MPN – What's new in the morphological classification, grading of fibrosis and the impact of novel drugs

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Disclosure of speaker’s interests

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Research funding & Consulting: Novartis, Incyte
the WHO classification emphasizes the identification of distinct clinicopathological entities, rather than just being a "cell of origin" classification

stresses an “integrated approach” to disease definition by incorporation of key available information including morphology, molecular and cytogenetic findings, immunophenotype, and clinical features

the work of a large number of hematopathologists, but developed with the active advice and consent of clinicians
Modification of the *BCR/ABL*-negative MPN classification according to WHO 2016/2017

- **Polycythemia vera (PV)**
  - lowering of hemoglobin or hematocrit thresholds
  - role of BM morphology as a major criterion

- **Essential thrombocythemia (ET)**
  - differentiation of "true ET" from prefibrotic/early primary myelofibrosis (prePMF)
  - emphasizing the lack of reticulin fibrosis at onset

- **Primary myelofibrosis (PMF)**
  - definition of minor clinical criteria in prePMF
  - histomorphological features of prePMF

- inclusion of new molecular findings
Overlapping clinical and molecular features in MPN

- **PMF**
  - Anemia
  - ↑ LDH
  - Leukoerythroblastosis
  - Megakaryocytic and granulocytic proliferation & myelofibrosis

- **CALR or MPL**
  - Clonal marker

- **ET**
  - ↑ PLTs
  - Predominant megakaryocytic proliferation without atypia

- **PV**
  - Trilineage proliferation (panmyelosis)

- **JAK2**
  - Splenomegaly
  - Symptoms
  - ↑ Hb
  - ↑ HCT
  - ↓ EPO
Survival and impact of age at diagnosis in MPN

## WHO 2016/2017 criteria for MPN

<table>
<thead>
<tr>
<th></th>
<th>PV</th>
<th>ET</th>
<th>prePMF</th>
<th>PMF</th>
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<tbody>
<tr>
<td><strong>Major criteria</strong></td>
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<tr>
<td></td>
<td>- Hb &gt; 16.5 g/dL in men, Hb &gt; 16.0 g/dL in women OR, Hct &gt; 49% in men, Hct &gt;48% in women OR, increased red cell mass</td>
<td>- PLT ≥ 450 x 10⁹/L</td>
<td>- BM biopsy with megakaryocytic proliferation and atypia, without reticulin fibrosis &gt;grade 1</td>
<td>- BM biopsy with megakaryocytic proliferation and atypia, reticulin and/or collagen fibrosis grade 2/3</td>
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<tr>
<td></td>
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<td></td>
<td>- not meeting WHO criteria for other MPN subtype</td>
<td>- not meeting WHO criteria for other MPN subtype or MDS, or other</td>
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<tr>
<td></td>
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<td></td>
<td>- JAK2, CALR or MPL mutation</td>
<td>- JAK2, CALR or MPL mutation or presence of other clonal markers* or absence of reactive myelofibrosis**</td>
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<tr>
<td></td>
<td>- BM biopsy showing trilineage proliferation (panmyelosis)</td>
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<td></td>
<td>- JAK2 mutation</td>
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<tr>
<td><strong>Minor criteria</strong></td>
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<td>at least one of the following:</td>
<td>at least one of the following:</td>
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<tr>
<td></td>
<td>- subnormal serum EPO level</td>
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<td>a. anemia</td>
<td>a. anemia</td>
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<td></td>
<td>b. leukocytosis &gt;11K/uL</td>
<td>b. leukocytosis &gt;11K/uL</td>
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<td></td>
<td>c. splenomegaly</td>
<td>c. splenomegaly</td>
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<td>d. LDH increase</td>
<td>d. LDH increase</td>
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<td></td>
<td></td>
<td>e. leukoerythroblastosis</td>
<td>e. leukoerythroblastosis</td>
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<tr>
<td><strong>Diagnosis</strong></td>
<td>all three major criteria, or the first two major criteria and the minor criterion</td>
<td>all four major criteria or the first three major criteria and one of the minor criteria</td>
<td>all three major criteria, and minor criteria</td>
<td>all three major criteria, and minor criteria</td>
</tr>
</tbody>
</table>

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

**Bone marrow fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

The current genetic landscape concerning phenotypic driver mutations in MPN

**ET**
- JAK2 (60%)
- CALR (20%-25%)
- MPL (3%-6%)

**PMF**
- JAK2 (60%)
- CALR (20%-30%)
- MPL (5%-8%)

**PV**
- JAK2 (>95%)

All MPN-associated mutations directly (JAK2), indirectly (MPL) or through complex mechanisms (CALR) result in abnormal activation of JAK/STAT and other signaling pathways.

