CIMF-1 without leuko-erythroblastosis from intermediate stage CIMF-1 and CIMF-2 with leuko-erythroblastosis of the peripheral blood (table 3)72,73. Clinicians should realise that the overall survival curves of patients with early stage CIMF-0 or CIMF-1 and no leuko-erythroblastosis will be still as good as for newly diagnosed PV patients, and that the life expectancy of patients with CIMF-2 and CIMF-3 with leuko-erythroblastosis, splenomegaly and anemia will be significantly shortened (table 7).

A recent report of 116 PVSG-defined ET patients and reclassified based on WHO bone marrow cri-
teria confirmed that such cohort of patients with the clinical picture of ET in fact comprises true ET in 19%, CIMF-0 in 21%, CIMF-1 in 37%, CIMF-2 in 12%, early PV in 8%, and unclassified MPD in 3%74. Median age of true ET and CIMF-0 patients was 54 and 52 years respectively, which is 7 to 14 years younger compared to CIMF-1 (59 years) and CIMF-2 (66 years), which points to the unexplored question whether true ET will progress to PV of CIMF after very long-term follow-up74. Thromboembolic events were equally frequent in ET, CIMF-0 and 1, but relevant life threatening events including acute myeloid leukemia, advanced CIMF and second malignancies were more frequent in CIMF-1 and 2 during long-term follow-up74.

In ECMP-defined true ET, progression into myelofibrosis grade 1 or 2 was not seen five years after diagnosis in two recent studies, but data on very long-term follow-up of more than 10 to 15 years are lacking75,76. Kvasnicka and Thiele calculated that the estimated risk of transformation within 3 years into clinically defined IMF (with anemia, leuko-erythroblastosis and extramedullary hematopoiesis) was 2.2% in PVSG-defined ET and 2.8% in WHO-defined CIMF-0 and -1 (nearly 1% per year)71. In a large series of 195 PVSG-defined ET patients and a median follow-up of 7.2 years, evolution into CIMF-2/3 (classic IMF with anemia, leuko-erythroblastosis and splenomegaly) occurred in 2.7% at 5 years, 8.3% at 10 years and 15% at 15 years (6.7% after a median of 8.3 years)77. In another retrospective study of 322 PVSG-defined ET, the cumulative risks of CIMF-2/3 and leukemia were 3.8% and 1.4% at 10 years, and 19.9% and 8.1% at 20 years respectively28. In these two studies of PVSG-defined ET patients, the overall survival was similar to that of the age-matched control population in the first decade, but significantly worse beyond the first decade of the disease. Applying ECMP criteria to PVSG-defined ET patients at time presentation will separate patients with true ET from early PV and CIMF-0 mimicking ET and therefore surely will become of prognostic importance to distinguish true ET from prefibrotic CIMF-0 and early fibrotic CIMF-1 in the context of new prospective clinical management studies.

Diagnostic work-up of patients with thrombocythemia in various MPD

Clinical features suspicious for thrombocythemia in various MPDs (true ET, early PV and CIMF-0) include a sustained increased platelet counts (>400 x10⁹/l) in the absence of any cause for reactive thrombocytosis20,23,24,72,73. The presence of giant platelets in a peripheral blood smear is indicative for MPD and precludes reactive thrombocytosis. Sustained increase of platelet counts (>400 x10⁹/l) associated with slight splenomegaly on echogram (>12cm), increased leukocytes (>12 x10⁹/l) or LAP score with normal ESR is highly suspicious of myeloproliferative thrombocythemia. Clinical manifestations of thrombocythemia in various MPDs consist of microvascular circulation disturbances including atypical and typical TIAs, ocular ischemic attacks, erythromelalgia, splanchnic or cerebral vein thrombosis41. Clinicians and pathologists should realise that PVSG-defined ET includes true ET, early PV mimicking ET, and thrombocythemia associated with CIMF-0 or CIMF-1 when ECMP criteria are applied (tables 1, 2, 3 and 4). The presence of numerous abnormal enlarged or giant mature megakaryocytes with hyperlobulated nuclei and preserved nuclear/cytoplasmic ratio or the presence of pleomorphic small and enlarged or giant megakaryocytes with hypolobulated cloudlike nuclei, and/or the evidence of several clusters of enlarged megakaryocytes are the pathognomonic clues to the diagnosis of prefibrotic MPD (ET, PV or CIMF)3,19-33. The diagnostic work-up of patients with ET and thrombocythemia associated with CIMF according to ECMP criteria28,72,73 is based on positive criteria in peripheral blood and bone marrow (figure 5). These include:

