Eosinophilia: A Diagnostic Approach and Test Utilization Strategies for Bone Marrow Evaluation

American Society for Clinical Pathology 2014 Annual Meeting

Presented by: Matthew T. Howard, MD
Assistant Professor
Division of Hematopathology
Mayo Clinic, Rochester, MN 55901
Email: howard.matthew@mayo.edu
# Table of Contents

I. Introduction:  
   a. Normal absolute count of eosinophils in peripheral blood and bone marrow  
   b. Normal cytologic aspects of eosinophils  
   c. Normal function of eosinophils  
   d. Harmful consequences of excessive eosinophil tissue infiltration  

II. Definitions of Eosinophilia:  
   a. Criteria for mild, moderate and marked eosinophilia in peripheral blood  

III. Causes of Eosinophilia  
   a. Reactive/non-clonal (a.k.a. secondary)  
   b. Clonal (a.k.a. primary)  

IV. Genetics Underlying Clonal Eosinophilia:  

V. Therapeutic Implications of Key Genetic Alterations in Neoplastic Eosinophilia  
   a. Imatinib sensitive genetic alterations  
   b. Imatinib insensitive genetic alterations  

VI. Algorithmic Approach to the Diagnostic Workup of Eosinophilia in Bone Marrow (page 13)  

VII. References (page 17)
I. Introduction

A. Normal numbers: Eosinophils are a normal constituent of the peripheral blood and bone marrow, accounting for <0.5 x 10^9/L of cells in the blood and 1-5% of cells in the bone marrow.

B. Cytologic features: Eosinophils are morphologically distinctive on a Wright-stained smear given their prominent, large, bright orange cytoplasmic granules. Hence, they are rarely a morphologic diagnostic dilemma. Mature eosinophils have 2-3 lobed nuclei.

C. Function: Eosinophils derive from the granulocyte-monocyte precursor cell in the bone marrow and subsequently migrate to extramedullary tissues to perform important functions in normal immune hemostasis. They are recruited to ingest and remove foreign material such as parasites and allergens, for example.

D. Consequences: In some instances, the release of excess eosinophilic granule proteins including major basic protein and peroxidase may incite exuberant tissue inflammation resulting in unwanted and harmful tissue destruction or fibrosis. This latter phenomenon may occur in association with both reactive and clonal causes of eosinophilia and requires aggressive treatment to avert the particularly devastating consequences in the myocardium and central nervous system.

II. Definitions of Eosinophilia (absolute counts in the peripheral blood)

A. Criteria for degree of eosinophilia:

1. Mild eosinophilia: 0.5 - <1.5 x 10^9/L
2. Moderate eosinophilia: 1.5 – 5.0 x 10^9/L
3. Marked eosinophilia: > 5.0 x 10^9/L
III. Causes of Eosinophilia

A. Secondary/Non-clonal/reactive eosinophilia (Table 1)

   1. General comments
      a. The eosinophilia is often the result of released eosinophilic cytokines (e.g. IL-3, IL-5) which promote eosinophil proliferation.
      b. Secondary eosinophilia is by far the most common.
      c. “Hypereosinophilia” in and of itself is not a diagnosis and refers merely to an excess of eosinophils. The etiology is not specified and may be primary or secondary in nature.
      d. “Idiopathic hypereosinophilia” is the term used when no etiology has been recognized and there is no associated direct end-organ damage.
      e. “Idiopathic hypereosinophilic syndrome” (IHES) is the term used when no etiology has been recognized and there is associated direct end-organ damage.

