Advances in the Diagnosis of Myeloproliferative Neoplasms

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Myeloproliferative neoplasms

- Clonal hematopoietic stem cell disorders
- “Overexuberant” production of one or more hematopoietic cell types
- Erythroid and granulocytic elements generally appear normal, without dysplasia
- Treated differently from other myeloid neoplasms

Discolosures

- Consulting income from Promedior, Inc.
Chronic myeloproliferative neoplasms (WHO 2016)

- Chronic myeloid leukemia, Ph+
- Polycythemia vera
- Essential thrombocytopenia
- Primary myelofibrosis
- Rare entities
  - Chronic neutrophilic leukemia
  - Chronic eosinophilic leukemia/hypereosinophilic syndrome
  - Myeloproliferative neoplasm, unclassifiable

Genetically defined eosinophilic neoplasms

- BCR-ABL
- CSF3R
- JAK2
- MPL
- CALR
- PDGFRA
- PDGFRB
- FGFR1
- PCM1-JAK2

Diagnostic issues with MPN

- Distinguishing MPN from reactive conditions that can produce elevated counts
- Separating MPN from other myeloid neoplasms (MDS and MDS/MPN)
- Providing a specific diagnosis
  - Requires integration of clinical and molecular genetic data with morphology
  - Important in predicting prognosis and dictating therapy
- Recognizing signs of progression

CML

- Hematopoietic stem cell neoplasm associated with \( BCR-ABL1 \) fusion gene
- Patients present with neutrophilic leukocytosis with morphologically normal and maturing granulocytic elements
- Natural history is that of genetic instability, with progressive accumulation of blasts culminating in acute leukemia

The Philadelphia chromosome

- Structurally abnormal chromosome 22
- \( BCR-ABL \) fusion proteins
  - p190
  - p210
  - p230
Sequelae of *BCR-ABL1*

- Growth advantage with progressive colonization of marrow at the expense of normal cells
  - Marked hypercellularity (typically 90-100%)
- Overproduction of granulocytes, eosinophils, and basophils, underproduction of erythroids
- Increased small, hypolobated megakaryocytes
- Decreased cell retention in marrow
  - Leukocytosis with circulating immature myeloid forms
  - Basophilia and eosinophilia
  - Often thrombocytosis
Natural course of CML

- Patients may survive many years with relatively few symptoms
- Inexorably progress to an acute leukemia with loss of differentiation
  - Termed ‘Blast crisis’ or ‘Blast phase’
  - Blast crisis phenotype
    - 70% myeloid (≥20% BM/PB myeloblasts)
    - 30% B-lymphoid (any BM/PB lymphoblasts raises strong suspicion)

CML in the 20th century

- Therapies generally ineffective at delaying progression
- Blast crisis very aggressive with short survival
- Bone marrow transplant offered only cure
Role of pathology in the current era of CML management

- **At the time of initial diagnosis of CML**
  - Get the diagnosis right!
  - Provide prognostic information
- **At later timepoints**, determine any progression and evaluate for other pathologic processes while on therapy

**CML in the 21st century**

- Treated very effectively with tyrosine kinase inhibitors (TKI)
  - Imatinib mesylate, nilotinib, dasatinib, bosutinib, ponatinib
- Disease progression no longer inevitable
- Patterns of disease evolution closely linked to responsiveness (versus resistance) to TKI therapy

**Tyrosine kinase inhibitors**

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**Criteria for accelerated phase**

- Any one or more of the following hematologic/cytogenetic criteria or response-to-TKI criteria:
  - Persistent or increasing WBC (>30 x 10^9/L), unresponsive to therapy
  - Persistent or increasing splenomegaly, unresponsive to therapy
  - Persistent thrombocytopenia (<30 x 10^9/L), unresponsive to therapy
  - Persistent thrombocytopenia (<100 x 10^9/L) unrelated to therapy
  - 20% or more blasts in the peripheral blood
  - 10-19% blasts** in the peripheral blood
  - Resistance to the first TKI
  - Occurrence of two or more mutations in BCR-ABL1 during TKI therapy