**WHO criteria for Polycythemia vera (PV)**

**Major criteria:**
1. Hb $> 16.5$ g/dL in men, Hb $> 16.0$ g/dL in women OR, Hct $> 49\%$ in men, Hct $> 48\%$ in women OR, Increased red cell mass
2. **Bone marrow biopsy** showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic and megakaryocytic proliferation with pleomorphic megakaryocytes (differences in size)
3. Presence of **JAK2** mutation

**Minor criterion:**
Subnormal serum EPO level

Diagnosis of PV requires meeting either all three major criteria, or the first two major criteria and the minor criterion

Arber et al., Blood (2016) 127:2391-2405
WHO criteria for Polycythemia vera (PV)

**Major criterion:**

Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
WHO 2016 criteria for PV

- In cases with sustained absolute erythrocytosis (Hb levels >18.5 g/dL, Hct >55.5 % in men or >16.5 g/dL, 49.5% in women, bone marrow biopsy may not be necessary for diagnosis if major criterion 3 and the minor criterion are present.

- However, only by performing a bone marrow biopsy an initial myelofibrosis (up to 20%) may be detected that indicates a more rapid progression to overt myelofibrosis (post-PV MF).
Progression in PV stratified by the presence of initial BM fibrosis at diagnosis

Barraco et al., Blood Cancer J (2017) 7:e538

<table>
<thead>
<tr>
<th>Grade at diagnosis</th>
<th>Incidence per 100 pts./yrs.</th>
<th>cumulative incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 yrs.</td>
<td>10 yrs.</td>
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<tr>
<td>MF-0</td>
<td>0.8</td>
<td>1.3</td>
</tr>
<tr>
<td>≥ MF-1</td>
<td>2.2</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Progression to overt MF (post-PV MF)
JAK2 allele burden in PV correlates with risk of transformation to post-PV MF and blast phase (sAML)

Passamonti et al., Leukemia (2010) 24:1574-1579
Silver et al., Leuk Res (2011) 35:177-182
The rate of transformation from JAK2-mutated ET to PV is influenced by an accurate WHO-defined clinico-morphological diagnosis.

- ET JAK2+ [n=268] 5%
- ET wt [n=422] 1%

Barbui et al., Leukemia, 2015, 29:992-993
Young patients (< 40 years) with early PV have a high incidence of thrombosis

- might be associated with less aggressive treatment
- less frequent use of phlebotomies and cytoreduction in early PV
- identification of early PV in young JAK2-mutated patients is important in order to reduce risk and optimize treatment

### Incidence of Thrombosis

<table>
<thead>
<tr>
<th>Incidence of Thrombosis</th>
<th>ET JAK2 pos</th>
<th>early PV</th>
<th>overt PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR % pts/yr</td>
<td>1.36 (0.99 - 1.88)</td>
<td><strong>3.01 (1.75 - 5.18)</strong></td>
<td>1.99 (1.22 - 3.25)</td>
</tr>
<tr>
<td>risk at 5 years</td>
<td>0.06 (0.04 - 0.09)</td>
<td><strong>0.11 (0.04 - 0.24)</strong></td>
<td>0.08 (0.03 - 0.16)</td>
</tr>
<tr>
<td>risk at 10 years</td>
<td>0.15 (0.11 - 0.21)</td>
<td><strong>0.28 (0.16 - 0.47)</strong></td>
<td>0.23 (0.14 - 0.37)</td>
</tr>
</tbody>
</table>

Dynamics of the disease process in PV

**Early PV** → **Manifestation** → **Transformation**

- **Evolution of high-risk disease features**
  - increased need for phlebotomy
  - leukocytosis, thrombocytosis
  - splenomegaly
  - symptoms
  - thrombotic events

- **Resistance/intolerance to standard therapy (HU)**
  - increased need for phlebotomy
  - leukocytosis, thrombocytosis
  - progressive splenomegaly
  - cytopenia
  - burdensome symptoms

- **~10%-15% mimicking “ET”**
- **JAK2 + EPO ↓↓**
- **Splenomegaly & BM Fibrosis**
  - 10 - 15 yrs.

