WHO 2016/17 update on Myeloproliferative Neoplasms

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THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

The 2016 revision of the World Health Organization classification of lymphoid neoplasms

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THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia

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# 2016/17 Classification of Myeloproliferative Neoplasms

<table>
<thead>
<tr>
<th>Classification</th>
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<tbody>
<tr>
<td>Chronic Myeloid Leukaemia (CML), BCR-ABL-positive</td>
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<tr>
<td>Chronic Neutrophilic Leukaemia (CNL)</td>
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<tr>
<td>Polycythaemia Vera</td>
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<tr>
<td>Primary Myelofibrosis (PMF)</td>
</tr>
<tr>
<td>- PMF Prefibrotic/early stage</td>
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<tr>
<td>- PMF Overt fibrotic stage</td>
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<tr>
<td>Essential Thrombocythaemia (ET)</td>
</tr>
<tr>
<td>Chronic Eosinophilic Leukaemia, NOS</td>
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<tr>
<td>MPN, unclassifiable</td>
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Talk outline

• CML BCR-ABL1 positive

• CNL (and aCML) – insights in the genomics and changes in classification

• Classical BCR/ABL1 negative MPNs
  • New criteria for PV, ET and PMF
  • Rationale for the changes

• New insights in the pathophysiology of MPNs
<table>
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<tr>
<th>CML, accelerated phase</th>
<th>Any 1 or more of the following criteria or response-to-TKI criteria:</th>
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<tbody>
<tr>
<td><strong>Persistent WBC &gt;10 x10^9/L, unresponsive to Tx</strong></td>
<td><strong>Provisional response-to-TKI criteria</strong></td>
</tr>
<tr>
<td><strong>Persistent or increasing splenomegaly, unresponsive to Tx</strong></td>
<td><em>Hematological resistance to 1st TKI (or failure to achieve a complete hematological response to the 1st TKI)</em> or</td>
</tr>
<tr>
<td><strong>Persistent Plt &gt;1000 x10^9/L unrelated to Tx</strong></td>
<td><em>Any haematological, CG or molecular indications of resistance to 2 sequential TKIs or</em></td>
</tr>
<tr>
<td><strong>20% or more Baso in PB</strong></td>
<td><em>Occurrence of 2 or more mutations in BCR-ABL1 during TKI therapy</em></td>
</tr>
<tr>
<td><strong>10%-19% blasts† in the PB and/or BM</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Additional clonal chromosomal abn in Ph+ cells at diagnosis</strong> that include major route abnormalities (2nd Ph, +8, iso 17q, +19), complex karyotype or abn 3q26.2</td>
<td></td>
</tr>
<tr>
<td><strong>Any new chromosomal abn in Ph+ cells that occurs during Tx</strong></td>
<td></td>
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</tbody>
</table>

†Finding of bona fide lymphoblasts in PB even <10% should prompt concerns for imminent blast phase
Large clusters or sheets of abnormal MGK associated with marked fibrosis may be considered a presumptive evidence of AP
Chronic Neutrophilic Leukaemia
and atypical CML BCR/ABL1 negative
Chronic Neutrophilic Leukaemia (CNL)

- Hepatosplenomegaly
- WBC $\geq 25$ (<10% immature)
- No granulocytic or MEG’s dysplasia
- No increase in Mo Ba Eo
- Most have normal CG
- Blastic transformation (med. time to transformation 21/12)

Atypical CML BCR-ABL1neg (aCML)

- Hepatosplenomegaly
- WBC 13 $\geq$ (>10% immature)
- Granulocytic +/- MEG’s dysplasia
- <2% Baso <10% Mono
- 20%-88% abn. CG +8, del(20q)
- Blastic transformation in 40% at 18/12
CNL
Neutrophilia with bands and toxic changes

aCML
Neutrophilia with left shift and dysplasia

Features overlap

No significant megakaryocytic dysplasia

Megakaryocytic dysplasia
CSF3R, SETBP1 and ETNK1

CSF3R

• Mutated in 80% of pts with severe congenital neutropenia
• 2013 Maxson et al. CSF3R mutated in 89% of CNL and 44% of aCML
• Majority are membrane proximal CSF3R T618I
• Subsequently less frequent in strictly WHO-defined aCML in <10% (Pardanini et al. Meggendorfer et al. Wang et al.)

