Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN): Diagnosis and Recent Advances

Sonam Prakash, MBBS
Associate Clinical Professor
Laboratory Medicine, UCSF
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Outline of Talk

- Overview of myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
- Diagnosing the various subtypes of MDS/MPN: case based approach
- WHO 2016 updates for MDS/MPN*


What are MDS/MPN

- Myeloid neoplasms with clinical, laboratory and morphologic features that overlap between myelodysplastic syndromes (cytopenias, morphologic dysplasia) and myeloproliferative neoplasms (increased peripheral counts and marrow cellularity)
- Splenomegaly: significant subset of cases (30-40%)
- Karyotype: normal or abnormalities in common with MDS
- Negative for BCR/ABL1, PDGFRA, PDGFRB, FGFR1 or PCM1-JAK2 rearrangement
- Genes mutated in myeloid neoplasms present in a high proportion of cases: can be helpful for diagnosis in difficult cases

Disclosures

I have nothing to disclose
Subtypes of MDS/MPN

- Chronic myelomonocytic leukemia (CMML)
- Atypical chronic myeloid leukemia, BCR-ABL1–negative (aCML)
- Juvenile myelomonocytic leukemia (JMML)
- Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
- Myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U)

When Do We Consider a Diagnosis of MDS/MPN

- Increased peripheral counts with associated cytopenias and morphologic dysplasia
  - Peripheral blood smear must be reviewed
  - Increased monocytes [≥1 x 10⁹/L] with or without increase in granulocytes: CMML, JMML
  - Increased granulocytes: aCML
  - Increased platelets with anemia: MDS/MPN-RS-T

Case 1

- 74 y.o. male with leukocytosis, anemia and thrombocytopenia
- No splenomegaly
- No history of recent infections

Case 1: Peripheral Blood

WBC 33.5 x10⁹/L, HGB 6.9 g/dl, MCV 98 fl, PLTS 28 x10⁹/L; neutrophils 25% (8.23), lymphocytes: 16% (5.37), monocytes: 54% (18.09), immature granulocytes 5% (1.79), rare blasts.
Case 1: Marrow

Dyserythropoiesis

Dysgranulopoiesis

3% blasts, G:E 8:1, no significant dysplasia in megakaryocytes

Increased monocytic cells

Naphthyl butyrate esterase

Hypercellular marrow

Granulocytic hyperplasia

Additional Studies

- Flow cytometry on the marrow:
  - 4% myeloid blasts, 30% monocytic cells
- Cytogenetics
  - 46,XY,del(20)(q11.2q13.3)[20]
  - FISH negative for BCR-ABL1
Summary of Case 1

- **Proliferative features**
  - WBC 33.5, monocytosis [54%, absolute 18 x 10^9/L]
  - Hypercellular marrow with granulocytic hyperplasia and increased monocytes

- **Dysplastic features**
  - Anemia, thrombocytopenia
  - Dysplasia in erythroid precursors and granulocytes

- **Other findings**
  - Blasts <5% in marrow, rare blasts in peripheral blood
  - Del(20q)

Diagnostic Criteria for Chronic Myelomonocytic Leukemia (CMML) (WHO 2016)

- Persistent PB monocytosis ≥1 X10^9/L, **WITH** monocytes accounting for ≥10% of the white blood cell count ✔
- Not meeting WHO criteria for BCR-ABL1-positive CML, primary myelofibrosis, polycythemia vera or essential thrombocythemia ✔
- No evidence of PDGFRα, PDGFRβ or FGFR1 rearrangement or PCM1-JAK2 (should be specifically excluded in cases with eosinophilia)
- Fewer than 20% blasts in the blood and bone marrow ✔
- Dysplasia in one or more myeloid lineages (✔). If myelodysplasia is absent or minimal, the diagnosis of CMML may still be made if the other requirements are met and
  - an acquired clonal cytogenetic or molecular genetic abnormality is present in hematopoietic cells ✔
  - OR
  - the monocytosis (as previously defined) has persisted for at least 3 months and all other causes of monocytosis have been excluded