- **post-PV MF (MF-2/3)**
  - ~ 20%
- **post-PV MF with blastic transformation**
  - < 10%
HU Resistance/Intolerance and Disease Progression in PV

- resistance and intolerance to HU occurs in 11%-13%
- resistance to HU is associated with a 5.6-fold increase in the risk of death (HR, 5.6; 95% CI, 2.7%-11.9%; p < 0.001)
- resistance to HU is associated with an 6.8 fold increased risk of transformation into acute leukemia or myelofibrosis (HR, 6.8; 95% CI, 3.0%-15.4%; p < 0.001)
- both resistance to HU and lack of WBC response are prognostically important

Alvarez-Larrán et al., Blood 2012;119:1363-1369
BM morphology in progressive PV at time of treatment failure (HU resistance/intolerance)

Post-PV myelofibrosis (MF)

Heterogeneity of BM features

- MDS like features
- Increased blast count
- Toxic hypoplasia

Kvasnicka et al., USCAP 2017
WHO criteria for essential thrombocythemia (ET)

**Major criteria:**

1. Platelet count equal to or greater than $450 \times 10^9$/uL
2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis and very rarely minor increase in reticulin fibers.
3. Not meeting WHO criteria for BCR-ABL1+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of JAK2, CALR or MPL mutation

**Minor criteria:**

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

**Diagnosis of ET requires meeting all four major criteria or the first three major criteria and one of the minor criteria**

Arber et al., Blood (2016) 127:2391-2405
WHO criteria for ET

Major criterion

**Bone marrow biopsy** showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left-shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor increase in reticulin fibers.
CALR mutated ET patients have lower rates of thrombosis

CALR mutated and ‘wild-type’ patients may be at a very low risk of thrombosis, and the effect of the CALR mutation may be particularly evident in younger patients.

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>JAK2</td>
<td>1.78</td>
<td>1.06-3.18</td>
</tr>
<tr>
<td>MPL</td>
<td>1.65</td>
<td>1.70-3.92</td>
</tr>
<tr>
<td>CALR</td>
<td>0.74</td>
<td>0.33-1.00</td>
</tr>
</tbody>
</table>

P-value = 0.004
P-value adjusted for age = 0.02
P-value adjusted for thrombosis history = 0.01
P-value adjusted for age, CV risk and thrombosis history = 0.02

Morphological criteria (major criteria) for the diagnosis of prePMF and ET according to WHO

<table>
<thead>
<tr>
<th>ET</th>
<th>prePMF</th>
</tr>
</thead>
</table>

![Image of morphological criteria for ET and prePMF]
WHO criteria for prePMF

**Major criteria:**
1. Megakaryocytic proliferation and atypia, **without reticulin fibrosis > grade 1** and accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation and often decreased erythropoiesis
2. Not meeting WHO criteria for ET, PV, BCR-ABL1+ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2, CALR* or *MPL* mutation or in the absence of these mutations, presence of an other clonal marker* or absence of minor reactive myelofibrosis**

* in the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (**ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, SF3B1**) are of help in determining the clonal nature of the disease.
**bone marrow fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

Arber et al., Blood (2016) 127:2391-2405
WHO criteria for prePMF

Megakaryocytic proliferation and atypia
(small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei and dense clustering)

Histopathology of hematopoiesis
Megakaryocyte changes are accompanied by an increased age-adjusted bone marrow cellularity, granulocytic proliferation and often decreased erythropoiesis

(reticulin fibrosis grade 0 or 1)
[prePMF / MF-0]
[prePMF / MF-0]
[prePMF / MF-0]
Morphological features helpful in distinguishing ET from prePMF*

<table>
<thead>
<tr>
<th></th>
<th>ET</th>
<th>prePMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity§</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>M/E ratio</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Megakaryocyte dense clusters $$</td>
<td>Rare</td>
<td>Frequent</td>
</tr>
<tr>
<td>Megakaryocyte size</td>
<td>Large</td>
<td>Variable</td>
</tr>
<tr>
<td>Megakaryocyte nuclear lobulation</td>
<td>Hyperlobated</td>
<td>Bulbous/hypolobated</td>
</tr>
<tr>
<td>Reticulin fibrosis grade 1**</td>
<td>Rare</td>
<td>More frequent</td>
</tr>
</tbody>
</table>