1. Thrombocythemia patients should fulfil the peripheral blood (clinical) criteria for the diagnosis of thrombocythemia irrespective of bone marrow features (tables 2 and 3).

2. The screening for JAK2V617F as a first intention diagnostic test is very helpful in the diagnostic work-up of patients with suspected thrombocythemia in various MPDs, but only half of ET and CIMF patients carry this mutation.

3. Pretreatment bone marrow biopsy will allow clinicians and pathologists to diagnose the early stages of MPDs including JAK2V617F positive and JAK2 wild type thrombocythemia. The ECMP criteria classify the PVSG-defined ET (table 1) as: true ET (table 2); early PV mimicking ET (table 4); early stage CIMF-0 or CIMF-1 without features of leukoerythroblastosis and extramedullary hematopoiesis (table 3); and intermediate CIMF-1, 2 and 3 with features of leucoerythroblastosis79,80 (table 7).

4. The ECMP criteria distinguish thrombocythemia in various MPDs from thrombocythemia...
associated with Ph1-chromosome and bcr/abl positive chronic myeloid leukemia (CML) or myelodysplastic syndromes (MDS) including the so-called 5q-syndrome, which clearly differs from refractory anemia with ringed sideroblasts and significant thrombocytosis (RARS-T) (figure 5). Among 9 RARS-T patients in a recent study, 6 showed the presence of JAK2V617F mutation.

Comparing the laboratory features of JAK2V617F positive (in granulocytes) and JAK2 wild type PVSG-defined ET patients in the PT-1 study showed that JAK2V617F positive ET is characterized by higher values for hemoglobin, hematocrit, neutrophil counts, LAP score, by lower values for serum EPO levels, serum ferritin and MCV, and by increased cellularity of the bone marrow in biopsy material. This observation confirms the ECMP concept that JAK2V617F and EEC positive ET patients represent an early PV mimicking ET (“forme fruste” PV, stage 1 PV, table 5) 24,56,72,73. As compared to JAK2V617F positive ET (early PV), JAK2 wild type ET patients had significantly higher platelet counts, normal serum EPO levels, a typical bone marrow picture of true ET, no features of early PV, and are at lower risk for the development of thrombotic complications. These data are in line with the hypothesis that JAK2V617F positive and JAK2 wild type ET patients at diagnosis represent two distinct entities with a related pathophysiology in the JAK-2/STAT signalling pathway but different molecular etiology similar to the gain of function mutation in the TPO or MPL genes causing hereditary ET.

Diagnostic work-up of patients with polycythemia vera

Suspected polycythemia (PV) with characteristic PV features include increased hematocrit (>0.51), increased erythrocytes (>6 x10^12/l), slight splenomegaly, increased leukocytes (>12 x10^9/l) or LAP score with normal ESR, increased platelets (>400 x10^9/l). PV patients usually show the presence of large platelet in peripheral blood smear. PV patients frequently present with headache, TIAs, erythromelalgia, splenchnic or cerebral vein thrombosis and microcytosis of erythrocytes due to iron deficiency. Patients with congenital or acquired erythrocytosis lack the clinical and laboratory features of MPD are usually asymptomatic. The presence of JAK2V617F has a sensitivity of about 95% and positive predictive value of 100% for the diagnosis of PV in the context of absolute erythrocytosis (hematocrit >0.51 in males and >0.48 in females) and excludes congenital and secondary erythrocytosis (figure 6). Subsequent red cell mass measurement will distinguish apparent from absolute erythrocytosis but does not differentiate between PV and congenital or secondary erythrocytosis. In contrast, bone marrow histology not only differentiates trilineal hypercellularity in PV (figures 2 and 3) from isolated increase of erythropoiesis (figure 3) in congenital polycythemia and secondary erythrocytosis, but also significantly contributes to phenotyping and staging of PV patients.