SETBP1 and ETNK1

• Recurrent mutations in aCML (Piazza et al 2013 Gambacorti et al 2015)
• SETBP1 in 15-32% of aCML
• ETNK1 in 9% of aCML (in 1/3 of these co-exist with SETBP1)
• In CNL occurs together with CSF3R in ~50%
# Genomics of CNL and aCML

<table>
<thead>
<tr>
<th>Type mutations</th>
<th>Gene</th>
<th>CNL</th>
<th>aCML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signaling</td>
<td>CSF3R</td>
<td>90%</td>
<td>&lt;10%</td>
</tr>
<tr>
<td></td>
<td>SETBP1</td>
<td>~50%</td>
<td>15%-32%</td>
</tr>
<tr>
<td></td>
<td>ETNK1</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>JAK2/CALR/MPL</td>
<td>0</td>
<td>&lt;4%</td>
</tr>
<tr>
<td>Epigenetic modifiers</td>
<td>ASXL1</td>
<td>30%-60%</td>
<td>30%-60%</td>
</tr>
<tr>
<td></td>
<td>TET2</td>
<td>30%</td>
<td>40%</td>
</tr>
<tr>
<td>Spliceosome</td>
<td>U2AF1</td>
<td>21%</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>SRSF2</td>
<td>10%</td>
<td>40%</td>
</tr>
</tbody>
</table>

P<.001
5. Presence of CSF3R mutation

OR

In the absence of CSF3R, persistent neutrophilia (3/12), splenomegaly and no identifiable cause of reactive neutrophilia incl. absence of plasma cell dyscrasia
• Although rare CSF3R present in aCML
• Hybrid cases with morphology between aCML and CNL
• Reported in patients with pre-existing MDS or MPN-U transforming to aCML/CNL-like disorder

It is unclear whether
1. CSF3R+ aCML is a distinct entity or a disease evolution from CNL
2. All patients with CSF3R placed in single category?

Maxson et al Blood 2017
Dao et al ASH 2015
BCR-ABL negative classical MPNs

Polycythaemia vera
Essential thrombocythaemia
Primary myelofibrosis
Essential thrombocythemia (ET)

Polycythemia vera (PV)

<5%  
<10%  
20-30%  

Myelofibrosis (MF)

de novo

Similar complications: 
Bleeding 
Thrombosis 
Splenomegaly

acute leukemia
• 1951 William Dameshek: Proposes lumping CGL, PV, Idiopathic Myeloid Metaplasia Megakaryocytic Leukemia and Erythroleukaemia into a group of myeloproliferative disorders, proliferative activity due to an undiscovered stimulus
• 2005 JAK2 V617F mutation proved Dameshek’s concept
• 2006 MPL mutation in ET and PMF
• 2007 JAK2 exon 12 mutation in PV
• 2008 4th edition of WHO classification
• 2013 CALR mutation in ET and PMF
Other New data since 2008

• In **Polycythemia vera** underdiagnosis when applying the 2008 WHO threshold values for Hb and Hct

• In **Essential thrombocythemia** the distinction between "true ET" and prefibrotic/early primary myelofibrosis has prognostic implications

• In **Primary Myelofibrosis** identification of additional clonal markers with an impact on prognosis
Polycythaemia vera
Under-diagnosis when applying the 2008 WHO values for Hb (M >185 F> 165)

- JAK2-mutated patients with Hb 160-185 g/L (M) and 150-165 g/L (F) who display PV-characteristic BM morphology have incidence of thrombosis similar to overt PV
- In JAK2-mutated patients best cutoff to discriminate ET from PV
  M: Hb 165 g/L Hct 49%  F: 160 g/L 48%
The role of morphology

\[ mPV = PV \]

\[ \neq ET \]
<table>
<thead>
<tr>
<th>PV</th>
<th>2008 Both major and 1 minor or 1st major and 2 minor</th>
<th>2016/17 All 3 major or 1st 2 major and one minor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major</td>
<td>1. Hb &gt;185(M) &gt;165(F) or other evidence of increased RC volume*</td>
<td>1. Hb &gt;165(M) &gt;160(F) or Hct &gt;49(M) &gt;48(F)</td>
</tr>
<tr>
<td></td>
<td>2. JAK2+ (either exon 14 or 12)</td>
<td>2. BM hypercellularity for age, panmyelosis with pleomorphic MEG**</td>
</tr>
<tr>
<td>Minor</td>
<td>1. BM trilineage myeloproliferation (panmyelosis)</td>
<td>1. Subnormal EPO</td>
</tr>
<tr>
<td></td>
<td>2. Subnormal EPO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Endogenous Ery colony growth</td>
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</table>