* based on a representative BM biopsy (> 1.5 cm)
§ age matched cellularity
** according to WHO grading

$$ WHO definition of a megakaryocyte cluster: 3 or more megakaryocytes lying strictly adjacent - without other hematopoietic cells lying in between
## Frequency of minor criteria in 954 patients with prePMF and ET

<table>
<thead>
<tr>
<th>Parameter</th>
<th>cut-off</th>
<th>prePMF [n=706]</th>
<th>ET [n=248]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>M $\leq$ 13 g/dL F $\leq$ 12 g/dL</td>
<td>36.4 %</td>
<td>22.6 %</td>
</tr>
<tr>
<td>Spleen</td>
<td>$\geq$ 1 cm</td>
<td>43.6 %</td>
<td>26.6 %</td>
</tr>
<tr>
<td>LDH</td>
<td>$\geq$ 220 U/L</td>
<td>84.4 %</td>
<td>45.2 %</td>
</tr>
<tr>
<td>Blasts</td>
<td>$\geq$ 1 % (Myeloblasts + Erythroblasts)</td>
<td>6.2 %</td>
<td>1.2 %</td>
</tr>
<tr>
<td>WBC</td>
<td>$\geq$ 11 x 10^9/L</td>
<td>51.3 %</td>
<td>33.1 %</td>
</tr>
</tbody>
</table>

Kvasnicka et al., unpublished data
WHO criteria for prePMF

**Minor criteria:**

Presence of **at least one** of the following, confirmed in two consecutive determinations:

a. Anemia not attributed to a comorbid condition
b. Leukocytosis >11K/µL
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range

Diagnosis of prePMF requires meeting all three major criteria, and minor criteria.
ET vs. prePMF: Why does it matter?

- Differences in the biology of ET and prePMF
  - prePMF has higher JAK2V617F and MPLW515L allele burden than ET
  - alterations of megakaryocyte differentiation and function in vitro distinguishes prePMF from ET patients
  - high mobilization of circulating endothelial colony forming cells (ECFCs) is characteristic of prePMF
  - different gene signature in prePMF

- Clinical differences between ET and prePMF
  - clinical presentation
  - risk of transformation
  - outcome prediction

- Therapeutic considerations
  - slow-down of disease progression in prePMF with a new molecularly targeted therapy

Barosi, Best Practice & Research Clinical Haematology, 2014, 27, 129-140
Differences in mutation load in WHO defined ET and early PMF (prePMF)

Palandri et al., Leukemia (2015) 29:1344-1349
Survival in ET and prePMF

Relative survival in ET and prePMF

ET and pre-fibrotic MF vs Europe*
Age- and sex-adjusted actuarial survival curves


*EUROSTAT 2008
(crude death rates, all causes of death, EU 27 countries)
Disease progression in ET and prePMF

<table>
<thead>
<tr>
<th>Event</th>
<th>No. of Events</th>
<th>% of Events</th>
<th>Incidence per 100 Patient-Years</th>
<th>IRR</th>
<th>P</th>
<th>5-Year Cumulative Incidence (%)</th>
<th>10-Year Cumulative Incidence (%)</th>
<th>15-Year Cumulative Incidence (%)</th>
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</thead>
<tbody>
<tr>
<td>Thrombosis</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>ET</td>
<td>109</td>
<td>12</td>
<td>1.7</td>
<td>1.1</td>
<td>.57</td>
<td>8.7</td>
<td>16.2</td>
<td>21.5</td>
</tr>
<tr>
<td>Early/prefibrotic PMF</td>
<td>26</td>
<td>15</td>
<td>1.9</td>
<td></td>
<td></td>
<td>6.6</td>
<td>17.9</td>
<td>25.4</td>
</tr>
<tr>
<td>Transformation to overt myelofibrosis</td>
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<td></td>
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<tr>
<td>ET</td>
<td>32</td>
<td>4</td>
<td>0.5</td>
<td>2.0</td>
<td>.04</td>
<td>0.2</td>
<td>0.8</td>
<td>9.3</td>
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<tr>
<td>Early/prefibrotic PMF</td>
<td>14</td>
<td>8</td>
<td>1</td>
<td></td>
<td></td>
<td>2.3</td>
<td>12.3</td>
<td>16.9</td>
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<tr>
<td>Leukemic transformation</td>
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<td></td>
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<tr>
<td>ET</td>
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<td>1</td>
<td>0.1</td>
<td>5.2</td>
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<td>0.2</td>
<td>0.7</td>
<td>2.1</td>
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<tr>
<td>Early/prefibrotic PMF</td>
<td>9</td>
<td>5</td>
<td>0.6</td>
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<td></td>
<td>1.5</td>
<td>5.8</td>
<td>11.7</td>
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<td>Death</td>
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</tr>
<tr>
<td>ET</td>
<td>87</td>
<td>10</td>
<td>1.3</td>
<td>2.1</td>
<td>.0002</td>
<td>3.0</td>
<td>14.8</td>
<td>24.6</td>
</tr>
<tr>
<td>Early/prefibrotic PMF</td>
<td>40</td>
<td>22</td>
<td>2.7</td>
<td></td>
<td></td>
<td>8.6</td>
<td>24.4</td>
<td>56.1</td>
</tr>
</tbody>
</table>

