Opportunities for Optimal Testing in the Myeloproliferative Neoplasms

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DISCLOSURES:

Relevant Financial Relationship(s)
None

Off Label Usage
None
WHO Classification

Chronic Myeloid Neoplasms

MDS/MPN
- CMML
- JMML
- Atypical CML

MDS
- RA
- RARS
- RCMD
- RAEB-1
- RAEB-2
- 5q- Syndrome
- MDS-unclassified

MPN
- Chronic myelogenous leukemia

BCR-ABL1?
- Pos
- Neg

Classic

MDS/MPN
Non-classic
- Chronic eosinophilic leukemia
- Hypereosinophilic syndrome
- Chronic neutrophilic leukemia
- MPN-unclassified

Mast cell disorders

- Polycythemia vera
- Essential thrombocytocemia
- Primary myelofibrosis
Myeloproliferative Neoplasms (MPNs)

- Malignancy of hematopoietic stem cells
- Effective hematopoietic proliferation
  - Hypercellular bone marrow with elevated counts or organomegaly
- Fibrosis is common
- No (minimal) dysplasia
- Classified on the basis of dominant cell line involved
Imatinib responsiveness in CML is one of the original genetic success stories of cancer treatment!

In 2013: CML is a genetic evaluation.
## Recommendations for cytogenetic and molecular monitoring in CML

| At diagnosis | • Chromosome banding analysis (CBA)  
|             | • FISH (in case of Ph negativity to identify variant, cryptic translocations)  
|             | • Qualitative PCR (identification of transcript type) |
| During treatment | • Quantitative real-time PCR (RQ-PCR) (international scale); every 3 months until an MMR (BCR-ABL ≤0.1%); then every 3 to 6 months  
|             | and/or  
|             | • CBA at 3, 6, and 12 months until a CCyR has been achieved, then every 12 months.  
|             | • If adequate molecular monitoring can be ensured, cytogenetics can be spared. |
| Failure, progression | • RQ-PCR  
|         | • Mutational analysis  
|         | • CBA |
| Warning | • Molecular and cytogenetic tests to be performed more frequently.  
|         | • CBA of marrow cell metaphases recommended in case of myelodysplasia or CCA/Ph– with chromosome 7 involvement. |

- Whenever possible, both cytogenetic and molecular tests are recommended until a CCyR and an MMR are achieved. Then RQ-PCR alone may be sufficient.
- Mutational analysis by conventional Sanger sequencing is recommended in case of progression, failure, and warning.

_Blood._ 2013 Aug 8;122(6):872-84.

_European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013._

_Baccarani M et al._
Diagnostic Approach to the Classic MPNs

- Clinical findings
- Laboratory studies
  - CBC and diff, serum erythropoietin, LDH, iron studies, etc.
- Morphologic assessment of blood and bone marrow
- Genetic studies
  - Chromosome analysis
  - JAK2, MPL, etc.
  - CALR – ASH abstracts
Morphologic Assessment

• Goals:
  • Establish the diagnosis of a MPN
  • Subclassify the MPNs when possible
  • Determine the degree of fibrosis
  • Assess % blasts / transformation
Subclassifying MPNs by Morphology: A Practical Approach

• Acknowledge that you cannot accurately subclassify every MPN with absolute confidence

• Step 1: PV is the big “masquerader”
  • Diagnose or exclude PV on the basis of CBC, iron studies, serum Epo, JAK2, etc.

• Step 2:
  • Establish the diagnosis of those cases that are either straightforward ET or PMF

• Step 3:
  • Everything else is MPN, NOS
Possible MPN?

Morphology confirms MPN

Diagnose or exclude PV

“>15 to 20” year survivals

MPN, NOS. Clinical criteria needed

“3 to 7” year survivals
WHO Criteria for Diagnosis of PV

**Major criteria**
- Hemoglobin > 18.5 g/dL in men, > 16.5 g/dL in women, or evidence of increased red cell volume
- Presence of JAK2(V617F) or other functionally similar mutation (eg, JAK2 exon 12 mutation)

**Minor criteria**
- BM biopsy showing hypercellularity for age with trilineage myeloproliferation
- Serum erythropoietin level below the normal reference range
- Endogenous erythroid colony formation in vitro

Requires either both major criteria and 1 minor criterion or the first major criterion and 2 minor criteria.
Diagnostic Criteria for ET: Practical Approach

- WHO criteria
  - Sustained platelet count ≥ 450 x 10^9/L
  - Morphologic features of a MPN
  - Exclude PV, PMF, CML, MDS, CMML