*Hb or Hct >99th percentile of ref range for age/sex/altitude or Hb>170(M) >150(F) if sustained increase of 20g/l from baseline or elevated RCM >25% above mean normal

**BM examination not necessary for clinically overt PV, i.e. JAK2+ and Hb/Hct >185/52 M >165/48 F

BUT only by performing BMB an increase in fibrosis can be detected
Progression of PV stratified by BM fibrosis at diagnosis

Myelofibrosis-free survival in PV

Barnaco et al Blood Cancer 2017
Barbui et al Blood 2012
PMF and ET

Early/pre PMF or ET, why is it important to distinguish?
Differences in course and complications

- Increased risk of thrombosis, bleeding and myelofibrosis in pre-PMF vs ET
- More major bleeding with high platelet counts in pre-PMF
- Therapeutic consideration – slowing down disease progression in pre-PMF with a new molecularly targeted therapy

Barbui et al Blood 2012
Rupoli et al Diagn Pathol 2015
Finazzii et al Leukemia 2012
Difference in survival

10-year OS 89% ET 76% pre-PMF
15-year OS 80% ET 59% pre-PMF

Barbui et al JCO 2011
Thiele et al Blood 2011
<table>
<thead>
<tr>
<th><strong>ET</strong></th>
<th><strong>PMF (early-prefibrotic stage)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• no or only slight increase in age-matched cellularity</td>
<td>• marked increase in age-matched cellularity</td>
</tr>
<tr>
<td>• no significant increase in granulo- and erythropoiesis</td>
<td>• pronounced proliferation of granulopoiesis and reduction of erythroid precursors</td>
</tr>
<tr>
<td>• prominent large to giant mature megakaryocytes with hyperlobulated or deeply folded nuclei, dispersed or loosely clustered in the marrow space</td>
<td>• dense or loose clustering and frequent endosteal translocation of medium sized to giant megakaryocytes showing hyperchromatic, hypolobulated, bulbous, or irregularly folded nuclei and an aberrant nuclear/cytoplasmic ratio</td>
</tr>
<tr>
<td>• no or very rarely minor increase in reticulin fibers</td>
<td>• no or no significant increase in reticulin fibers</td>
</tr>
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</table>
Distinctive features of PMF

• Number of dense clusters are important

• In cases without clustering: cellularity, M:E ratio and nuclear features of the MEGs are important (hypolobulated “bulbous/clumsy nuclei”)

• The overall pattern is the key to the diagnosis, not the single parameter

WHO definition of a dense megakaryocyte cluster: 3 or more megakaryocytes lying strictly adjacent - without other hematopoietic cells lying in between
<table>
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<tr>
<th></th>
<th><strong>2008</strong></th>
<th><strong>2016/17</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ET</strong></td>
<td><strong>All 4 major criteria:</strong></td>
<td><strong>All 4 major or 1\textsuperscript{st} 3 major + the minor criterion</strong></td>
</tr>
<tr>
<td><strong>Major</strong></td>
<td>Plt $\geq 450$</td>
<td>Plt $\geq 450$</td>
</tr>
</tbody>
</table>
|                      | MGC proliferation with large and mature morphology | BMB: mainly MEG proliferation with large and mature morphology  
No significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis and very rarely minor increase in reticulin fibers (grade1) |
|                      | Not meeting WHO criteria for CML, PV, PMF, MDS or other MN | Not meeting WHO criteria for CML, PV, PMF, MDS or other MN |
|                      | JAK2 or other clonal marker or no evidence of reactive thrombocytosis | JAK2, CALR or MPL mutation |
| **Minor**            | Presence of a clonal marker or no evidence of reactive thrombocytosis |          |
JAK2 60-65%, CALR 20-25% MPL 4-5%, <1% MPL mutation outside exon 10

~10% of ET are ‘triple negative’ – 55% of them have polyclonal haematopoiesis and most likely do not have a true MPN

