Approaching myeloid neoplasms: diagnostic algorithms

Alexandar Tzankov
Content

• Integration of clinical and laboratory data
• Bone marrow evaluation approaching
  – Myeloproliferative neoplasms
  – Neoplasms with eosinophilia
  – Myelodysplastic syndromes
  – Myelodysplastic/myeloproliferative neoplasms
  – Acute leukemias
• Caveats and pitfalls
• Conclusion
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Integration of clinical and laboratory data

• First (and most important) part of an integrative diagnostic approach

• Careful study of the clinical question and history
  – Is there a hematological abnormality?
  – Does the patient have any symptoms?
  – Is there a specific medication or infectious history?
  – Does the patient have an underlying disorder (HIV, collagenosis)?
  – Does the patient have a previous history of cancer?
5q⁻ like effects of azathioprine
Reading hemograms

- Is there a cytopenia? Selective or pancytopenia?
  - prototypic of MDS, almost excludes cellular phase MPN
- Is there a cytosis?
  - prototypic of MPN
  - thrombocytosis – pronounced in ET, PMF, RARS-T, CML, 5q-
  - neutrophilia – pronounced in CML, CNL, occ. CMML
  - eosinophilia – prototypic in MLNE
  - basophilia – pronounced in CML
  - monocytosis – prototypic in CMML
- Are there anisopoikilocytosis, leukoerythroblastosis, makrocytosis, blasts in the peripheral blood?
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Bone marrow biopsy findings

• Cellularity
  – hypercellularity – common to all myeloid disorders
  – hypocellularity – hypocellular MDS/AML (spent MPN)

• Fibrosis
  – prototypic of PMF; exclude myeloma, renal failure, mastocytosis
  – poor risk MDS-f

• Osteosclerosis
  – prototypic of PMF; exclude myeloma, renal failure, mastocytosis

• Dilated sinuses
  – prototypic of PMF, may be seen after chemotherapy
Reticulin and collagenous fibrosis
Dilated sinuses with hematopoiesis in MPN
Bone marrow biopsy findings

- Abnormal megakaryocytes
  - increased in MPN, some MDS (RCMD), 5q-
    cave: consumption, inflammation, hepatopathy
  - clusters – prototypic of MPN
  - hyperlobated - prototypic of MPN
  - hypolobated – typical in MDS, 5q-

- Erythropoiesis
  - increased, dysplastic (and ineffective) in MDS
  - (relatively) decreased in CML, almost lacking in AML
  - bizarrely vascularized nodules in JAK2 translocated MDS-MPN
Clustered megs in a paraneoplastic state
Dysplastic megs in MDS
MDS-MPN, U with t(8;9), PCM1-JAK2 fusion
Bone marrow biopsy findings

• Abnormal myelopoiesis
  – increased in all myeloid neoplasias
    cave: stimulation (CSFs), inflammation, drugs
  – monocytoid islets – typical of CMML & poor prognosis MPN
  – ALIP – typical of MDS & transforming MPN

• Blasts
  – prototypic of AML
    cave: regenerative paratrabecular blasts

• Stainable iron
  – lacking in MPN, particularly PV
  – often increased in MDS
Neoplastic myelopoietic hyperplasia
Myelopoietic hyperplasia due to G-CSF
Monocytoid nodules in MPN
Integration of genetic findings (selection)

- **BCR-ABL1** fusion products proof CML (ALL)
- **PDGFRA, PDGFRB** and **FGFR1** rearrangements proof MLNE
- Isolated del(5q), inv(3) and t(6;9) proof MDS (AML)
- t(15;17), t(8;21) and inv(16) proof AML, even if blasts <20%
- **JAK2, CALR** and **MPL** mutations support MPN diagnosis
- **CSF3R** mutations support (define?) CNL and aCML
- **SF3B1** mutations 98% PPV for RARS, RCMD-RS or RARS-T
- **SRSF2** mutations support CMML or poor risk-MDS diagnosis
- **FLT3** and **NPM1** add prognostic information in AML
- Etc. ...
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Approaching MLNE

