BCR/ABL1-Negative Classical Myeloproliferative Neoplasms

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Outline

• Overview of various types of classical myeloproliferative neoplasms (MPNs), their clinical implications
• Diagnosing these MPNs, case based approach
• Proposed changes to WHO 2008 criteria
• Role of molecular testing

Classical Myeloproliferative Neoplasms

– Polycythemia Vera (PV)
  • Masked PV
– Primary Myelofibrosis (PMF)
  • Early/prefibrotic PMF
– Essential Thrombocythemia (ET)

– They all share propensity for thrombotic complications and disease progression including:
  • Myelofibrotic progression with development of extramedullary hematopoiesis
  • Leukemic transformation

Disclosure

• Sonam Prakash declares affiliation with Incyte Corporation:
  – Advisor for Hematopathology Publications Steering Committee
Why is this Distinction Important

– When diagnosed based on WHO criteria, these are distinct diseases that differ substantially regarding
  • Prognosis
  • Progression to myelofibrosis (MF)*
    – PV > ET
  • Risk of leukemic transformation
    – PMF > PV (<10% at 20 years) > ET (5% at 20 years)**
  • Risk of thrombosis
    – PV > ET*
– Different treatment modalities (appropriate risk-based therapy based on specific prognostic scoring system for each entity)*
  • PMF: Allogeneic SCT, JAK1/JAK2 inhibitors
  • PV: Phlebotomy, cytoreductive agents, interferons
  • ET: Aspirin, cytoreductive agents

Bone Marrow Morphology

- **Cellularity**
- 3 lineages:
  - Granulocytic : Erythroid ratio
  - Megakaryocytic morphology: distribution (scattered or clustered), individual morphology
  - Blasts
  - Dysplasia
- **Fibrosis:**
  - Reticulin
  - Trichrome
  - Osteosclerosis

Defining Hypercellularity in MPNs on Biopsy Sections

\[(100 - \text{age}) + 10\% = \text{upper limit of normal}\]

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>% Hematopoietic Area (normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>60-70</td>
</tr>
<tr>
<td>40-60</td>
<td>40-50</td>
</tr>
<tr>
<td>&gt;70</td>
<td>30-40</td>
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Megakaryocytic Morphology in MPNs

- ET
  - Scattered, giant (stag-horn)
- PV
  - Pleomorphism, no maturation defects
- PMF
  - Dense clusters, hypolobation (cloud-like), maturation defects, bizarre hyperchromatic nuclei

Grading of Marrow Reticulin Fibrosis (WHO 2008 Criteria)

- **MF-0** Scattered linear reticulin with no intersections (cross-overs) corresponding to normal bone marrow
- **MF-1** Reticulin with many intersections, especially in perivascular areas
- **MF-2** Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of collagen (thick reticulin fibers) and/or focal osteosclerosis
- **MF-3** Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of collagen (thick reticulin fibers), usually associated with osteosclerosis
Comprehensive Assessment of Stroma, Including Reticulin, Collagen and Osteosclerosis at Baseline

<table>
<thead>
<tr>
<th>Reticulin</th>
<th>Trichrome</th>
<th>Osteosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF-0</td>
<td>MF-1</td>
<td>MF-2</td>
</tr>
</tbody>
</table>

Differential Morphologic Features of MPNs in BM Biopsy Sections

<table>
<thead>
<tr>
<th>Feature</th>
<th>ET</th>
<th>Early PMF</th>
<th>PMF</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Cellularity (age matched)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>↑ Granulopoiesis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>↑ Erythropoiesis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>↑ Megakaryopoiesis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dense clusters</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Size: Small</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Giant</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hyperlobation (staghorn)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Hypolobation (cloud-like)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Maturation defects</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Reticulin fibrosis ≥MF-2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>
### Driver mutations in MPN: JAK2 V617F, MPL W515L and Calreticulin (CALR)