International Study on 1,104 Patients

Transformation to overt MF

Risk of leukemic transformation

Barbui et al., J Clin Oncol. 2011;29:3179-3184
Risk of thrombosis and bleeding in prePMF and ET

- Major bleeding associated with thrombocytosis is more often seen in prePMF.
- Low-dose aspirin exacerbates these hemorrhagic events.
- Venous thrombosis (mainly atypical, i.e. splanchnic & mesenterial) are more common in prePMF.
- Higher leukocyte count in prePMF correlates with an increased risk for arterial thrombosis.

**Risk of thrombosis**

- **prePMF**
  - Cumulative risk of thrombosis (at 15-years): 48%
  - p = 0.032

- **ET**
  - Cumulative risk of thrombosis (at 15-years): 17%

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Finazzi et al., Leukemia (2012) 26:716-719
Buxhofer-Ausch et al., Am J Hematol (2012) 87:669-672
Rupoli et al., Diagn Pathol (2015) 10:29
Features of prePMF and its distinction from ET

- higher JAK2 V617F allele burden
- higher numbers of circulating progenitor cells
- higher rate of progression to overt MF and greater incidence of blast phase and worse survival

- more bleeding at high platelet counts
- increased frequency of thrombotic events, particularly arterial
- factors associated with thrombosis in prePMF:
  - Platelet count > 450 x 10^9/L
  - Presence of ≥ 1 cardiovascular risk factor
  - Age > 60 y
  - JAK2 mutation
  - prior thrombosis

PrePMF: 3.43%
ET: 1.29%
patients/year, P=0.01
WHO criteria for overt PMF

Major criteria:
1. Presence of megakaryocytic proliferation and atypia, usually accompanied by either reticulin and/or collagen fibrosis grades 2 or 3
2. Not meeting WHO criteria for ET, PV, BCR-ABL1+ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of \textit{JAK2}, \textit{CALR} or \textit{MPL} mutation or in the absence of these mutations, presence of an other clonal marker* or absence of reactive myelofibrosis**

* in the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (\textit{ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, SF3B1}) are of help in determining the clonal nature of the disease.
** bone marrow fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

Arber et al., Blood (2016) 127:2391-2405
WHO criteria for overt PMF

Minor criteria:

Presence of at least one of the following, confirmed in two consecutive determinations:

a. Anemia not attributed to a comorbid condition
b. Leukocytosis >11K/uL
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
e. Leukoerythroblastosis

Diagnosis of overt PMF requires meeting all three major criteria, and minor criteria.
overt PMF (MF-3)

heterogeneity of morphological presentation
overt PMF (PMF-3)

New WHO grading for
- collagen deposition
- osteosclerosis
Grading of Fibrosis (according to WHO 2016)

**MF-0**  Scattered linear reticulin with no intersections (cross-overs) corresponding to normal bone marrow

**MF-1**  Loose network of reticulin with many intersections, especially in perivascular areas

**MF-2**  Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibers consistent with collagen and/or focal osteosclerosis

**MF-3**  Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of thick fibers consistent with collagen, usually associated with osteosclerosis

Arber et al., Blood (2016) 127:2391-2405
Assessment of myelofibrosis after therapy

- Fiber density should be assessed only in hematopoietic areas.
- Areas of prominent scleredema and/or scarring should be included in the overall grading of myelofibrosis.
- If the pattern is heterogeneous, the final score is determined by the highest grade present in at least 30% of the marrow area.
- Quality of the reticulin stain (Gomori or Gordon & Sweets) should be assessed by detection of normal staining in vessel walls as internal controls.
- Disregard lymphoid nodules and vessels and fibers framing adipocytes.