The detection of JAK2V617F in granulocytes with sensitive PCR techniques as to play a key-role as a first intention diagnostic test for erythrocytosis (hematocrit >0.51), because it simplifies the diagnostic work-up of PV (table 4, figure 6). The presence of the JAK2V617F mutation combined with increased hematocrit (>0.51), and EEC or low serum EPO is diagnostic for PV without the need of red cell mass measurement (table 4), but is not enough to define the broad spectrum of PV phenotypes according to EMCP criteria (table 6). Since EEC is time consuming and difficult to establish in many (non-specialized) laboratories, clinicians and pathologists tend to replace it by the wide spread available bone marrow histology assessment as a gold standard criterion for the diagnosis of PV and its differentiation from the EMCP-defined true ET and prefibrotic or early fibrotic CIMF (tables 3 and 4, figures 2 and 3).

Comparing 45 JAK2V617F heterozygous and 13 homozygous PV patients showed that homozygote JAK2V617F PV patients displayed significantly higher hemoglobin at time of diagnosis, increased incidence of pruritus, higher PRV-1 transcripts in their blood granulocytes, and higher rate of fibrotic transformation. These observations indicate that pretreatment and follow-up bone marrow histology examinations will be helpful for proper staging of PV patients for reasons of prognosis assessment and therapeutic implications (table 6). In the context of a prospective clinical study to discriminate between early and advanced PV and to monitor disease progression to post-PV myelofibrosis, bone marrow histology, cytogenetic analysis and JAK2V617F mutation detection in granulocytes, and EEC should always be performed.

Grading of myelofibrosis in myeloproliferative disorders (MPD)

Myelofibrosis (MF) itself is not a disease because reticulin and collagen fibrosis are produced by pol-
yclonal fibroblasts as the consequence of cytokines released from the clonal granulocytic and megakaryocytic proliferative cells in both PV and CIMF\textsuperscript{19-97}. The Baumeister scoring system of MF was developed on aspirated bone marrow samples, but proved to be not reliable for the proper grading of myelofibrosis in bone marrow biopsies by pathologist (table 8)\textsuperscript{98}. The Manoharan system used silver stain according to Gordon and Sweet and scored the degree of reticulin in bone marrow biopsy in a completely different (table 8)\textsuperscript{99}. A scoring system based on morphometric analysis (point intersection with an ocular grid) and quality of fibers (reticulin and collagen fibers) and the bone marrow fiber density (fine or course reticulin and some course bundles of collagen) have been proposed by Georgii et al\textsuperscript{19,63,64} and Thiele et al\textsuperscript{65-68}. All these different scoring systems for MF use different criteria for grading of reticulin and collagen, are subjective and not comparable by lack of strict criteria. A panel of experienced European pathologists and a USA expert reached a consensus on how to grade bone fibrosis in bone marrow biopsies of patients with CIMF or PV (EC, tables 3 and 8)\textsuperscript{100}. Grading of MF was simplified by using four easily reproducible categories that included differentiation between reticulin and collagen\textsuperscript{100}. According to defined standardized semiquantitative grading of reticulin and collagen fibrosis in the bone marrow, MF can reliably be graded at the pathological bone marrow level as 0 in prefibrotic, as 1 in early fibrotic, as 2 in classical fibrotic and as 3 in classi-

Conclusion: The diagnosis CIMF according to WHO bone marrow features does not distinguish between CIMF-0/CIMF-1 without leuko-erythroblastosisc versus CIMF-1/CIMF-2 with leuko-erythroblastosis when ECMP criteria are applied (tables 3 and 7) indicating the need to use clinical score assessment on top of bone marrow features for prognosis prediction.

References