- Peripheral blood eosinophilia >1.5x10^9/L
- Organ damage symptoms (heart, lung)
- Integrate molecular genetics
  - *PDGFRA, PDGFRB, FGFR1, t(5;14), inv(16), T-cell clonality*
  - if all negative and >5% BM blast → CEL/<%5 blasts → HES
- CMML-like presentation of *PDGFRB, t(5;12)* cases
  - Imatinib-sensitive
- CNL/CEL/CML/ALL-like presentation of *PDGFRA* cases
  - Imatinib-sensitive
- CEL/AML/ALL/ABL-like presentation of *FGFR1, t(8;13)* cases
  - Imatinib-resistant
Prototypic MLNE features
Diagnostic algorithm in eosinophilia

unexplained eosinophilia

- consider benign reactive causes, especially allergy, drug reaction, pneumonitis, infection
- consider malignant reactive causes, especially T-cell lymphoma (or abnormal T-cell subsets), cHL

conventional karyotype

- 8p11 or 5q31-33 rearrangement

MLNE

- PGFRA rearrangement by FISH

morphology consistent with CML but t(9;22) negative

- apparent CMML
- with eosinophilia
- apparent AML
- with eosinophilia
- apparent ALL
- T-ALL with eosinophilia
- B-ALL with eosinophilia

apparent SM, but KIT D816V negative

Foucar et al 2010
• Integration of clinical and laboratory data
• Bone marrow evaluation approaching
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Approaching MDS

• Peripheral blood cytopenia (± dysplastic features)
  – exclude non-MDS causes
  – macrocytic anemia - typical
  – thrombocytosis in 5q-

• Clinico-pathological correlations
  splenomegaly – not a typical feature

• Integrate molecular genetics

  cytogenetics ... (NGS) e.g. Leuk Res 2015;39:6-17
  TP53 testing in 5q- because of Lenalidomide resistance (?)
Bone marrow biopsy findings

• Hypercellularity, rarely hypocellularity

• Abnormal megakaryocytes (>10% significant)
  – increased
  – paratrabecular
  – small sized
  – hyperchromatic
  – hypolobated - prototypic of MDS, 5q-
  – nuclear separation (aspirate)
  – CD34+

• Erythropoiesis increased with colony formation loss
Bone marrow biopsy findings

- Abnormal myelopoiesis
  - ALIP – diagnostic and prognostic
- Blast increase
  - % critical for classification
  - consider counting out of non-erythroid cells in erythroid-predominant cases/gray zone to acute erythroid leukemia
- Fibrosis
  - prognostically relevant in MDS
- Increased stainable iron
Prototypic MDS features
Special cases

• Hypoplastic MDS
  – females and children more commonly affected
  – DD: AA, HCL, T-LGL, toxic myelopathy

• Therapy-related myeloid neoplasms
  – alkylating agents/RTX – long latency, complex karyotypes
    • multilineage dysplasia and fibrosis
  – topoisomerase II inhibitors – brief latency, *MLL* rearrangements
    • monocytic differentiation
  – distinction between AML, MDS and subtypes irrelevant
Hypoplastic MDS
Therapy-related myeloid neoplasm
Caveats and pitfalls

- Cytopenias with marrow hypercellularity
  - collagenoses (ALIP might be present)
  - chronic infections
  - HCL
  - T-LGL
  - constitutional and congenital disorders

- Cytopenias with dysplasia
  - vitamin $\text{B}_{12}$/folic acid deficiency
  - HIV-myelopathy
  - toxic myelopathy (azathioprine, chemotherapy, valproate...)
  - constitutional and congenital disorders
T-LGL “mimicking” MDS
Azacitidine-induced changes
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Approaching MDS-MPN

• Peripheral blood
  – at least one lineage “penia” and one “cytosis”
  – blasts <20%

• Clinico-pathological correlations
  – splenomegaly
  – extramedullar disease
  – neurofibromatosis type 1 (associated with JMML)

• Integrate molecular genetics

  other-disease-defining mutations preclude MDS-MPN
  
  BCR-ABL1-fusion, PDGFRA-, PDGFRB-, FGFR1-rearrangement, del(5q), inv(3)

  NRAS-mutations and monosomy 7 common in JMML

  ASXL1, CBL, SETBP1, TET2 and NRAS-mutations common in CMML
Bone marrow biopsy findings

• Hypercellularity

• Abnormal myelopoiesis
  – increased
  – ALIP
  – monopoietic increase (CD11c, **CD14**, CD68, CD163)
    • >20% CD14\(^+\) only in CMML/AMML (Rollins-Raval et al. 2012)
  – blasts <20%
  – plasmacytoid dendritic cell nodules (CD123)

• Dysmegakaryopoiesis

• Sea-blue or tingible-body histiocytes
Prototypic MDS-MPN features

Orazi et al. 2006
The waste-basket problem in MDS-MPN

Myelodysplastic/myeloproliferative disease with erythropoietic hyperplasia (erythroid preleukemia) and the unique translocation (8;9)(p23;p24): first description of a case

Simone Heiss MD, Martin Erdel PhD, Eberhard Günsilius MD, David Nachbaur MD, Alexandar Tzankov MD.