   2. Secondary to a non-neoplastic disorder (Table 1A)
      a. There are a significant number of non-neoplastic disorders that may be associated with a non-clonal eosinophilia; these greatly outnumber other etiologies for eosinophilia.
      b. These are quite diverse and may have been clinically investigated to varying degrees prior to coming to pathologic examination.
      c. The most common etiologies include allergic reaction, helminthic infection, drug/medication reaction and atopic disorder.
d. Lymphocytic variant of hypereosinophilic syndrome
   a. Definition: Lymphocytic disorder characterized by a nonmalignant, clonal, expansion of a T-cell population producing IL-5 in patients fulfilling HES diagnostic criteria
   b. Diagnostic criteria: hypereosinophilia, aberrant T-cell population and clonal T-cell receptor, absence of myeloid or lymphoid malignancy
   c. Phenotype: Most commonly surface CD3 negative, CD4 positive, CD5 positive and CD7 negative
   d. Clinical features: Affects males and females equally, cutaneous manifestations, malignant transformation to lymphoma may occur rarely
   e. Treatment: Corticosteroids first-line, interferon alpha, cytotoxic drugs, anti-IL5 monoclonal antibody
   e. See Table 1A for a list of underlying non-neoplastic disorders that may be associated with a non-clonal eosinophilia

3. Secondary to an underlying neoplastic disorder (Table 1B)
   a. There are a number of underlying neoplastic disorders that may be associated with a non-clonal eosinophilia.
      a. The most common disorders include hematopoietic neoplasms such as T-cell lymphoma and classical Hodgkin lymphoma.
b. Rarely, carcinomas may be associated with reactive eosinophilia.

b. Reactive eosinophilia secondary to underlying neoplasia may be quite exuberant and mask the underlying tumor (e.g. acute lymphoblastic leukemia, T-cell lymphoma).

c. See Table 1B for a list of underlying neoplastic disorders that may be associated with a non-clonal eosinophilia

B. Primary/Clonal eosinophilia (modified from WHO 2008, reference 2) (Table 2)

1. General comments
   a. Clonal eosinophilic syndromes derive largely from myeloid neoplasms although a subset of cases are clearly lymphoid (e.g. lymphoblastic leukemia/lymphoma)
   b. Clonal eosinophilic syndromes run the gamut from chronic myeloid disorders to acute leukemia
   c. A variety of diagnostic ancillary tests are necessary to sort through the vast array of possible underlying diagnoses
   d. The utilization of ancillary tests is absolutely predicated on the clinical and morphologic findings and should not be performed in every case.
      a. See our proposed algorithm for more details (Figure)
      b. These ancillary methods include, but are not limited to:
         i. Conventional karyotyping,
ii. Flow cytometry for aberrant T-cell population and T-cell receptor gene rearrangement

iii. Immunohistochemistry for tryptase and CD25,

iv. FISH for PDGFRα rearrangement detection and,

v. KIT D816V mutation

e. Recurring genetic abnormalities characterize a subset of these disorders (See Table 2 and handout section IV, page 8).

f. Targeted drug therapies are available for several of these disorders, adding to our growing knowledge and classification of hematologic disorders by their molecular underpinnings with an emphasis on potential specific treatment strategies.

2. See Table 2 for a list of clonal eosinophilic disorders
IV. Genetics Underlying Clonal Eosinophilia

A. General comments

1. The diagnostic and therapeutic importance of detecting specific genetic alterations in clonal disorders including eosinophils is becomingly recognized.

   a. The introduction of tyrosine kinase inhibitor therapy (TKI), targeting various tyrosine kinases, has resulted in therapeutic benefits for patients with a variety of diseases.

   b. TKI sensitive mutations: \textit{PDGFRA, PDGFRB} and \textit{BCR-ABL1}

   c. TKI insensitive mutations: \textit{FGFR1, KIT D816V}

B. Given the exquisite sensitivity of several disorders to Imatinib (first generation TKI), identification of specific genetic alterations is essential.

C. Bone marrow morphology in these various disorders may range from:

   1. Unremarkable aside from the proliferation of eosinophils,

   2. To remarkable for the presence of a distinct myeloid or lymphoid neoplasm and eosinophilia

D. \textit{PDGFRA} rearrangement

   1. Abnormalities involving 4q12

   2. Cytogenetically cryptic; requires FISH testing

   3. Most often submicroscopic deletion results in \textit{PDGFRA-FIP1L1} fusion

   4. Bone marrow is generally hypercellular with scattered, individually-distributed interstitial spindled mast cells; \textit{KIT} D816V mutation negative.
5. Associated clonal eosinophilia

6. Rare cases may be morphologically indistinguishable from classic systemic mastocytosis with multifocal compact dense aggregates of spindled mast cells

E. PDGFRB rearrangement

1. Translocations involving 5q31-q33; often t(5;12)(q31-33;p12); chromosomes necessary with FISH to subsequently confirm PDGFRB rearrangement

2. Associated clonal eosinophilia

3. Most cases morphologically resemble chronic myelomonocytic leukemia; less commonly atypical chronic myeloid leukemia, chronic eosinophilic leukemia and myeloproliferative neoplasm, NOS.