Thiele et al., Haematologica 2005
ELN consensus 2013
Problems of Reticulin Staining

Kvasnicka et al., Histopathology, 2016, 68:905-915

Pitfalls in grading of bone marrow fibrosis, collagen deposition, and osteosclerosis

<table>
<thead>
<tr>
<th>A. Reticulin</th>
<th>B. Collagen</th>
<th>C. Osteosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crushing artefacts and squeezing</td>
<td>Crushing artefacts and squeezing</td>
<td>Subcortical bias</td>
</tr>
<tr>
<td>Thickness of section (overestimation of fibers in thick sections)</td>
<td>Weak staining (lack of internal perivascular control)</td>
<td>Tangential section of cortical bone</td>
</tr>
<tr>
<td>Quality of staining (fragmentation of fibers)</td>
<td>Overstaining (nonspecific blue cytoplasm of haematopoietic cells, in particular megakaryocytes)</td>
<td>Squeezing and/or fragmentation of bone structure</td>
</tr>
<tr>
<td>Overstaining (distinct framing of adipocytes, black or grey background)</td>
<td>Van Gieson staining (visual underestimation of collagen)</td>
<td>Unrelated changes of bone structure (age dependent thickness of trabeculae does not impact grading)</td>
</tr>
<tr>
<td>Weak staining (lack of internal perivascular control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quality of silver nitrate solution (precipitation with occurrence of droplets)</td>
<td></td>
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</tr>
</tbody>
</table>
Grading of collagen

Grading of osteosclerosis

Grade 0  normal bone trabeculae (distinct paratrabecular borders)
Grade 1  focal budding, hooks, spikes or paratrabecular apposition of new bone
Grade 2  diffuse paratrabecular new bone formation with thickening of trabeculae, occasionally with focal interconnections
Grade 3  extensive interconnecting meshwork of new bone with overall effacement of marrow spaces

Comment on staining and grading:
- bone marrow core biopsy taken at right angle from the cortical bone of sufficient length (> 1.5 cm) without fragmentation
- H&E or silver impregnation

EUN consensus 2013
Grading of osteosclerosis

Grade 0
normal bone trabeculae (distinct paratrabecular borders)

Grade 1
focal budding, hooks, spikes or paratrabecular apposition of new bone

Grade 2
diffuse paratrabecular new bone formation with thickening of trabeculae, occasionally with focal interconnections

Grade 3
extensive interconnecting meshwork of new bone with overall effacement of marrow spaces

Comment on staining and grading:
- bone marrow core biopsy taken at right angle from the cortical bone of sufficient length (> 1.5 cm) without fragmentation
- H&E or silver impregnation
Comparison of survival among patients with PMF stratified by their mutational status

Prognostic implication of “triple-negative” cases

Prognostic implication of calreticulin type 1 or type 1-like variants

CALR type 1: 52-bp deletion (CALRdel52)
CALR type 2: 5-bp insertion (CALRins5)

Tefferi et al., Blood, 2014, 124, 2465-2466
Tefferi et al., Blood, 2014, 124, 2507-2513
Tefferi et al., Abstract #2801, ASH 2015
Kourie et al., Br J Haematol. 2016; DOI: 10.1111/bjh.14259
Number of somatically acquired mutations impacts prognosis in PMF

Presence of adverse variants/mutations (ASXL1, SRSF2, CBL, KIT, RUNX1, SH2B3, CEBPA)

Number of adverse variants/mutations

Vannucchi et al., Leukemia, 2013, 27:1861–1869
Guglielmelli et al., Leukemia, 2014, 28:1804-1810
Tefferi et al., Blood Advances (2016) 1:105-111
The type and the number of mutations determine the phenotype of PMF

- proliferation is driven mainly by signaling mutations (JAK2, CALR, and MPL) while most of the mutations in epigenetic regulators and spliceosome components lead to differentiation defects
- the heterogeneity of the disease and its prognosis are dependent on the respective levels of additional mutations

Vainchenker et al., F1000Res. 2016:5
Clinical impact of bone marrow fibrosis in PMF

- degree of fibrosis and its changes over time in patients with myelofibrosis is an area of ongoing research
- standard pharmacotherapy like HU has not been shown to result in bone marrow fibrosis improvement
- recent data suggest that degree of BM fibrosis is an independent prognostic factor in PMF in multivariate analysis