**Provisional** Response-to-TKI Criteria

- Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response (CHR) to the first TKI)

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**Additional diagnostic abnormalities in Ph+ cells at diagnosis that include:**

- "Major" hematologic abnormalities
- "Minor" hematologic abnormalities
- Complex karyotype
- Other abnormalities of 5q or 17q
Required at diagnosis

- Bone marrow biopsy and aspirate
  - Reticulin stain to assess baseline fibrosis level
  - Blast count (may be higher in marrow than blood)
- Full karyotype of bone marrow
  - Karyotype/FISH of blood may not pick up all abnormalities
- CBC and review of peripheral smear
  - Blast and basophil count

Caveats with CML diagnosis

- Relatively low M:E ratio in patients with hemoglobinopathies
- Prominent thrombocytosis mimicking ET
- Minimal or no myeloid left-shift in blood
- Monocytosis mimicking CMML
- Blast crisis mimicking AML or ALL
  - Splenomegaly, basophilia, cytogenetic clues help differentiate CML blast crisis from Ph+ AML or B-ALL
    - +8, double Ph, +19, i(17q)
  - Ph+ AML will be a new genetically-defined AML subtype in the 2016 WHO update

Erythroid-rich CML

73 yo man
WBC 13.3, HGB 14.0, PLT 1,483
56% polys
28% lymphs
4% monos
9% basos
2% eos
Chronic neutrophilic leukemia

- Rare MPN with leukocytosis (>25 x 10⁹/L)
  - No dysplasia (hypogranulation) of neutrophils
  - Splenomegaly
  - <10% immature myeloid cells in blood
  - No BCR-ABL1 rearrangement
  - No significant basophilia or eosinophilia
- 83-89% have CSF3R mutation

46, XY, t(9;22)(q34;q11.2) in all 20 metaphases
RT-PCR showed p230 BCR-ABL1 transcript

Chronic neutrophilic leukemia: peripheral blood smear

WBC 36.7 x 10^9/L
HCT 40.0% (MCV 98 fl)
PLT 253 x 10^9/L

82% polys, 14% lymphs, 2% metas, 2% myelos

Chronic neutrophilic leukemia: bone marrow aspirate

Chronic neutrophilic leukemia: bone marrow biopsy
Pathologic differential diagnosis of neutrophilia

<table>
<thead>
<tr>
<th>Disease</th>
<th>Peripheral counts</th>
<th>Neutrophil morphology</th>
<th>Genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML, BCR-ABL1+</td>
<td>↑Granulocytes</td>
<td>Normal</td>
<td>t(9;22); BCR-ABL1</td>
</tr>
<tr>
<td>Atypical CML, BCR-ABL1-</td>
<td>↑Granulocytes</td>
<td>Dysplastic</td>
<td>SETBP1 mutation(30%)</td>
</tr>
<tr>
<td>Chronic neutrophilic leukemia</td>
<td>↑Granulocytes</td>
<td>Normal</td>
<td>CSF3R mutation (90%)</td>
</tr>
<tr>
<td>Primary myelofibrosis</td>
<td>Leukoerythroblastic</td>
<td>Normal</td>
<td>JAK2, MPL, or CALR mutations (90%)</td>
</tr>
</tbody>
</table>

Algorithm for workup of persistent neutrophilia

Possible reactive causes excluded?

- BCR-ABL +
- CML

Significant granulocytic left-shift and dysplasia?