- Normal to slightly hypercellular bone marrow
- Neutrophils <12 x 10^9/L
- No granulocytic hyperplasia in bone marrow
- No reticulin fibrosis
- No marked megakaryocytic hyperplasia or striking atypia
Mayo Clinic Series of 605 ET Patients

Adverse risk factors
- Age < 60 years
- Lower than normal hemoglobin
- WBC $\geq 15 \times 10^9/L$

Gangat et al. Leukemia 2006
Diagnostic Criteria for PMF: Practical Approach

- WHO criteria
  - Morphologic features of a MPN
  - Exclude PV, ET, CML, MDS, CMML
  - LEBR; increase in LDH; anemia; splenomegaly

- Present with cytopenias or neutrophilia or thrombocytosis

- Hypercellular bone marrow - with or without fibrosis

- Granulocytic hyperplasia

- Prominent megakaryocyte hyperplasia and marked megakaryocytic atypia
Possible MPN? \rightarrow Morphology confirms MPN \rightarrow Diagnose or exclude PV

“>15 to 20” year survivals \rightarrow MPN, NOS. Clinical criteria needed \rightarrow “3 to 7” year survivals
## JAK2 V617F: Positive Assay

<table>
<thead>
<tr>
<th>(+)</th>
<th>(-)</th>
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<tbody>
<tr>
<td>• PV 95%</td>
<td>• Healthy volunteers</td>
</tr>
<tr>
<td>• PMF ~ 50%</td>
<td>• Secondary polycythemia</td>
</tr>
<tr>
<td>• ET ~ 50%</td>
<td>• “Apparent” polycythemia</td>
</tr>
<tr>
<td>• Acute MF ~ 20%</td>
<td>• Reactive thrombocytosis</td>
</tr>
<tr>
<td>• MDS 0-5%</td>
<td>• Reactive leukocytosis</td>
</tr>
<tr>
<td>• AML 0-5%</td>
<td>• Solid tumor</td>
</tr>
<tr>
<td>• “Idiopathic” thrombosis</td>
<td>• Lymphoid disorders</td>
</tr>
<tr>
<td></td>
<td>• CML</td>
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</table>
JAK2 V617F Mutation Assay

- **Positive result:**
  - Confirms the presence of a myeloid disorder
  - Strongly favors MPN over MDS diagnosis
  - Cannot distinguish between MPN’s

- **Negative result**
  - In general, diagnostically not helpful
  - Argues against a diagnosis of PV or PPMF

- **Assay sensitivity**
  - Sequencing: ~ 20%
  - Quantitative AS-PCR: <0.1%
JAK2 Exon 12 Mutations

- JAK2 V617F (allele specific PCR)
  - PV: 95% / PMF: 50% / ET: 50%

- JAK2 Exon 12 (Sanger sequencing)
  - 5% PV
  - ~0% PMF and ET
  - Less sensitive than V617F PCR (~20% vs <<1%)
    - Not an appropriate JAK2 screening assay
  - Use for suspected PVs with negative V617F
    - Erythrocytosis, low/normal serum erythropoietin, normal LDH, and JAK2 V617F negative by PCR
MPL Exon 10 Sequencing

- *MPL* mutations present in <10% of ET and PMF
- Mutations not present when there are *JAK2* mutations
- Not useful in straight-forward cases of classic MPN (clinical findings and morphology are PV, PMF, or ET)
- Useful when there is a clinical suspicion of a MPN but the bone marrow morphology is equivocal
  - Fibrosis without megakaryocyte clusters
  - Thrombocytosis and/or neutrophilia without megakaryocyte clusters
- May assess blood if BM biopsy is not possible and there is a high clinical suspicion of a MPN
# MPNs: Distinctive Genetic Abnormalities

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cytogenetics</th>
<th>Molecular genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML</td>
<td>t(9;22)(q34;q11.2)</td>
<td><strong>ABL1-BCR</strong></td>
</tr>
</tbody>
</table>
| PV      | +8, +9, del(20q), del(13q), del(9p) | **JAK2 V617F (95%)**
|         |              | **JAK2 other (5%)** |
| ET      | Rare abnormalities | **JAK2 V617F (40-50%)**
|         |              | **MPL Exon 10 mutation (5%)** |
| PMF     | +8, +9, +1q, del(20q), del(13q)(q12-q22), der(6)t(1;6) | **JAK2 V617F (40-50%)**
|         |              | **MPL Exon 10 mutation (10%)** |
Emerging Value of Point Mutations in PMF as Prognostic Markers

• ASXL1, SRSF2, and EZH2 mutations predict shortened survival

• Only ASXL1 mutations appear to be significant in the context of the International Prognostic Scoring System (IPSS)

• Leukemia-free survival in PMF was negatively affected
  • European data: IDH1/2, SRSF2, and ASXL1 mutations
  • Mayo cohort: IDH1 and SRSF2 mutations.