<table>
<thead>
<tr>
<th></th>
<th>JAK2</th>
<th>CALR</th>
<th>MPL</th>
<th>Triple Negative</th>
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<tbody>
<tr>
<td>PV</td>
<td>&gt;95%</td>
<td>-</td>
<td>-</td>
<td>Very rare</td>
</tr>
<tr>
<td>PMF and ET</td>
<td>50-60%</td>
<td>20-30%</td>
<td>5-10%</td>
<td>10-15%</td>
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### Case 1
- 45 year old woman presented with DVT
- CBC: Hgb 16.8 g/dL, WBC 5.5 x 10(9)/L, Plts 320 x 10(9)/L
- **JAK2V617F** mutation positive on peripheral blood
- No splenomegaly on physical exam

### PV criteria WHO 2008

- **Major Criteria**
  - Erythrocytosis
    - Hemoglobin >18.5/16.5 g/dL in men/women
    - Hemoglobin >17/15 g/dL in men/women, with sustained increase of 2 g/dL over baseline
    - Increased red cell mass (>25% above normal)
    - Hemoglobin or hematocrit >99% percentile
  - JAK2 mutation (V617F or Exon 12)

- **Minor Criteria**
  - Bone marrow showing typical PV histology
  - Decreased serum EPO levels
  - Endogenous erythroid colony formation

Diagnosis requires both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria
Diagnosis for Case 1

• Polycythemia Vera

Early stage PV can mimic ET or PMF

• In early stage PV the first major WHO criterion for diagnosing PV is often not fulfilled and they may be diagnosed (erroneously) as ET or PMF
• 50% ET and 60% of PMF patients carry the JAK2 mutation
• If borderline increase in hemoglobin, PV needs to be excluded particularly in cases with low MCV, low ferritin or absent stainable BM iron
• Red cell mass usually allows identification as PV; however, it is not available in all institutions

Case 2

• 53 year old woman
• Portal vein thrombosis in 2005. Five years later, superior mesenteric vein thrombosis (while on anticoagulant)
• CBC at the time of the biopsy (2010): Hb 16.2g/dL; MCV 82.7 fL; RBC 4.7 x10(9)/L; Hct 46.9%. WBC 3.9 x10(9)/L with a normal differential count. Platelets 460 x10(9)/L
• JAK2V617F mutation positive on peripheral blood
• Normal /borderline low EPO level (4 U/L)
• No splenomegaly

Courtesy of Attilio Orazi MD, WCMC
Summary of Case 2

- Thrombosis
- JAK2 positive
- Elevated Hgb (16.2) but not meeting WHO criteria for PV (16.5)
- Thrombocytosis
- Marrow with typical PV histology
  - Hypercellular with panmyelosis
  - Increased megakaryocytes with pleomorphism without abnormal nuclear maturation

PV criteria WHO 2008

- Major Criteria
  - Erythrocytosis
    - Hemoglobin >18.5/16.5 g/dL in men/women
    - Hemoglobin >17/15 g/dL in men/women, with sustained increase of 2 g/dL over baseline
    - Increased red cell mass (>25% above normal)
    - Hemoglobin or hematocrit >99% percentile
  - JAK2 mutation (V617F or Exon 12)

- Minor Criteria
  - Bone marrow showing typical PV histology
  - Decreased serum EPO levels
  - Endogenous erythroid colony formation

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

- Early phase of polycythemia vera (masked PV)
- Follow-up
  - Treated elsewhere with HU plus 2-4 phlebotomies per year for hematocrit control
  - After three years, seen at Cornell where a repeat BM confirmed PV with MF-1 and a mildly increased spleen size

The Clinical Importance of Recognizing PV “Early”


Proposed Revision to WHO PV Criteria

Major Criteria:

1. Erythrocytosis
   - Hemoglobin >16.5/16.0 g/dL in men/women or Hematocrit >49/48% in men/women
   - Hemoglobin >17/15 g/dL in men/women, with sustained increase of 2 g/dL over baseline
   - Increased red cell mass (>25% above normal)
   - Hemoglobin or hematocrit >99%

