ABSTRACT

Hypereosinophilic syndromes are a heterogeneous group of disorders with low incidence, characterized by eosinophil blood count higher than 1.5x10^9/L persistent for at least 6 months in the absence of a reactive cause and with signs and symptoms of organ involvement due to the eosinophilia itself or to the release of eosinophilic mediators. Recent advances in molecular biology allowed a better understanding which led to a new classification of the hypereosinophilic syndromes corresponding to molecular abnormalities, offering the possibility of new therapeutic approaches. The utility of imatinib therapy in some of these new identified clinical and pathological entities dramatically improved the prognosis of these patients, justifying the continuous efforts to discover new therapeutic agents.

Key words: Hypereosinophilia, prognostic, molecular abnormalities, therapeutic perspective

Abbreviations:

AR – rheumatoid arthritis  
ATLL – adult T cell leukemia/lymphoma  
CEL – chronic eosinophilic leukemia  
CMML – chronic myelomonocytic leukemia  
CML – chronic myeloid leukemia  
aCML – atypical chronic myeloid leukemia  
FGFR1 – fibroblast growth factor receptor 1  
FISH – fluorescence in situ hybridization  
GM-CSF – granulocyte-macrophage colony-stimulating factor  
HES – hypereosinophilic syndromes  
CHF – congestive heart failure  
IL-3 – interleukin 3  
IL-5 – interleukin 5  
LAL – acute lymphoblastic leukemia  
LAM – acute myeloblastic leukemia  
LES – systemic erythematous lupus  
MDS – myelodisplastic syndrome  
MI – idiopathic myelofibrosis  
MPN – myeloproliferative neoplasm  
MPN/MDS – myeloproliferative neoplasm/myelodisplastic syndrome  
PDGFRA/B – platelet derived growth factor receptor alpha/beta  
PV – polycythaemia vera  
RT-PCR – reverse transcriptase polymerase chain reaction  
SM – systemic mastocytosis  
TE – essential thrombocythaemia  
TKI – tirosin-kinase inhibitors
The eosinophil granulocytes are mature leukocytes differentiated from CD 34+ myeloid precursors cells. Production and maturation of eosinophils take place in the bone marrow and is promoted by cytokines: GM-CSF, IL-3 and especially IL-5 (a product of CD4+ T-lymphocytes), which appears to be the dominant eosinophil growth and survival factor. These cytokines are also responsible for the inhibition of eosinophilic apoptosis (1).

Following maturation, eosinophils circulate in blood and migrate to inflammatory sites in tissues, or to sites of helminthic infection in response to certain chemokines (eotaxin-1, eotaxin-2, RANTES), and leukotrienes (leukotriene B4). At the infectious sites, eosinophils are activated by Type 2 cytokines released from a specific subset of helper T cells (Th2).

Eosinophils are normally found in the bone marrow, at the junction between the cortex and medulla of the thymus, in the lower gastrointestinal tract, respiratory structures, ovary, uterus, spleen, and lymph nodes. The presence of eosinophils in other organs is associated with disease (2).

When examined by standard blood-staining techniques, the mature eosinophil is 12-17 micrometers in size, with bilobed nucleus and abundant cytoplasm filled with large reddish-orange (eosinophilic) granules that do not cover the nucleus (FIGURES 1 and 2).

Eosinophils play an important role in fighting helminth colonization, viral and fungal infections, they are important mediators of allergy (including asthma pathogenesis). Eosinophils are also involved in fibrin removal during inflammation, postpubertal mammary gland development, oestrus cycling, allograft rejection and neoplasia. They have also recently been implicated in antigen presentation to T cells.