• Mutational profiling for ASXL1, EZH2, SRSF2, and IDH identifies PMF patients who are at risk for premature death or leukemic transformation.

Leukemia. 2013 Sep;27(9):1861-9.
Mutations and prognosis in primary myelofibrosis.
Vannucchi AM et al.
**CSF3R Mutations in CNL**

- Truncation mutations of the receptor cytoplasmic domain for colony-stimulating factor 3 (CSF3R) frequent in severe congenital neutropenia

- Missense mutations affecting the extracellular domain (exon 14) in hereditary neutrophilia and chronic neutrophilic leukemia (CNL)

- CSF3R T618I occurred exclusively in WHO-defined CNL with a mutational frequency of 83% (10 of 12 cases)

- CSF3R mutations not seen in aCML or MGUS-associated neutrophilia

- CSF3R T618I absent among 170 patients with PMF or CMML

CSF3R T618I is a highly prevalent and specific mutation in chronic neutrophilic leukemia. Pardanani A et al.

Leukemia. 2013 Sep;27(9):1870-3.
CSF3R Mutations in CNL: Two Types

- Point mutations in the extracellular domain of *CSF3R* (membrane proximal mutations)
  - *CSF3R* T618I is the most common mutation in CNL
  - Strongly activates the JAK/signal transducer and activator of transcription pathway
  - Thus, are sensitive to JAK kinase inhibitors such as ruxolitinib
- Nonsense or frame-shift mutations that lead to premature truncation of the receptor’s cytoplasmic tail (truncation mutations)
  - Downstream signaling operates predominantly through SRC kinases
  - Exhibits drug sensitivity to SRC kinase inhibitors such as dasatinib

Common Test Utilization Issues in MPNs

- JAK2 V617F studies in concurrent blood and marrow
- JAK2 exon 12 sequencing studies without JAK2 V617F studies
- Both JAK2 exon 12 sequencing and JAK2 V617F studies ordered at the same time
- MPL exon 10 sequencing studies in bonafide cases of MPNs
- Multiple BCR-ABL assays in obvious classic MPN
- Flow cytometry requests in MPNs
- Mast cell or eosinophil studies (KIT, PDGFR-A or PDGFR–B, etc.) in classic MPNs
JAK2 V617F Testing on PB & BM

- All JAK2 V617F tests performed on MCR patients from 2006 to 2009 were reviewed (n=1624).
- 267 patients with concurrent PB and BM studies were identified

<table>
<thead>
<tr>
<th>JAK2V617F</th>
<th>PB+</th>
<th>PB-</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM+</td>
<td>137</td>
<td>2*</td>
</tr>
<tr>
<td>BM-</td>
<td>2*</td>
<td>126</td>
</tr>
</tbody>
</table>

- *Mutation burden at lower limit of detection
- *BM diagnoses in discrepant cases
  - 1 MDS/CMPN, unclassified
  - 3 normal bone marrow
- *No change in dx, management, outcome
- **Action: cancel duplicate tests**
JAK2 Exon 12 Sequencing Data (n = 92)

- Mutation present (n=20)
  - 16 positive V617F
  - 4 with non-V617F mutation (all had PV lab features)*

- Failed study (n=3)
  - none with PV lab features

- No mutation (n=69)
  - 58 negative V617F
    - 19: PV lab features*
    - 39: other MPN and non-PV CBC
    - 4 V617F + by PCR
    - 7 had no V617F done

*Appropriate testing 23 of 92 cases (25%)
MPL Exon 10 Sequencing: (n = 60)

- Mutation present (n=7)
  - 7 negative V617F
  - 4 classic MPN
  - 3 PB studies only*
- No mutation (n=53)
  - 10 positive V617F
  - 40 negative V617F
  - 5 classic MPN
  - 16 normal BM or other disease processes
  - 12 borderline BMs*
  - 7 PB studies only*
  - 3 with no V617F done

*Appropriate testing 22 of 60 cases (37%)
Myeloproliferative Neoplasm:
A Diagnostic Approach to Peripheral Blood Evaluation