Milosevic Feenstra et al Blood 2016
Cabagnols et al Blood 2016
Harrison and Vanucci Blood 2016
<table>
<thead>
<tr>
<th>PMF</th>
<th>2008</th>
<th>All 3 major + 2 minor</th>
<th>2016/17</th>
<th>All 3 major and at least one minor (confirmed in 2 consecutive determinations)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major</strong></td>
<td></td>
<td></td>
<td></td>
<td>Pre-PMF</td>
</tr>
<tr>
<td></td>
<td>1. MEG proliferation and atypia, accompanied by fibrosis; if no fibrosis, increased cellularity, granulocytic proliferation and decreased erythropoiesis</td>
<td></td>
<td>1. MEG proliferation and atypia without &gt;1 gr 1 fibrosis plus increased for age cellularity, granul. proliferation and often decreased Ery PMF</td>
<td>1. MEG proliferation and atypia accompanied by either reticulin and/or collagen fibrosis grades 2 or 3</td>
</tr>
<tr>
<td></td>
<td>2. Not meeting criteria for CML, PV, MDS or other MN</td>
<td></td>
<td>2. Not meeting criteria for CML, PV, ET, MDS or other MN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. JAK2 or other clonal marker or no evidence of reactive fibrosis</td>
<td></td>
<td>3. JAK2, CALR or MPL or in the absence, presence of another clonal marker* or exclusion of reactive fibrosis</td>
<td></td>
</tr>
<tr>
<td><strong>Minor</strong></td>
<td>Anaemia</td>
<td>Anaemia not attributable to another condition</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>WBC $\geq 11 \times 10^9$/l</td>
<td>Palpable splenomegaly</td>
<td></td>
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<tr>
<td></td>
<td>Increased LDH</td>
<td>LDH above upper normal limit</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leukoerythroblastosis</td>
<td>Leukoerythroblastosis</td>
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In the absence of JAK2/CALR/MPL finding mutations in ASXL1, EZH2, TET2, IDH1,2, SRSF2, SF3B1 is helpful to determine clonality.
“Triple negative” PMF

- Triple neg. PMF similar to MDS-F
- Fibrosis can be driven by mutation in tumor-suppressor genes EZH233 or SRSF2 and so can be present in MN other than PMF
- These patients should ideally have myeloid panel NGS
Summary modifications in the 2016/2017 WHO

• Polycythaemia vera
  ➢ Lower Hb and Hct thresholds
  ➢ BM morphology as a major criterion

• Essential Thrombocythaemia
  ➢ Differentiating true ET from prefibrotic MF, emphasis on the lack of reticulin fibrosis at onset
  ➢ Inclusion of CALR and MPL

• Primary myelofibrosis
  ➢ Pre-PMF - better definition of morphological features and minor clinical criteria
  ➢ Inclusion of CALR, MPL and other molecular findings
Are the changes bringing us closer to the truth?
In their typical forms PV, ET and PMF are distinct.

In many cases continuum/progression between the subtypes is observed and boundaries cannot be well established.

Introduction of transitional entities prefibrotic PMF and masked PV.

Open questions
How the same mutation (JAK2) produces 3 different phenotypes?
How identical phenotypes are produced by different mutations?
What determines the phenotype of MPN?
MPN-restricted driver mutations

JAK2 V617F mutation
- Activate signaling through 3 receptors EPOR, MPL and G-CSFR – erythrocytosis, thrombocytosis and neutrophilia

MPL mutations
- R for TPO
- Mutations have a gain of function effect with either TPO-independent growth or TPO-hypersensitivity

CALR mutations
- Mutations activate MPL mainly and at a low level G-CSFR and result in thrombocytosis

All 3 MPN mutations directly (JAK2) or indirectly (MPL and CALR) result in inappropriate activation of JAK/STAT and other signaling pathways
The 3 driver mutations are mutually exclusive

W. Vainchenker et al Blood 2017
The mechanism of JAK2 activation may define 2 main types MPN

**Driven by JAK2V617F**
- Associated with proliferation of the 3 cell lineages
- Leads to ET, PV or PMF
- Associated with higher HB, higher WBC and lower Plt and older age

**Driven by CALR and MPL mutations**
- Associated with proliferation of megakaryocytic lineage mainly
- Leads to ET and PMF
- CALR+ ET and MF: younger age, higher Plt count, lower WBC count and better survival and in CALR+ET: lower HB and rate of thrombosis

W. Vainchenker et al Blood 2017
How the same JAK2 mutation result in 3 MPNs?
Effect of ‘gene dosage’

Increased JAK2 signaling leads to more of a polycythaemic phenotype

- JAK2 homozygosity found in 1/3 of PV but rare in ET
- Correlation b/w the size of the homozygous clones and Hb concentration in PV and ET; JAK2+ ET with greater allele burden may represent a form fruste of PV
- JAK2 exon 12 mutations result in stronger signaling compared to JAK2V617F and are found in PV only
However