Atypical chronic myeloid leukaemia, BCR-ABL1 negative

J.W. Vardiman
J.M. Bennett
B.J. Bain
R.D. Brunning
J. Thiele

Some cases of t(8;9)(p22;p24) with the PCM1-JAK2 fusion gene have been reported as “aCML” (259, 1833) but data currently available suggest they have eosinophilia and lack myelodysplasia and may be better regarded as chronic eosinophilic leukaemia. Meticulous description of the morphology of atypical myeloid proliferations associated with various genetic defects will be necessary to assign them to appropriate categories.
Deciphering molecular mechanisms

- **RARS-T**
  - 2001 provisional $\rightarrow$ 2008 distinctive MDS-MPN $\rightarrow$ 2015 ?

- **SF3B1** - novel molecular diagnostic tool in MDS
  - 98% PPV for RARS, RCMD-RS or RARS-T
Caveats and pitfalls

• Non-neoplastic disorders
  – alcoholism
  – chronic viral infections
  – collagenoses
  – growth factor application/growth-factor producing neoplasms

• Other myeloid neoplasms
  – MDS, MLNE, MPN ... AML
Integration of clinical and laboratory data

Bone marrow evaluation approaching
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Caveats and pitfalls

Conclusion
Approaching AML

- **Peripheral blood**
  - blasts >20%
  - blasts <20% and t(15;17), t(8;21), inv(16) (±eosinophilia)

- **Clinico-pathological correlations**
  - splenomegaly
  - extramedullar disease
  - previous chemotherapy or radiation exposure
  - constitutional disorder (e.g. Down syndrome)
  - hematological disorder (e.g. Mono-MAC syndrome)
  - application of growth factors

- **Integrate aspirate’s, FACS’ and molecular genetics’ results**
**Bone marrow biopsy findings**

- Hypercellularity, rarely hypocellularity
- Blast enumeration
  - in cases of fibrosis/necrosis or marked hypocellularity - trephines
  - CD34, but promyelocytes CD34⁻ (and HLA-DR⁻), $NPM1^{\text{mut}}$ CD34⁻
  - MPOX
  - CD33, CD117, HLA-DR – valuable complementary markers
  - CD11c (CD4, CD56) for monoblasts
  - E-cadherin for erythroblasts
    - to be subtracted if >50% and non-erythroid AML
  - CD61 for megakaryoblasts
  - Cave: PAX5, CD19 and CD79a – t(8;21)⁺ and CD2 in t(15;17)⁺ cases
The advantage of E-cad to glycophorins
Prototypic AML features
Caveats and pitfalls

- Non-neoplastic disorders
  - growth factor application/growth-factor producing neoplasms
- Hypocellular AML \( \rightarrow \) trephines!
- AML with fibrosis/necrosis –trephines!
- Difficulties in DD erythroid AML(M6a)/MDS-related AML
  - if dysplasia prominent and/or in 2 lineages and/or MDS-typical cytogenetic changes \( \rightarrow \) AML with MDS-related changes
- Difficulties in DD AMML/microgranular APL
  - strong MPOX\(^+\), CD2\(^+\) (and CD34\(^+\)), ↑ leukocytosis \( \rightarrow \) consider APL
- Other neoplasms
  - ALL – CD3 for T-/CD19 and another B-cell marker for B-lineage
“Evolution” (04.2008)
• Integration of clinical and laboratory data

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• Caveats and pitfalls

• Conclusion
Conclusion

- Study the clinical question and history
- Read the hemogram
- Integrate clinical and laboratory data
- Always consider reactive changes
  - actively verify/falsify this possibility
- Systematically assess
  - cellularity and fibrosis
  - all hematopoietic lineages incl. mast cells
  - apply accessory techniques (pheno-/genotypization)
- Verify/falsify differential diagnoses
- Be aware of pitfalls
- Don’t worry giving a descriptive report if unconfident
Clustered hyperlobated megs in MPN
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Approaching MPN

• Peripheral blood “cytosis”
  – exclude non-MPN causes
  – basophilia – typical of CML, can be seen in other MPN
  – leukoerythroblastosis – prototypic of MPN
  – eosinophilia → exclude MLNE
  – monocytosis → exclude CMML

• Clinico-pathological correlations
  splenomegaly? LDH? EPO?