Clinical suspicion of myeloproliferative neoplasm

Peripheral blood testing begins with:
- Complete blood count (CBC)
- EPO / Erythropoietin (EPO), Serum
- JAK2 / JAK2 V617F Mutation Detection, Blood
- BADX / BCR-ABL, RNA Detection, Reverse Transcription-PCR (RT-PCR), Qualitative, Diagnostic Assay
  OR MBCR / BCR/ABL Translocation 9:22, FISH (q-FISH)

JAK2 V617F Mutation

POSITIVE
- CBC
- EPO
- Clinical findings

NEGATIVE OR EQUIVOCAL

POSITIVE for BCR/ABL
- Chronic myelogenous leukemia

NEGATIVE OR EQUIVOCAL
- Chronic myelogenous leukemia

PV
- Not supportive of PV
- Bone marrow study indicated

PV possible
- Not supportive of PV
- Bone marrow study indicated

JAK2 / JAK2 Exon 12 and Other Non-V617F Mutation Detection, Blood

HIGH
- Clinical suspicion for ET or PMF

LOW
- No further testing

Bone marrow study indicated

If bone marrow study must be avoided, order MPLB / MPL, Exon 10 Mutation Detection, Blood

Bone marrow study indicated

Legend:
- PV: Polycythemia vera
- ET: Essential thrombocythemia
- PMF: Primary myelofibrosis
- MPN, Myeloproliferative neoplasm
Myeloproliferative Neoplasm:
A Diagnostic Approach to Peripheral Blood Evaluation

NEGATIVE OR EQUIVOCAL

- CBC
- EPO
- Clinical findings
Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation

Clinical suspicion of myeloproliferative neoplasm

Bone marrow testing begins with
- 7016 / Hematopathology Consultation, Wet Tissue
- BM / Chromosome Analysis, Hematologic Disorders, Bone Marrow
- JAK2M / JAK2 V617F Mutation Detection, Bone marrow
- BADX / BCR/ABL, mRNA Detection, Reverse Transcription-PCR (RT-PCR), Qualitative, Diagnostic Assay
  OR MBCR / BCR/ABL, Translocation 9:22, FISH (D-FISH)

Bone marrow morphology: MPN?

- NO
  - Erythrocytosis?
    - YES
      - REVP / Erythrocytosis Evaluation
      - Erythrocytosis
        - Complete blood count (CBC)
        - EPO / Erythropoietin (EPO), Serum
        - JAKXm / JAK2 Exon 12 and Other Non- V617F Mutation Detection, Bone Marrow
      - PV possible
        - Not supportive of PV
    - NEGATIVE
      - ET
      - PMF
      - MPN, not otherwise specified (ET or PMF)
  - YES
    - Positive for JAK2 V617F Mutation
    - Complete blood count (CBC)
    - Clinical findings
    - Bone marrow features
    - EPO / Erythropoietin (EPO), Serum

- YES

- EQUIVOCAL

- Chronic myelogenous leukemia

Positive for BCR/ABL

- Negative or Equivocal for JAK2 V617F Mutation
  - Clinical and morphologic suspicion of MPN
    - HIGH
      - No further testing
    - LOW
      - No further testing

- Negative for JAK2 V617F Mutation
  - Not supportive of PV
  - PV possible
  - PV

POSITIVE

- ET
- PMF
- MPN, not otherwise specified (ET or PMF)

NEGATIVE

- PV
- ET
- PMF
- MPN, not otherwise specified (ET or PMF)

Legend
- PV: Polycythemia vera
- ET: Essential thrombocythemia
- PMF: Primary myelofibrosis
- MPN: Myeloproliferative neoplasm
Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation

Clinical suspicion of myeloproliferative neoplasm

- BM Morph is equivocal
  - Negative or Equivocal for JAK2 V617F Mutation
    - Clinical and morphologic suspicion of MPN
      - HIGH
        - MPLM/MPL Exon 10 Mutation Detection, Bone Marrow
          - POSITIVE
            - ET
            - PMF
            - MPN, not otherwise specified (ET or PMF)
          - NEGATIVE
            - No further testing
      - LOW
        - No further testing
Link to algorithms

www.mayomedicallaboratories.com/articles/resources/index.html
Initial Tests For Patients With Suspected MPNs

- Bone marrow aspirate and biopsy
- Chromosome analysis
- *BCR/ABL* Diagnostic PCR to quickly confirm or exclude CML (FISH can also be used)
- *JAK2* V617F (either PB or BM; not both)
- Then follow the algorithm
Summary

• There are lots of opportunities to develop a test utilization approach in the MPNs

• Use a sequential testing approach that brings together clinician, pathologist, and laboratory

• Understand the benefits and the limitations of genetic testing
Questions & Discussion