Diagnostic Approach for Eosinophilia and Mastocytosis

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DISCLOSURES:

Relevant Financial Relationship(s)
None

Off Label Usage
None
Molecular Classification of Myeloproliferative Neoplasms

Molecularly defined

MPD-eos

MPD-eos

Molecularly characterized with therapeutic validation

CML

BCR-ABL

MPD-eos

MPD-eos

MPNs with recurrent genetic markers

PV

JAK2

SM

KIT

SCLL

FGFR1

Molecularly assigned without therapeutic validation

ET

JAK2 /CALR /MPL

PMF

JAK2 /CALR /MPL

CNL

CSF3R

JMML

RAS/PTPN11/NF1GFR1

Clinicopathologically assigned

CEL

HES

CBL

MPN-NOS

CMML*

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MPD-eos $^{PDGFRA}$

MPD-eos $^{PDGFRB}$

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CMML $^{RAS/PTPN11/NF1/GFR1}$

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Clinicopathologically assigned

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CMML$^*$
Increased Eosinophils or Increased Mast Cells?

Malignant

Tumor burden?

Imatinib-responsive disease?

Benign
Increased Eosinophils

Secondary / Reactive
- Drugs, allergies
- Helminth, fungal
- Allergy, inflammatory
- Neoplasia (HL, PTCL, MPN, MDS)
- Mast cell disease

Clonal
- Molecular or cytogenetic abnormality
- BM morphologic features of a myeloid disorder

Idiopathic
- If AEC > 1500 x 6 months and end-organ damage

Primary

Clonal
- IPH of T-cells
- Clonal T-GR

CEL

HES

LV-HES
Increased Eosinophils

Malignant

- Atypical eosinophils or mast cells?
- Mast cell infiltrate – bx / IHC?
  - Confirmatory studies
  - Bone marrow, genetics, etc.

Tumor Burden

- Amount of marrow involvement
- Associated hematologic disease?

Clinical:
- Splenomegaly, pancytopenia, osteoporosis
- End-organ damage

Imatinib-responsive?

PDGFR-A or PDGFR-B present?
- Imatinib-responsive
  - KIT D816V present?
  - Imatinib non-responder

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Assess Morphology

• Increased eosinophils and precursors only
  • Normal bone marrow with increased eosinophils
  • Take care in assessing eosinophil cytology
  • Abnormal forms may be found in secondary causes
  • Eosinophil cytology alone should not be used to indicate clonal eosinophilic disease
Genetics of the Eosinophilias

- **PDGFRA**
  - *FIP1L1-PDGFR* fusion
  - del(4q12)

- **PDGFRB**
  - *PDGFRB* with variable translocation partners
  - t(5;var)(q31~q33;var)

- **FGFR1**
  - *FGFR1* with variable translocation partners
  - t(8;var)(p11;var)
Genetics of the Eosinophilia

- **PDGFRα**
  - *FIP1L1-PDGFRα* fusion
  - del(4q12)

- **PDGFRβ**
  - *PDGFRβ* with variable translocation partners
    - t(5;var)(q31~q33;var)

- **FGFR1**
  - *FGFR1* with variable translocation partners
    - t(8;var)(p11;var)
Chronic eosinophilic leukemia, not otherwise specified (CEL, NOS)

- Persistent eosinophil count greater than $1.5 \times 10^9/L$ (greater than 6 months)
- No $BCR-ABL1$, $PDGFR-A$, $PDGFR-B$, $FGFR1$, or $inv(16)$ abnormalities
- Less than 20% blasts
- Clonal cytogenetic abnormality, or increase in blasts but less than 20%
  - Something to “hang your hat on” as malignant
Idiopathic Hypereosinophilias

- Idiopathic Hypereosinophilia
  - Persistent eosinophil count greater than $1.5 \times 10^9$/L (greater than 6 months)
  - No identifiable secondary causes
  - No myeloid neoplasms or mast cell disease
  - No phenotypically aberrant T-cell population
- Idiopathic Hypereosinophilic Syndrome
  - Everything above, and end-organ tissue damage due to eosinophils
Idiopathic HES
Lymphocytic variant of hypereosinophilic syndrome (LV-HES)

- Cytokine-driven eosinophilia due to overproduction of eosinophil growth factors by T-cells
- CD3(-), CD4(+), CD5(+), CD7(-) phenotype
- Cutaneous manifestations
- Often responsive to steroid therapy
Lymphocytic variant of hypereosinophilic syndrome (LV-HES)

- Morphology subtle
  - Eosinophilia
  - Small lymphocytes in peripheral blood
  - Slight nuclear irregularities
  - Difficult to identify without lymphocytosis
  - Minimal lymphoid infiltrate in marrow
- Flow cytometric identify unique phenotype
- Clonal T-cell receptor gene rearrangement
sCD3(-), CD2+, CD4+, CD5+, CD7(-)
Increased eosinophils only –
No other disease process

Remember to order:
• Chromosomes
• FISH for PDGFR-A
• PB flow with T-cell panel
• PB T-cell GR
• IHC for tryptase / CD25
• KIT D816V

Not necessary to do:
• BCR-ABL-1
• JAK2, CALR
• PDGFR-B FISH
• FGFR1 FISH
• Mast cell flow studies
Bone Marrow Evaluation for Blood Eosinophilia

- History of sustained elevation: >1.5 × 10^9/L

Assess Morphology

BM with only increased eosinophil precursors

- **DON'T DO:**
  - JAK2 V617F
  - BCR/ABL FISH or PCR
  - FISH for PDGFRB
  - FISH for FGFR1
  - Mast cell flow cytometry