Some patients with PV do not carry homozygous clones and some patients with ET do (although generally small)

JAK2 homozygosity is neither necessary nor sufficient to cause PV. Mechanisms and mutations other than the JAK2 dosage contributes to the determination of PV or ET phenotype.
The role of “nonrestricted” (to MPN) mutations
TET2

• TET2 the most commonly mutated gene among all 3 MPNs (~15% of MPNs)

• Similar to other epigenetic regulators TET2 is linked to **self-renewal and block of differentiation**

• JAK2 drives proliferation but does not confer advantage at HSC level (does not result in a self-renewal advantage); alone insufficient to initiate disease

CA Ortmann et al NEJM 2015
J. Grinfeld et al Haematologica 2017
The role of mutation order

JAK2 first patients present at a younger age and are more likely to present as PV

If TET2 1st => dominant ‘single mutant’ subclone i.e. TET2 only because of the greater self-renewal capacity but no overproduction of downstream progeny => JAK2 clone constrained => ET phenotype

If JAK2 1st => excess differentiated cells but no self-renewal advantage, when 2nd TET2 => larger ‘double mutant’ subclones as well as larger JAK2 homozygous clones => expansion of Ery progenitors => PV phenotype

and have increased risk of thrombosis (both a. and v.)

Ortmann et al NEJM 2015
Order matters

S. Seton-Rogers Nature Reviews Cancer 2015
The majority of PV and ET have only one mutation in a MPN driver gene (JAK2 CALR MPL). The majority of PMF (>80%) have 2 or more mutations (50% ≥3). Spliceosome mutations (SRSF2, U2AF1, SF3B1) are strongly associated with PMF or MDS/MPN. Epigenetic regulators (ASXL1 and EZH2) common in PMF and MDS/AML, significant association with fibrosis and worse survival in MPN.
The type and number of mutations determine the phenotype of MPN

- Proliferation is driven mainly by signaling mutations
- Most mutations in epigenetic and spliceosome components lead to differentiation defects
- PMF is not a pure MPN
- The heterogeneity of the PMF and its prognosis depend on the number of additional mutations and prognosis is poor if MDS features are prominent

\[
\begin{align*}
\geq 3 \text{ mutations} & \quad 2 \text{ mutations} \\
1 \text{ mutation} & \quad \text{Myelodysplasia}
\end{align*}
\]
Is Primary Myelofibrosis really primary?

- PMF exhibits features of a late stage disease
  - High clonal burden
  - More CG abnormalities
  - Increased progression to acute leukaemia
- PMF and MF transformation from PV or ET are indistinguishable
- In mouse models JAK2 and CALR mutations induce PV-like or ET-like phenotype but not Primary MF
- Genetically PMF is not a pure MPN but a mixed MPN/MDS
- ”Primary” MF may actually reflect an accelerated phase from a preceding and undiagnosed MPN
- Prefibrotic MF may represent a transitional point between ET and MF
Other determinants of MPN phenotype

- **Germline determinants**
  - Gender: ET more common in F, PMF in M (?hormones)
  - Predisposition to MPN:
    - Germline mutations in RBBP6
    - SNPs in TERT and MECOM
    - JAK2 haplotypes 46 and 1

- **BM microenvironment and inflammation**
  - TNFα, IL-6, FGF produces by BM stroma and promote growth of MPN clones
  - Secretion of proteases disrupt the CXCR4 axis => greater mobilisation of HSCs
  - Clonal MEGs and Mono secrete cytokines that stimulate angiogenesis => fibrosis
  - Concurrent inflammatory conditions

- **Age**
  - most frequent somatic mutations linked to aging (DNMT3A, TET2, ASXL1 and JAK2) implicated in MPN

- **Iron deficiency**
  - may constrain Ery-poiesis and promote Plt production skewing towards ET phenotype

Jones et al. Ther Adv Hematol. 2013
J. Grinfeld Haematologica 2017
The traditional division into 3 MPNs does not reflect the underlying biological complexity.
ET

PV

JAK2 + additional mutations

(P)MF

JAK2/CALR/MPL + additional mutations

JAK2
“...we find it difficult to draw any clear-cut dividing lines; in fact, so many ‘transition forms’ exist that one may with equal reasonableness call a single condition by at least two different terms.”

“...it can be anticipated that gene mutational analysis is likely to become a fundamental component of innovative diagnostic approaches and prognostic models in MPN.”