F. FGFR1 rearrangement

1. Abnormalities involving 8p11; chromosomes necessary with FISH to subsequently confirm FGFR1 rearrangement

2. Associated clonal eosinophilia

3. Cases have a heterogeneous presentation ranging from myeloproliferative neoplasm, acute myeloid leukemia to acute lymphoblastic leukemia/lymphoma.

G. BCR-ABL1 genetic fusion [associated with t(9;22)(q34;q11.2)]
1. Genetic fusion underlies all cases of suspected chronic myelogenous leukemia (CML)

2. Morphology shows mild to moderate clonal eosinophilia in some cases in association with the typical hypercellular bone marrow with granulocytic hyperplasia and left shift, basophilia, lack of dysplasia and small monolobated megakaryocytes.

H. **CBFB-MHY11** (associated with inv16)
   1. Subtype of Acute Myeloid Leukemia
   2. Associated clonal eosinophilia
   3. Favorable prognosis

I. **RUNX1-RUNXIT1** fusion [associated with t(8;21)(q22;q22)]
   1. Subtype of Acute Myeloid Leukemia
   2. Associated clonal eosinophilia
   3. Favorable prognosis

J. Other
   1. **PCM1-JAK2** fusion and **ETV6-FLT3** fusions have also been described.
      a. Small molecule inhibitors of **JAK2** and **FLT3** could potentially be considered in those cases.
V. Therapeutic Implications of Key Genetic Alterations in Neoplastic Eosinophilia

A. General comments

1. The discovery of molecular alterations underlying various hematopoietic disorders has led to the elucidation of targeted drug therapies that aid in disease management.

2. Goal: The aim of therapy in the group of CEL, IHES and PDGFRα, PDGFRβ and FGFR1-associated eosinophilias is to abrogate eosinophil-mediated damage.

3. Tyrosine kinase inhibitors (TKIs) are the primary drug group. This includes Imatinib and second and third-generation TKIs such as dasatinib, nilotinib, etc.

4. For the lymphocytic variant of HES, corticosteroids are first-line therapy.

5. In eosinophilias with a more aggressive disease course (e.g. AML), then cytotoxic treatments and transplantation come into consideration.

6. Imatinib is overall a fairly safe drug.

B. Imatinib sensitive molecular alterations

1. BCR-ABL1 fusion, PDGFRα rearrangements and PDGFRβ rearrangements

   a. Given the exquisite sensitivity of these molecular events to Imatinib, their identification is of vital therapeutic importance.

   b. Imatinib is considered the drug of choice.

   c. Imatinib is sufficient to induce long-term remission.
d. While acquisition of Imatinib resistance is known for CML (BCR-ABL1 fusion), very few instances have been identified in PDGFRA-rearranged disease.

e. PDGFRA alterations are typically cytogenetically cryptic and therefore require FISH for detection.

f. PDGFRB alterations are typically cytogenetically evident and therefore FISH is only required for confirmation of a cytogenetic abnormality.

g. BCR-ABL1 genetic fusion can be suspected from the karyotype t(9;22)(q34;q11.2). Rarely BCR-ABL1 fusion is cryptic therefore utilization of an additional detection technique such as FISH is suggested.