Diagnosis

- Chronic myelomonocytic leukemia
  - CMML-1 by WHO 2008 criteria

Cases with monocytosis, minimal dysplasia, normal cytogenetics and no increase in blasts

- Diagnosis of CMML would require:
  - Presence of molecular genetic abnormality in hematopoietic cells
  - OR
  - Persistent monocytosis for at least 3 months and all other causes of monocytosis have been excluded
Somatic Mutations in CMML

- Most commonly mutated genes: SRSF2, TET2, and/or ASXL1 (>80% of cases)
- Other mutations: SETBP1, NRAS/KRAS, RUNX1, CBL, and EZH2
- In the proper clinical context, can be used to support a diagnosis of CMML
  - Many of these mutations can be age-related, must be interpreted cautiously
- ASXL1 and NPM1: Poor prognosis in CMML

WHO 2016 Updates in CMML

- Subtypes of CMML (prognostic significance)
  - Based on blast percentage:
    - CMML-0: <2% blasts in PB and <5% blasts in BM
    - CMML-1: 2 to 4% blasts in PB and/or 5 to 9% blasts in BM
    - CMML-2: 5 to 19% blasts in PB, 10 to 19% in BM, and/or presence of Auer rods

Prognosis in CMML

- Kaplan–Meier curve of survival of CMML 0 versus CMML 1 versus CMML II.


Prognosis in CMML

- Kaplan–Meier curves of cumulative AML evolution of CMML 0, I and II.

WHO 2016 Updates in CMML

- Subtypes of CMML (prognostic significance)
  - Based on WBC count
    - **Proliferative type**: WBC ≥13 x 10(9)/L
    - **Dysplastic type**: WBC <13 x 10(9)/L

Other Things to Remember in CMML

- Rare cases of MPN with associated monocytosis may simulate CMML.
  - A previous documented history of MPN excludes CMML.
  - The presence of classical MPN features in the bone marrow and/or of MPN associated mutations (JAK2, CALR or MPL) tend to support MPN with monocytosis rather than CMML.
- Important to distinguish blast equivalents (myeloblasts, monoblasts and promonocytes) from abnormal monocytes.
Differential Diagnosis of CMML

• Reactive monocytosis: more common than CMML
• Acute myeloid leukemia vs CMML-2
  — Important to distinguish abnormal monocytes from blast equivalents
• Chronic myelogenous leukemia, particularly CML with p190 fusion protein: BCR/ABL must be evaluated
• Lymphoid/myeloid neoplasms with PDGFA, PDGFRB and FGFR1 rearrangements vs. CMML with eosinophilia
• Myelodysplastic syndrome vs. low count CMML:
  — Monocytosis ≥1 X10^9/L, with monocytes accounting for ≥10% of the white blood cell count: CMML

Case 2

• 60 y.o. man who was found to have anemia and thrombocytopenia on a CBC prior to cataract surgery. Within a few weeks he developed marked leukocytosis and was referred to UCSF
• Presence of night sweats, diarrhea and elevated LFTs
• Also found to have hepatosplenomegaly and lymphadenopathy
• No reactive etiology for leukocytosis identified

Case 2: Peripheral Blood

WBC 79.5 x10^9/L, HGB 8.3 g/dl, MCV 89 fl, PLTS 30 x10^9/L; neutrophils 64% (50.88), lymphocytes: 13% (10.33), monocyte 7% (6.16), immature granulocytes 14% (11.13), blasts: 2% (1.59), basophils 0.

Case 2: Marrow (dry tap)

Hypercellular marrow
Marked granulocytic hyperplasia
Decreased megakaryocytes
Virtually absent erythropoiesis
No increase in blasts by CD34 and CD117
Case 2: Marrow

Reticulin: MF2
No collagen fibrosis

Additional Studies

- Karyotype: Normal
- FISH and PCR for BCR-ABL1: Negative
- No evidence of PDGFA, PDGFRB, or FGFR1 rearrangement
- Flow cytometry on marrow: No immunophenotypically abnormal population, no increase in blasts
- Mutational panel for myeloid neoplasms: Negative
- Cervical lymph node biopsy: Extramedullary involvement by myeloid neoplasm