2. Bone marrow showing typical PV histology

3. JAK2 mutation

Minor

- Decreased serum EPO levels
- Endogenous erythroid colony formation

PV diagnosis requires meeting either all three major criteria, or the first two major criteria plus the minor criterion.
Case 3

- 55 year old male who was found to have the following CBC on a routine exam:
  - Hgb 11.4 g/dL, WBC 12.4 x 10^9/L, Plts 290 x 10^9/L.
- Physical exam: moderate splenomegaly
- JAK2 positive, BCR/ABL negative on PB

Summary of Case 3

- Hypercellular marrow with granulocytic hyperplasia, increased atypical megakaryocytes in tight clusters, marked reticulin fibrosis
- JAK2 positive
- Anemia and splenomegaly

WHO 2008 Diagnostic Criteria for PMF

- Major criteria:
  - **Megakaryocytic proliferation** and atypia with fibrosis.
    - If without reticulin fibrosis accompanied by **increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis**
  - **Not meeting** the WHO criteria for CML, PV, ET, MDS or other myeloid neoplasm
  - Presence of JAK2, or MPL mutation OR absence of reactive causes of BM fibrosis
- Minor criteria:
  - Anemia
  - Palpable splenomegaly
    - LDH increased to above upper limit of normal of institutional reference range
    - Leukoerythroblastosis

Diagnosis requires meeting all three major criteria plus 2 of the minor criteria.
Diagnosis for Case 3

• Primary myelofibrosis (MF-3)

Case 4

• 55 year old man
• History of mild thrombocytosis for couple of years
• No splenomegaly/organomegaly
• CBC (Nov 2007) showed:
  – Hgb 12.6 g/dL
  – Hct 37.6 %
  – MCV 88.5 fL
  – WBC 6.5 x10^9/L (N, 68; Ly 18; Mo 11; Eo 2, Ba 1)
  – Plts. 483 x10^9/L
  – PB smears showed mild anisopoikilocytosis and thrombocytosis. No teardrops or leukoerythroblastosis.
• To investigate the possibility of MPN, bone marrow examination was performed in November 2007

55 y/o; 90% cellular; dry tap

Courtesy of Attilio Orazi MD, WCMC
Other results (Case 4)

- LDH 248 U/L (96-200 U/L)
- *JAK2* V617F mutation present
- Red Cell Mass within normal range
- EPO normal
- Flow cytometry of BM unremarkable
- Normal karyotype
- *BCR-ABL1* absent (FISH)

WHO Diagnostic Criteria for PMF

**Major criteria:**
- Megakaryocytic proliferation and atypia with fibrosis.
  - If without reticulin fibrosis accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis
  - Not meeting the WHO criteria for CML, PV, ET, MDS or other myeloid neoplasm
  - Presence of JAK2, or MPL mutation OR absence of reactive causes of BM fibrosis

**Minor criteria:**
- Anemia
- Palpable splenomegaly
- LDH increased to above upper limit of normal of institutional reference range
- Leukoerythroblastosis

Diagnosis requires meeting all three major criteria plus 2 of the minor criteria
**Diagnosis (Case 4):**
Favor early stage primary myelofibrosis

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**Follow-up**
- Fall 2011 (4 years after initial BM) LUQ pain
- Worsening anemia
- Splenomegaly
  - 5 cm below costal margin
- Abdominal CT
  - Confirmed splenomegaly 21.7 x 11.7 x 18.5 cm
  - Non-occlusive thrombus in superior mesenteric vein
Diagnosis on this follow-up biopsy
Primary Myelofibrosis (MF-2)

Proposed Updates to WHO Diagnostic Criteria for PMF and early PMF

Major criteria:
1. Megakaryocytic proliferation and atypia. Without reticulin fibrosis (≤ MF-1), and accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis.
2. Not meeting the WHO criteria for CML, PV, ET, MDS or other myeloid neoplasm
3. Presence of JAK2, CALR or MPL mutation