ORDER:
- IHC for tryptase & CD25
- KIT D816V
- Chromosomes
- FISH for PDGFRB
- PB flow with T panel
- PB TCR rearrangement

- Clonal myeloid cytogenetic abnormality present
  - t(5;12)(q31-33;p12) or related?
  - Yes: Order tests appropriate for underlying disease; use pertinent algorithm if available
  - No: Translocation/insertion involving 8p11.2, FGFR1

PDGFRA abnormal (i.e. CHIC2 deletion)

- Yes: Follow mastocytosis algorithm
  - Yes: Diagnosis: MPN with eosinophilia associated with PDGFRB rearrangement
  - No: Diagnosis: Myeloid neoplasm with PDGFRB rearrangement

- No: Other clonal cytogenetic abnormality

- Yes: Diagnosis: Eosinophilic leukemia
  - Yes: Diagnosis: Chronic eosinophilic leukemia
    - 1. Reactive eosinophilia
    - 2. Idiopathic hyper eosinophilia
    - 3. Idiopathic HES

- No: Diagnosis: Lymphocytic Variant HES
  - Diagnosis: Eosinophilia associated with a phenotypically abnormal T cell population
  - Diagnosis: Eosinophilia associated with clonal TCR rearrangement of uncertain significance
  - Refer to rest of algorithm
Increased Mast Cells

Malignant

- Spindled mast cells – asp & bx
- Mast cell infiltrate – bx / IHC
- Confirmatory studies

Tumor Burden

- Amount of marrow involvement
- Associated hematologic disease

Clinical:
- Splenomegaly, pancytopenia, osteoporosis

Imatinib-responsive?

KIT D816V present?
- Imatinib non-responder
Current SM Diagnostic Criteria (WHO 2008)

- **Major**
  - Multifocal dense aggregates of mast cells (>15 mast cells)

- **Minor**
  - >25% of mast cells are spindled or atypical
  - Activating point mutation at 816 codon in *KIT*
  - Mast cells express CD2 and/or CD25
  - Serum total tryptase ≥20 ng/ml (unless there is AHNMD)

- Diagnosis requires either 1 major and 1 minor or at least 3 minor.
3 Decision Points in the Mast Cell Evaluation Algorithm

Necessary: Clinical suspicion for mastocytosis

Bone marrow (BM) examination

1. Morphologically obvious mast cell (MC) aggregates with otherwise normal BM
2. Morphologically obvious MC aggregates with associated myeloid abnormality
3. Morphologically normal BM
BM: Obvious SMCD: Aggregates with **no** AHNMD

- **KIT** D816V
- IHC: Tryptase and CD25

- Tryptase-positive mast cells

**Need 1 minor criterion:**
- Spindled mast cells,
- **KIT** D816V mutation and/or
- CD25 co-expression

**Do not do:**
- Chromosomes
- **BCR-ABL**
- **JAK2, CALR**
- PDGFR-A FISH
- PDGFR-B FISH
- FGFR1 FISH
- Flow Cytometry
- **T-Cell GR**
BM: Obvious SMCD with AHNMD

Establish diagnosis of SMCD: Follow the “Obvious SMCD Algorithm”

Characterize the myeloid abnormality (AHNMD)

?MPN: Follow MPN algorithm

?MDS: Follow MDS algorithm

?Eosinophilia: Follow eosinophilia algorithm
What do we do when we have…

• Clinical suspicion for mastocytosis

• **But:** morphologically normal BM
BM: Normal morphology with no obvious mast cell aggregates

- **KIT** D816V
- IHC: Tryptase and CD25
- If CD25 equivocal, perform flow

Do not do:
- Chromosomes
- BCR-ABL
- JAK2, CALR
- PDGFR-A FISH
- PDGFR-B FISH
- FGFR1 FISH
- Flow
- *T-cell GR*

Tryptase-positive mast cell aggregates

Yes

Follow “Obvious SMCD algorithm”

No

Spindled mast cells?

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Spindled mast cells by tryptase IHC?

- **Yes**
  - **KIT D816V mutation** AND **CD25 positive mast cells**
    - **Yes**: Diagnosis: SMCD
    - **No**: Diagnosis: Clonal mast cells of uncertain significance

- **No**
  - **KIT D816V positive**
    - **Yes**: Diagnosis: Clonal mast cells of uncertain significance
    - **No**: Diagnosis: Normal; no features of mastocytosis

- **Yes**
  - **KIT positive**
    - **CD25 negative**: Diagnosis: Clonal mast cells of uncertain significance
  - **KIT negative**
    - **CD25 positive**: Diagnosis: Slightly increased phenotypically abnormal spindled mast cells of uncertain significance
  - **KIT negative**
    - **CD25 positive**: Diagnosis: Slightly increased spindled mast cells of uncertain significance
  - **KIT negative**
    - **CD25 negative**: Diagnosis: Clonal mast cells of uncertain significance
Potential caveats in the diagnosis

- Hemodilute aspirate for KIT mutation (false negative)
- Small and/or otherwise suboptimal BM biopsy (false negative)
- Inadequate communication between clinician and pathologist re concern for mastocytosis (ancillary studies not done)
- Suboptimal performance of ancillary studies (false negative)
- Serum tryptase not particularly useful for the pathologist as an isolated data point
Increased Eosinophils and/or Mast Cells

Malignant

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Questions & Discussion