Summary of Case 2

- Proliferative features
  - Leukocytosis (79.5K) with neutrophilia, increased immature granulocytes (14%)
    - Monocytes <10%
  - Hypercellular marrow with granulocytic hyperplasia
- Dysplastic features
  - Anemia, thrombocytopenia
  - Dysgranulopoiesis
- Other findings
  - 2% blasts in PB, no increase in marrow blasts
  - Moderate reticulin fibrosis
  - Normal cytogenetics, flow cytometry, mutational panel

Criteria for atypical chronic myeloid leukemia, BCR-ABL1-negative (aCML)

- ✔ Peripheral blood leukocytosis due to increased numbers of neutrophils and their precursors (promyelocytes, myelocytes and metamyelocytes) comprising ≥10% of leukocytes
- ✔ Dysgranulopoiesis, which may include abnormal chromatin clumping
- ✔ No or minimal absolute basophilia; basophils usually <2% of leukocytes
- ✔ No or minimal absolute monocytosis; monocytes <10% of leukocytes
- ✔ Hypercellular BM with granulocytic proliferation and granulocytic dysplasia, with or without dysplasia in the erythroid and megakaryocytic lineages
- ✔ Less than 20% blasts in the blood and bone marrow
- ✔ No evidence of PDGFA, PDGFRB, or FGFR1 rearrangement, or PCM1-JAK2
- ✔ Not meeting WHO criteria for BCR-ABL1-positive chronic myeloid leukemia, primary myelofibrosis, polycythemia vera or essential thrombocytopenia
Diagnosis

- Atypical chronic myeloid leukemia, *BCR-ABL1* negative.
- Clinical Course:
  - Treated with hydrea, multiple chemotherapeutic agents
  - Progressed to AML 4 months after initial diagnosis
  - Developed neutropenic sepsis, DIC and passed away 5 months after diagnosis

What if Some of the Features for aCML are Borderline

- Dysgranulopoiesis is present but not marked
- Borderline increase in immature granulocytes
  - aCML or chronic neutrophilic leukemia (CNL) or MDS/MPN-U

WHO 2016 Updates for aCML

- *SETBP1* and/or *ETNK1* mutations present in up to a third of cases.
- MPN-associated driver mutations (*JAK2, CALR, MPL*) are typically absent in aCML.
  - A previous history of MPN, classical MPN morphology in the bone marrow and/or MPN-associated mutations (*JAK2, CALR or MPL*) tend to exclude a diagnosis of aCML
- The presence of a *CSF3R* mutation is uncommon in aCML
  - If present, chronic neutrophilic leukemia should be excluded

Salient Features of CMML, aCML and CNL

<table>
<thead>
<tr>
<th></th>
<th>Chronic myelomonocytic leukemia (CMML)</th>
<th>Atypical chronic myeloid leukemia (aCML)</th>
<th>Chronic neutrophilic leukemia (CNL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WBC</strong></td>
<td>Variable</td>
<td>Increased</td>
<td>Markedly increased ≥25 × 10⁹/L</td>
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<tr>
<td><strong>Monocytes</strong></td>
<td>≥1 x 10⁹/L and ≥10%</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
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<tr>
<td><strong>Neutrophils</strong></td>
<td>Variable</td>
<td>Increased</td>
<td>Markedly increased</td>
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<tr>
<td><strong>Immature granulocytes</strong></td>
<td>Variable</td>
<td>≥10%</td>
<td>&lt;10%</td>
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<tr>
<td><strong>Platelets</strong></td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
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<tr>
<td><strong>Hemoglobin</strong></td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>Dysplasia</strong></td>
<td>Variable</td>
<td>Dysgranulopoiesis</td>
<td>Absent</td>
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<tr>
<td><strong>Cyto genetics</strong></td>
<td>+8, chr 7 abnormalities, i(17q)</td>
<td>+8, del(20q), +13</td>
<td>+8, +9, +21, del(20q)</td>
</tr>
<tr>
<td><strong>Somatic mutations</strong></td>
<td><strong>SRSF2, TET2, ASXL1</strong></td>
<td><strong>SETBP1, ETNK1</strong></td>
<td><strong>CSF3R</strong></td>
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Case 3