C. Imatinib insensitive molecular alterations

1. KIT D816V mutation (seen in mastocytosis), FGFR1 rearrangements

2. In FGFR1-rearranged disease, the clinical course is typically quite aggressive and requires the use of intensive chemotherapy and transplantation.
VI. Algorithmic approach to the Bone Marrow Workup of Eosinophilia (see flow chart on following page)

A. Goal: Diagnostic testing strategy to exclude clonal disorders of eosinophilia

B. Initial testing is predicated on peripheral blood /bone marrow morphologic findings and clinical history/presentation/symptomatology.

C. Diagnoses are driven by ancillary testing findings, in conjunction with morphology and clinical history.
Persistent Eosinophilia

$> 1.5 \times 10^9/L$

Assess morphology

Morphologic/clinical features of other disease:
- Mastocytosis, CML, MCL, MDS, AML, Hodgkin lymphoma, T cell lymphoma, skin lesions, lymphadenopathy, splenomegaly, etc.

Increased eosinophilia and precursors only

Order:
- IHC for tryptase/CD25
- KIT D816V
- Chromosomes
- FISH for PDGFRα
- PB flow with T-panel
- PB TCR

Do Not Order:
- JAK2 V617F
- BCR/ABL FISH or PCR
- FISH for PDGFRα or FGFR1
- Mast cell flow

Workup as appropriate for other disease; if morphologic features of myeloid neoplasia present, order chromosomes and FISH for PDGFRα in addition to other testing.

Clonal myeloid cytogenetic abnormality?

Negative for PDGFRα/ PDGFRβ/ FGFR1
- Diagnose as other disease

Positive for PDGFRα/ PDGFRβ/ FGFR1
- Myeloid neoplasm with PDGFRα/ PDGFRβ/ FGFR1 abnormality*

Other clonal abnormality
- CEL, NOS

Abnormal T-cell phenotype and clonal T-cell gene rearrangement?

No clonal abnormality
- Diagnosis: Lymphoctic Variant HES

Spindled mast cells/ KIT D816V mutation
- Work up as mastocytosis

1. Reactive eosinophilia
2. Idiopathic Hypereosinophilia
3. Idiopathic HES

* Bone marrow often shows abnormalities other than increased eosinophils and precursors
Table 1: Secondary (a.k.a. reactive, non-clonal) Causes of Eosinophilia

A. Secondary to a non-neoplastic disorder
   - Infection: Helminths, protozoa, fungi, others
   - Allergic Disorders: Allergies, atopia, asthma, hypersensitivity
   - Drug/Medication Reaction
   - Other rare disorders:
     - Primary immunodeficiency: hyper-IgE syndrome, Omenn syndrome, autoimmune lymphoproliferative syndrome (ALPS)
     - Secondary immunodeficiency/chronic irritation/inflammation: status post transplantation, bullous pemphigoid, sarcoidosis, radiation

B. Secondary to an underlying neoplastic disorder
   - Hematopoietic neoplasms: T-cell lymphomas, Classical Hodgkin lymphoma, rarely, acute lymphoblastic leukemia/lymphoma (B and T); typically associated with t(5;14)(q35;q32); rarely, some B-lineage non-Hodgkin lymphomas
   - Non-hematopoietic neoplasms: Carcinoma (e.g. lung, gastrointestinal tract), sarcoma
   - Other: Eosinophilia secondary to clonal proliferation of T-cells (not lymphoma or leukemia) currently termed the lymphocytic variant of hypereosinophilia
Table 2: Primary (a.k.a. clonal) Causes of Eosinophilia

- Chronic Eosinophilic Leukemia, Not Otherwise Specified
- Myeloid and lymphoid neoplasms with eosinophilia and rearrangements of 
  \textit{PDGFRA, PDGFRB} and \textit{FGFR1}
- Acute myeloid leukemia (uncommon)
  - AML with inv(16)
- Myeloproliferative Neoplasm
  - Chronic myelogenous leukemia, \textit{BCR-ABL1} positive
  - Systemic mastocytosis
- Myelodysplastic syndrome (less than 5\% of cases have eosinophilia)
- Myelodysplastic/myeloproliferative neoplasm lacking rearrangements of 
  \textit{PDGFRA, PGFRB} and \textit{FGFR1}
References:


2. WHO 2008


The author of this document has no financial disclosures to report.