- Atypical CML, BCR-ABL1-
- JAK2 mutation and typical bone marrow findings?
- Primary myelofibrosis

Secondary reactive neutrophilia

- Usually self-limited

- Treant with TKI immediately to prevent progression
- Poor prognosis, difficult to treat
- Several treatment options
- May respond to ruxolitinib

Eosinophilia

- Reactive
  - Allergy, drug, parasitic or other infections
- Paraneoplastic (non-neoplastic eosinophils stimulated by tumor cytokines)
  - Hodgkin lymphoma
  - T-cell lymphomas
    - May be a very small clonal T-cell population (without overt T-cell lymphoma)
  - Systemic mastocytosis
  - ALL with t(5;14)
    - Translocation of IL-3 gene

Neoplastic eosinophilias

- Eosinophils are part of a myeloid stem cell neoplasm
  - CML
  - AML with inv(16)
  - Rare MDS cases
- “Primary” eosinophilias
  - Rearrangement of PDGFRα
  - Rearrangement of PDGFRβ
  - Rearrangement of FGFR1
  - PCM1-JAK2 rearrangement (new entity in 2016)
  - Chronic eosinophilic leukemia, not otherwise specified
Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB, or FGFR1

- Share similar molecular and biologic features
  - Appear to involve pluripotent stem cell with both lymphoid and myeloid differentiation capacity
  - Translocations activate genes encoding tyrosine kinases
  - Eosinophilia is characteristic
- Most entities respond to Gleevec and related tyrosine kinase inhibitors

Myeloid/lymphoid neoplasms with PDGFRA rearrangement

- Patients present with chronic eosinophilia and elevated serum tryptase levels
- Bone marrow mast cells are increased, but are diffuse and not aggregated
- Small interstitial deletion at 4q12 fuses FIPL1 gene to PDGRFA, a tyrosine kinase
  - Cannot be detected by cytogenetics, must request FISH or PCR
  - Should be sought in all cases of idiopathic hypereosinophilia
  - Excellent response (100%) to Gleevec and related TKI

Myeloid/lymphoid neoplasm with PDGFRA rearrangement

Myeloid/lymphoid neoplasms with PDGFRB rearrangement

- Usually resemble CMML
  - Persistent monocytosis
  - Eosinophilia (almost always)
  - Dysplasia in one or more myeloid lineages
- Rearranged PDGFRB at 5q33 (multiple partners)
  - t(5;12) ETV6-PDGFRB most common
- TKI inhibits these fusion proteins and these patients respond to Gleevec therapy
- Evaluation for PDGFRB rearrangement is indicated for cases of CMML with eosinophilia
Myeloid/lymphoid neoplasms with \textit{FGFR1} rearrangement

- Most commonly t(8;13) ZNF198-FGFR1 fusion, but several other partners
- Aggressive disease characterized by
  - T-cell lymphoblastic lymphoma
  - Bone marrow myeloproliferative disease with eosinophilia
- Does not respond to currently available TKIs, but new inhibitors are being developed.

45 year old woman with leukocytosis. WBC 100,000  34% polys, 26% bands, 6% lymphs, 9% eos, 3% metas, 9% myelos.

Karyotype of both bone marrow and lymph node: 46, XX, t(8;13)(p12;q12)(ZNF198-FGFR1)

Diagnosis: Myeloid and lymphoid neoplasm with \textit{FGFR1} rearrangement
Myeloid neoplasms with t(8;9)(p22;q24); PCM1-JAK2

- Eosinophilia, erythroid predominance with left-shift, prominent lymphoid aggregates
- Fibrosis often present, mimicking PMF
- Can rarely present as T- or B-ALL
- Respond to JAK2 inhibitor ruxolitinib
- Added to the group of genetically defined eosinophilic leukemias as a provisional entity

Chronic eosinophilic leukemia (CEL), not otherwise specified

- Persistent blood eosinophilia >1,500/mm^3 with increased bone marrow eosinophils
- Exclusion of all reactive, paraneoplastic, and specific cytogenetic causes of eosinophilia
- Evidence of clonality
  - Clonal cytogenetic abnormality present
  - Increased bone marrow (>5%) or peripheral blood (>2%) blasts (but <20% blasts)
- Classified as hypereosinophilic syndrome if clonality cannot be proven

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**Chronic eosinophilic leukemia, NOS**

**Chronic eosinophilic leukemia, NOS**

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Algorithm for workup of persistent eosinophilia >1.5 x 10^9/L