- 54 y.o woman presented with dizziness and shortness of breath.
- CBC Hgb 10.8, MCV 89, WBC 9.0, platelets 980K.
- Normal iron studies
- No history of prior therapy for anemia
- On PB: JAK2 V617F positive; BCR-ABL1 negative
- No splenomegaly
- Clinical impression: essential thrombocythemia (ET)
- She had a bone marrow to confirm the diagnosis

Case 3: Peripheral Blood

WBC 9.3 x10E9/L, HGB 9.8 g/dl, MCV 88 fl, PLTS 910 x10E9/L; neutrophils 74% (6.87), lymphocytes 20% (1.86), monocytes 3% (0.28), eosinophils 2% (0.18), basophils 1% (0.9). No circulating blasts.

Case 3: Marrow

Increased megakaryocytes
Megaloblastic changes in erythroids

60% cellularity
Large atypical megakaryocytes

G.E 1:2
Megaloblastic erythroid precursors
Blasts <5%
No significant dysplasia in granulocytes
Case 3: Marrow

>15% ring sideroblasts

Case 3: Additional Studies

- Normal karyotype
- Flow cytometry: no increase in blasts, no immunophenotypically abnormal cell population

Summary of Case 3

- Proliferative features:
  - Thrombocytosis (Plt 910K)
  - Increased megakaryocytes with morphology similar to that seen in essential thrombocythemia
  - Positive for JAK2 mutation
- Dysplastic features:
  - Anemia
  - Dyserythropoiesis with >15% ring sideroblasts
- Additional findings:
  - Normal karyotype
  - No increase in blasts

Diagnostic Criteria for Myelodysplastic/Myeloproliferative Neoplasm with Ring Sideroblasts and Thrombocytosis (MDS/MPN-RS-T)

- Anemia associated with erythroid lineage dysplasia with or without multilineage dysplasia
- >15% ring sideroblasts, <1% blasts in peripheral blood and <5% blasts in the bone marrow
- Persistent thrombocytosis with platelet count ≥450 x 10^9/L
- Presence of a SF3B1 mutation or, in the absence of SF3B1 mutation, no history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myeloproliferative features
- No BCR-ABL1 fusion gene, no rearrangement of PDGFRA, PDGFRB or FGFR1 or PCM1-JAK2; no t(3;3)(q21;q26), inv(3)(q21q26) or del(5q)
- No preceding history of MPN, MDS (except MDS-RS), or other type of MDS/MPN

≥15% ring sideroblasts required even if SF3B1 mutation is detected

**A diagnosis of MDS/MPN-RS-T is strongly supported by the presence of SF3B1 mutation together with a mutation in JAK2 V617F, CALR or MPL genes**
Myelodysplastic/Myeloproliferative Neoplasm with Ring Sideroblasts and Thrombocytosis (MDS/MPN-RS-T)

- Formerly called refractory anemia with ring sideroblasts and associated thrombocytosis
- Was a provisional category in 2008 WHO under MDS/MPN-U
- WHO 2016 update: recognized as a distinct subtype of MDS/MPN

Biological Explanation for MDS/MPN-RS-T

- Mutations in the spliceosome gene SF3B1 (~85% of cases): dysplastic features, anemia, presence of ring sideroblasts
- Mutations in JAK2 V617F (50% of cases) or less frequently (<5%) in CALR, or MPL genes: proliferative features, thrombosis

Clinical Course and Prognosis of MDS/MPN-RS-T

- Overall good prognosis: in between MDS-RS and ET
- Thrombotic events (20%)
- Leukemic transformation (2%)
- Proposed prognostic indicators:
  - Unfavorable:
    - Age >80 years, SF3B1wt, JAK2wt
    - Abnormal karyotype, ASXL1 and/or SETBP1 mutations, HB < 10 gm/dl.