Survival and BM Fibrosis

<table>
<thead>
<tr>
<th>Survival</th>
<th>Relative survival N=726</th>
<th>5 yrs.</th>
<th>10 yrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMF-0</td>
<td>178</td>
<td>91</td>
<td>78</td>
</tr>
<tr>
<td>PMF-1</td>
<td>281</td>
<td>85</td>
<td>71</td>
</tr>
<tr>
<td>PMF-2</td>
<td>128</td>
<td>81</td>
<td>67</td>
</tr>
<tr>
<td>PMF-3</td>
<td>139</td>
<td>60</td>
<td>35</td>
</tr>
</tbody>
</table>

Kvasnicka & Thiele, Semin Thromb Hemost, 2006, 32, 219-230
IPSS and Grading of MF in PMF

The WHO grading of BM fibrosis significantly impacts clinical based prognostication of patients with PMF

<table>
<thead>
<tr>
<th>Hazard ratios for combined IPSS and MF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>MF-0</td>
</tr>
<tr>
<td>MF-1</td>
</tr>
<tr>
<td>MF-2</td>
</tr>
<tr>
<td>MF-3</td>
</tr>
</tbody>
</table>

Gianelli U et al, Mod Pathol 2012; Vener et al, Blood 2008
Prognostic impact of bone marrow fibrosis in PMF

- Impact of BM fibrosis is significant in low & int-1 IPSS risk categories

Prognostic impact of bone marrow fibrosis in PMF

- no correlation between fibrosis grade and phenotypic driver mutations
- frequency of HMR pts increased progressively with grade of fibrosis
- significant association between grade of fibrosis and ASXL1 and EZH2
Disease evolution in Myelofibrosis

Evolution → Manifestation → Transformation

**Initial stage**
- pronounced thrombocytopenia
- no blasts
- no or borderline anemia
- no or borderline splenomegaly
- normal or borderline increased LDH

**Bone marrow fibrosis / Osteosclerosis**
- decrease in platelet counts
- leukoerythroblastosis
- anemia
- splenomegaly
- increased LDH

**Clinical presentation**

**Grade of myelofibrosis**
- MF-0
- MF-1
- MF-2
- MF-3

**Morphological progression**

† Bone marrow fibrosis / Osteosclerosis
† Accumulation of dysplastic features *(MDS-like morphology)*

?? JAK2 allele burden
?? Other Mutations
- ASXL1, SRSF2,
- EZH2, TET2, DNMT3A,
- CBL, IDH1/2

Complex karyotype
Acquired new mutations

Progression of MF
<table>
<thead>
<tr>
<th>BM changes in PMF following HU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control of Myeloproliferation</td>
</tr>
<tr>
<td>Regression of BM fibrosis</td>
</tr>
<tr>
<td>Molecular response</td>
</tr>
<tr>
<td>Associated risk of MDS/AML</td>
</tr>
</tbody>
</table>
**Baseline BM fibrosis**
- MF grade 3
- osteosclerosis grade 3
- variable cellularity

**192 weeks post ruxolitinib**
- major morphological remission
- normal cellularity
- BM fibrosis grade 0
BM changes in PMF following JAK Inhibitors

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Control of Myeloproliferation</td>
<td>++</td>
</tr>
<tr>
<td>Regression of BM fibrosis</td>
<td>&gt;30%</td>
</tr>
<tr>
<td>Molecular response</td>
<td>11%</td>
</tr>
<tr>
<td>Associated risk of MDS/AML</td>
<td>-/-</td>
</tr>
</tbody>
</table>
Molecular Risk & Time to Treatment Failure in Patients Treated with a JAK Inhibitor

- Spleen response (≥50%) was inversely correlated with the number of mutations.
- Patients with ≥3 mutations also had a shorter time to treatment discontinuation and shorter overall survival than those with fewer mutations.

**Patel et al., Blood (2015) 126:790-797**
Summary and Conclusion

- in PV morphology of the BM is up-graded to a major criterion and the diagnostic thresholds for Hb and Hct were lowered
- disease progression in PV is heterogeneous and significantly associated with HU resistance
- in ET differentiation from prePMF is underscored by standardization of BM features and by the lack of reticulin fibrosis (< 5%) at onset
- in prePMF minor clinical criteria are more clearly defined
- number of somatically acquired mutations impacts prognosis in PMF

- in addition to clinical data and mutation profile, BM findings will be maintained as major diagnostic criteria