- Reactive eosinophilia
- Abnormal T-cell clone
- Other lymphoma with eosinophilia
- Clonal eosinophilia due to CML or AML
- Systemic mastocytosis

Screen for secondary causes of eosinophilia

Evaluate peripheral blood & bone marrow

Cytogenetics

Myeloid/lymphoid neoplasm with PDGFRα, PDGFRβ, or FGFR1

Other clonal abnormality or increased blasts

CEL HES

Gotlib J. Curr Opin Hematol 2010;17;117, Johnson R, George TI. Surgical Pathology 2013;6(4):767. Courtesy of Tracy George, University of New Mexico

The JAK2-associated MPNs

- Polycythemia vera (PV)
  - Increased red cell production, panmyelosis, and abnormal megakaryocytes
  - May progress to a fibrotic phase or rarely AML
- Essential thrombocythemia (ET)
  - Sustained thrombocytosis and increased large, atypical megakaryocytes
  - Only rarely progress to a fibrotic phase or AML
- Primary myelofibrosis (PMF)
  - Variable counts at presentation with progressive increase in splenomegaly and marrow fibrosis

The spectrum of megakaryocyte morphology

MDS and CML

Normal/Reactive

Myeloproliferative

The non-CML MPN: Deregulation of the JAK/STAT pathway

Cytokines

Cyclin D1

FGFB, VEGF

Polycythemia vera (2016)

- Increased red cell production
  - Hemoglobin >16.5/16.0 g/dL in men/women or hematocrit >49/48% in men/women
- Bone marrow showing typical PV histology
  - Hypercellular for age
  - Panmyelosis with increased erythroids and megakaryocytes
  - Spectrum of small, medium, and large megakaryocytes with bulbous and hyperlobated nuclei
- JAK2 mutation (98%) or decreased serum EPO levels
“Masked” polycythemia vera

• Some patients have bone marrow findings typical of PV, but do not meet 2008 WHO hemoglobin levels
  — Male ≥18.5 g/dL, female ≥16.5 g/dL
• These patients appear to behave clinically like typical PV and have PV-like morphology
  — Often present with thrombocytosis mimicking ET
• Required hemoglobin/hematocrit levels have been reduced in 2016 update to correctly diagnose these patients


WHO Essential thrombocythemia criteria (2016)

1. Platelet count ≥450 x 10^9/uL
2. Bone marrow biopsy showing typical morphology of ET and no or (rarely) minor increase in reticulin fibers.
3. Not meeting WHO criteria for CML, PV, PMF, MDS, or other myeloid neoplasms

AND

Presence of JAK2, CALR or MPL mutation
or
Presence of another clonal marker
or
Absence of evidence for reactive thrombocytosis

Causes of reactive thrombocytosis

• Non-neoplastic hematologic conditions:
  — Acute blood loss
  — Acute hemolytic anemia
  — Iron-deficiency anemia
  — Treatment of B12 deficiency
  — Rebound effect after treatment for ITP or ethanol-induced thrombocytopenia
• Inflammatory conditions:
  — Rheumatoid disorders
  — Vasculitides
  — IBD
  — Celiac disease
  — POEMS syndrome
• Tissue damage
  — Trauma, MI, thermal burns
• Infections
• Exercise
• Allergic/medication reactions
• Asplenia
Neoplastic hematologic conditions that can present with thrombocytosis

- Other MPNs
  - Polycythemia vera, early stage
  - Primary myelofibrosis, early stage
  - Chronic myelogenous leukemia
- Myelodysplastic syndrome:
  - MDS with isolated del(5q)
  - Refractory anemia with ring sideroblasts and thrombocytosis (RARS-T)

Typical ET morphology

- Normocellular for age
- Increased megakaryocytes with minimal clustering
- Megakaryocytes are large forms
  - Predominantly ‘staghorn’ nuclei with complex lobation and abundant cytoplasm
- Reticulin is not increased
Essential thrombocythemia

‘Myelofibrosis’