• Integrate molecular genetics
  \textit{BCR-ABL1, JAK2, MPL, CALR, CKIT, CSFR3} ...
Bone marrow biopsy findings

• Hypercellularity, except for ET
• Abnormal megakaryocytes
  – increased
  – clustered – prototypic of $BCR-ABL1^-$ MPN
  – hyperlobated - prototypic of $BCR-ABL1^-$ MPN
  – small to micromegakaryocytes - prototypic of CML
• Abnormal myelopoiesis
  – increased
  – monocytoid islets – poor prognosis MPN
  – ALIP - transforming MPN
• Relative decrease of erythropoiesis – typical of CML
Bone marrow biopsy findings

- **Fibrosis**
  - prototypic of MPN and prognostically relevant
  - rarely seen in ET → reconsider PMF
- **Osteosclerosis** - prototypic of PMF and SM
- **Dilated sinuses** - prototypic of PMF
- **Spindled mast cell aggregates** – major criterion of SM
- **Decreased stainable iron**, particularly in PV
- **Sea blue histiocytes** - in all high turnover-disorders
Prototypic MPN features
Revised criteria for **BCR-ABL1⁺ MPN**

Table 4. 2014 proposed revision for World Health Organization (WHO) diagnostic criteria for **BCR-ABL1-negative myeloproliferative neoplasms**

<table>
<thead>
<tr>
<th>Polycythemia vera (PV)ᵃ</th>
<th>Essential thrombocytethemia (ET)ᵇ</th>
<th>Primary myelofibrosis (PMF)ᶜ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major criteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Hemoglobin &gt; 16.5 g/dl (men) &gt; 16 g/dl (women) or hematocrit &gt; 49% (men) &gt; 48% (women)</td>
<td>Platelet count ≥450 x 10⁹/l</td>
<td>Presence of JAK2, CALR or MPL mutation</td>
</tr>
<tr>
<td>2 BM trilineage myeloproliferation with pleomorphic megakaryocytes</td>
<td>Megakaryocyte proliferation with large and mature morphology</td>
<td>Not meeting WHO criteria for CML, PV, ET, MDS or other myeloid neoplasm</td>
</tr>
<tr>
<td>3 Presence of JAK2 mutation</td>
<td>Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm</td>
<td>Presence of JAK2, CALR or MPL mutation</td>
</tr>
<tr>
<td>4 Presence of JAK2, CALR or MPL mutation</td>
<td>Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive thrombocytosis</td>
<td>Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive bone marrow fibrosis</td>
</tr>
</tbody>
</table>

**Minor criteria**

1 Subnormal serum erythropoietin level
2
3

Abbreviations: BM, bone marrow; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome. ᵃPV diagnosis requires meeting either all three major criteria or the first two major criteria and one minor criterion. ᵇET diagnosis requires meeting all four major criteria or first three major criteria and one minor criterion. ᶜPMF diagnosis requires meeting all three major criteria or the first two major criteria and all three minor criteria. ᵈSmall-to-large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering. In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (that is, prefibrotic PMF). ᵉDegree of abnormality can be borderline or marked and institutional reference range should be used for lactate dehydrogenase level.

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Caveats and pitfalls

- Leukemoid reactions
  - lack basophilia and molecular aberrations
- Rebound reactive “cytoses” and “myeloses”
  - peripheral consumption
  - para-neoplastic/para-inflammatory
  - medication (EPO, THPO, G-CSF)
  - Fe-deficiency
- MDS, MDS-MPN
  - 5q−, RARS-T
- Autoimmune myelofibrosis, chronic renal failure, gray platelet syndrome, Mb. Paget, previous biopsy site, SM
Fibrosis – misleading out of context
Osteosclerosis – misleading out of context
CNL – rare and prone to misinterpretation

- Neutrophilia and organomegaly
- Bone marrow hyperplasia with complete maturation
- Dilated sinuses
- Granulocytic phagocytosis by histiocytes
- CSF3R mutations - disease defining
- Myelomas – accompanied by CNL-like changes
- Other DD: p230 variant CML, other MPN, CMML, aCML