Minor criteria:
Minor criteria I:
Presence of at least one of the following confirmed in two consecutive determinations:
- Anemia not attributed to a comorbid condition
- Leukocytosis >13K/uL - New criterion
- Palpable splenomegaly
- LDH increased to above UNL of institutional reference range
- Leukoerythroblastosis

Minor criterion II:
- Presence of a clonal marker or absence of reactive causes of BM fibrosis - New criterion

Diagnosis requires meeting all three major criteria plus one of the minor criteria group I, or the first two major criteria and one of the minor criteria group I and the minor criterion II.

Case 5
- 45 year old male in good health
- Thrombocytosis on routine exams. No apparent cause based on history and lab studies.
- A CBC shows HGB 14.1 g/dl, MCV 92 fl, WBC 8.2 x10E9/L, PLTS 785 x10E9/L; differential: neutrophils 64%, lymphocytes 26%, monocytes 8%, eosinophils 2%, basophils 0%.
- No splenomegaly
- PCR on peripheral blood was negative for JAK2 or MPL mutations or BCR/ABL rearrangement.

Normocellular bone marrow with increased megakaryopoiesis displaying hyperlobulated nuclei lacking atypia. No dense clusters of megakaryocytes. No fibrosis on reticulin.
WHO 2008 Diagnostic Criteria for ET

- Platelet count ≥450 x 10^9/μL
- Megakaryocyte proliferation with large and mature morphology; no significant increase in granulopoiesis or erythropoiesis
- Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm
- Presence of JAK2 or other clonal marker or no evidence of reactive thrombocytosis

Diagnosis of ET requires meeting all four criteria

Additional Studies on Case 5

- Positive for CALR Mutation
  - Positive in 20-30% PMF and ET
  - Mutually exclusive with JAK2
- Normal karyotype
- Diagnosis: Essential thrombocythemia

Proposed Updated Diagnostic Criteria for ET

**Major criteria:**
- Platelet count ≥450 x 10^9/μL
- Megakaryocyte proliferation with large and mature morphology
- Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm
- Presence of JAK2, CALR or MPL mutation

**Minor criterion:**
- 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 the one minor criterion

Role of Molecular Testing
Driver Mutations: Activation of JAK-STAT Pathway

- **JAK2**
  - 95% PV have JAK2 V617F (exon 14) mutation; 4% have JAK2 exon 12 mutation
  - 50-60% PMF and ET have JAK2 V617F mutation
- **CALR**
  - 20-30% PMF and ET have CALR mutations
- **MPL**
  - 5-10% PMF and ET have MPL exon 10 mutation
  - “Triple negative”
  - 10-15% PMF and ET. In these negative cases, mutational analysis for a broad panel of myeloid genes may be useful
- **JAK2, MPL** and **CALR** mutations are mutually exclusive

Prognostic Value of Molecular Testing

- **Driver mutations**
  - Clinical characteristics as well as survival varies depending on the type of driver mutation present
- **Other mutations (ASXL1, SRSF2, IDH1/2 and others)**
  - Negatively affect leukemia-free survival in myelofibrosis
  - At this time not included in routine diagnosis or prognostic evaluation

ET: Survival: the longest for triple-negative and shortest for MPL-mutated patients. Median survival: 19 years for JAK2- and 20 years for CALR-mutated cases (P=0.32)

Comparison of survival of 428 patients with PMF stratified by their mutations


Tefferi A et al. Blood 2014;124:2465-2466; differences between CALR mut. type 1 vs. type 2
## Conclusions

- Integrated diagnostic approach
- *CALR* mutational analysis is useful in *JAK2* negative cases
- Recognition of masked/prepolycythemic PV as well as early PMF is important
- Updates to WHO 2008 criteria will incorporate these diagnostic findings: significant changes to criteria for PV
- Additional mutational analysis might become prognostically useful in the near future

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