- Descriptive term meaning increased collagen deposition in the marrow
  - Measured by reticulin and trichrome stains
- Broad differential diagnosis
  - Any myeloid neoplasm
  - Lymphomas (especially hairy cell leukemia)
  - Metastatic tumors
  - Inflammatory conditions (autoimmune diseases and infections, especially HIV)
  - Primary myelofibrosis

Primary myelofibrosis

- Peripheral blood
  - Variable counts, often decrease as disease progresses
  - Leukoerythroblastic smear
    - Myeloid immaturity + nucleated red cells + teardrop cells
- Bone marrow
  - Hypercellular with increase in all elements
  - Increased, prominently clustered megakaryocytes with **bulbous and hyperchromatic** nuclei
  - Variable but progressive reticulin fibrosis
- Progressive splenomegaly
- Median survival shorter than PV and ET
### Early/prefibrotic primary myelofibrosis

- ET PMF (early-prefibrotic stage)
  - no or only slight increase in age-matched cellularity
  - no significant increase in granulo- and erythropoiesis
  - prominent large to giant mature megakaryocytes with hyperlobulated or deeply folded nuclei, dispersed or loosely clustered in the marrow space
  - no or very rarely minor increase in reticulin fibers

- PMF (early-prefibrotic stage)
  - marked increase in age-matched cellularity
  - pronounced proliferation of granulopoiesis and reduction of erythroid precursors
  - 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
  - no or no significant increase in reticulin fibers

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### Stages of Primary Myelofibrosis

**Evolution** — **Manifestation** — **Transformation**

- **Initial stage**
  - PMF blast phase
  - BM insufficiency

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

- **Parameters**
  - spleen size (cm)
  - erythro-myeloblasts (%)
  - haemoglobin (g/dL)
  - LDH (U/L)
  - thrombocytes (x10^9/L)

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### Primary myelofibrosis, fibrotic phase

- WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues; 4th edition

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*Courtesy of Hans Michael Kvasnicka, University Hospital, Frankfurt*
Primary myelofibrosis, fibrotic phase

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF-0</td>
<td>Scattered linear reticulin with no intersections (crossovers) corresponding to normal bone marrow</td>
</tr>
<tr>
<td>MF-1</td>
<td>Loose network of reticulin with many intersections, especially in perivascular areas</td>
</tr>
<tr>
<td>MF-2</td>
<td>Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of collagen and/or focal osteosclerosis</td>
</tr>
<tr>
<td>MF-3</td>
<td>Diffuse and dense reticulin with extensive intersections and coarse bundles of collagen, often associated with osteosclerosis</td>
</tr>
</tbody>
</table>

*Fiber density should be assessed in hematopoietic (cellular) areas.*
Importance of accurate diagnosis of MPN to inform prognosis and guide therapy

<table>
<thead>
<tr>
<th></th>
<th>PV</th>
<th>ET</th>
<th>PMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemic transformation</td>
<td>3% at 10 years</td>
<td>1% at 10 years</td>
<td>12-30% at 10 years</td>
</tr>
<tr>
<td>Fibrosis progression</td>
<td>15-25%</td>
<td>Rare</td>
<td>100%</td>
</tr>
<tr>
<td>Thrombosis, per 100 patients/year</td>
<td>5.5</td>
<td>1-3</td>
<td>2</td>
</tr>
<tr>
<td>Initial treatment</td>
<td>Phlebotomy +/- HU</td>
<td>None , aspirin +/- HU</td>
<td>Allo-SCT, JAK inhibitors, chemotherapy</td>
</tr>
</tbody>
</table>

Summary

- Myeloproliferative neoplasms have distinctive morphologies and distinctive genetic aberrations
  - Important to correctly diagnose the various MPN diseases, which have different patterns of progression and are treated differently
- The presence of dysplastic features in a patient with cytosis should suggest the possibility of an MDS/MPN overlap disease
  - Generally poorer prognosis than MPN diseases
- Eosinophilic myeloid disorders are characterized by recurrent genetic abnormalities and some are amenable to targeted therapies with TKIs