MDS/MPN-U

- Case has features of MPN and MDS but does not meet criteria for any of the defined MDS/MPN subtypes
- Should not be used for patients with previously diagnosed MPN who subsequently develop dysplastic features: aggressive phase of MPN
- Very few studies on MDS/MPN-U: poor prognosis with an OS of 12.4 months from presentation (n=85)*

Case 4

• 2 year old boy with failure to thrive, epistaxis and hepatosplenomegaly
• Past history significant for CMV infection and EBV PCR greater than 300,000 copies
• CBC showed monocytosis and thrombocytopenia
• Transferred to UCSF for further evaluation

Case 4: Peripheral Blood

WBC 14.1 x10^9/L, HGB 10.4 g/dl, MCV 90 fl, PLTS 32 x10^9/L; Neutrophils 13%, lymphocytes 29%, monocytes 55% (Abs 7.7), eosinophils 1%, immature granulocytes 2%, rare blast.

Case 4: Marrow

Dyserythropoiesis, Increased monocytic cells, Dysplastic megakaryocyte, <5% Blasts
Additional Studies

• Flow cytometry: No increase in blasts (2%); atypical monocyte population (39% of total events)
• Cytogenetics: Normal karyotype; BCR-ABL1 negative
• Hgb F: Increased
• Molecular studies: Positive for NRAS mutation

Summary of Case 4

• Proliferative features:
  – Monocytosis (55%, abs 7.7)
  – Increased monocytic cells in the marrow
• Dysplastic features
  – Mild anemia with dyserythropoiesis
  – Thrombocytopenia
    • Markedly decreased megakaryocytes (dysplastic)
• Other findings
  – Hepatosplenomegaly
  – Normal karyotype
  – Elevated Hgb F
  – NRAS mutation

Diagnostic Criteria for Juvenile Myelomonocytic Leukemia (JMML)

• I. Clinical and hematologic features (all 4 features mandatory) ✔
  – Peripheral blood monocyte count > 1x10^9/L ✔
  – Blast percentage in peripheral blood and bone marrow <20% ✔
  – Splenomegaly ✔
  – Absence of Philadelphia chromosome (BCR/ABL1 rearrangement) ✔
• II. Genetic studies (1 finding sufficient) ✔
  – Somatic mutation in PTPN11 or KRAS or NRAS ✔
  – Clinical diagnosis of NF1 or NF1 mutation
  – Germline CBL mutation and loss of heterozygosity of CBL
• III. For patients without genetic features, besides the clinical and hematologic features listed under I, the following criteria must be fulfilled: ✔
  – Monosomy 7 or any other chromosomal abnormality
    OR at least 2 of the following criteria:
    – Hemoglobin F increased for age ✔
    – Myeloid or erythroid precursors on peripheral blood smear ✔
    – GM-CSF hypersensitivity in colony assay
    – Hyperphosphorylation of STAT5

Case 4: Diagnosis

• Juvenile myelomonocytic leukemia (JMML)
Why is Diagnosing JMML Challenging

- **Reactive conditions** often present with monocytosis
- Morphologic dysplasia may be minimal and can also be seen in reactive conditions
- Many patients with JMML have a normal karyotype
- In a pediatric patient with sustained monocytosis, additional findings of circulating blasts, thrombocytopenia, and decreased marrow megakaryocytes are useful parameters to suggest additional work-up for JMML
  - Mutational panel for JMML may be helpful in difficult cases (~90% of patients carry mutations of **PTPN11**, **KRAS**, **NRAS**, **CBL** or **NF1**)

Do All Cases with Monocytosis with RAS Mutations Represent JMML

- Ras-associated autoimmune leukoproliferative disorder (RALD)*
  - Recently described indolent disease entity in patients with autoimmune lymphoproliferative syndrome (ALPS)
  - Clinical and laboratory features overlap with those of JMML
    - Persistent monocytosis often associated with leukocytosis
    - RAS (KRAS or NRAS) mutations in all patients
- Indolent clinical course suggests that these cases are distinct from JMML.
- Has only been described in the context of ALPS patients.


Conclusions

- Think about MDS/MPN in a patient with proliferative counts with dysplasia or cytopenia
- A peripheral blood smear must be reviewed
- Splenomegaly (if present) is helpful in this diagnosis
- When in doubt about the diagnosis, it is okay to be descriptive
- Molecular studies can be helpful in the appropriate clinical context