Following activation, the eosinophils degranulate and release their content: major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), eosinophil-derived neurotoxin (EDN), capable of inducing tissue damage. ECP and EDN are ribonucleases with antiviral activity. MBP induces mast cell and basophile degranulation, and is implicated in peripheral nerve remodeling. ECP creates toxic pores in the membranes of target cells allowing potential entry of other cytotoxic molecules to the cell, can inhibit proliferation of T cells, suppress antibody production by B cells, induce degranulation by mast cells, and stimulate fibroblast cells to secrete mucus. EPO stimulates reactive oxygen species and promotes oxidative stress causing cell death by apoptosis and necrosis (4). Other mediators released by eosinophils are growth factors (such as TGF beta, VEGF, and PDGF), cytokines (such as interleukin and TNF alpha), enzymes (elastase), leukotriens and prostaglandins.

In normal individuals eosinophils make up about 1-6% of white blood cells but the absolute eosinophil count (AEC) seems more correct and is preferred by most authors. The detection of more than 0.6x10^9/L eosinophils defines eosinophilia.

**Classification of eosinophilia** by its degree may be the initial clue for diagnosis (TABLE 1, (5)).

**Classification of eosinophilia** (6):
1. familial eosinophilia – autosomal dominant disorder with constant number of eosinophils and a benign evolution

![FIGURE 1. Mature eosinophil and precursors – MGG (40X). (3)](image1)

![FIGURE 2. Mature eosinophil –MGG (100X). (3)](image2)
TABLE 1. Causes of eosinophilia [adapted from M.L.Bridgen (5)]

<table>
<thead>
<tr>
<th>Likely causes</th>
<th>Less likely causes</th>
</tr>
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<tbody>
<tr>
<td><strong>Mild eosinophilia</strong> ((0.6 – 1.5 \times 10^9/L))</td>
<td></td>
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<tr>
<td>Allergic rhinitis</td>
<td>Neoplasm</td>
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<tr>
<td>Hay fever</td>
<td>Gastrointestinal disease</td>
</tr>
<tr>
<td>Extrinsic asthma</td>
<td>Skin disease</td>
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<tr>
<td>Drug reactions (penicillins, cephalosporins, anti fungal, anti epileptic, NSAID’s, gold compounds, allopurinol, anti diabetics, anticoagulants, anticancer drugs.)</td>
<td>Certain infections diseases (Brucellosis, Mycobacterium, Chlamydia pneumonia)</td>
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<tr>
<td>Parasitic infections (cestodes, protozoans)</td>
<td>Peritoneal dialysis</td>
</tr>
<tr>
<td>Fungal infections (aspergillosis, coccidiodymycosis)</td>
<td>Immunodeficiency state (Wiskott-Aldrich syndrome, isolated IgA deficiency syndrome, elevated IgE syndrome)</td>
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<tr>
<td>Occupational lung disease</td>
<td>Radiation therapy</td>
</tr>
<tr>
<td>Endocrinopathies: Addison disease</td>
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<tr>
<td><strong>Moderate eosinophilia</strong> ((1.5 – 5 \times 10^9/L))</td>
<td></td>
</tr>
<tr>
<td>Parasitic disease (nematodes, trematodes)</td>
<td>Polyarteritis nodosa</td>
</tr>
<tr>
<td>Intrinsic asthma</td>
<td>Autoimmune diseases (Crohn disease, scleroderma, SLE, RA, ulcerative colitis, vasculitis)</td>
</tr>
<tr>
<td>Neoplasm (lung, stomach, pancreas, colorectal, cervix, ovarian carcinomas and sarcomas, Hodgkin lymphoma, non-Hodgkin lymphoma, ALL, myeloproliferative syndromes, AML, MDS)</td>
<td>Syndromes of pulmonary infiltration with eosinophilia (Loeffler syndrome, chronic eosinophilic pneumonia, Churg-Strauss syndrome)</td>
</tr>
<tr>
<td>Drug reactions</td>
<td></td>
</tr>
<tr>
<td>Skin disorders (exfoliative dermatitis, dermatitis herpetiformis, psoriasis, pemphigus)</td>
<td>Myeloproliferative and lymphoproliferative neoplasms with PDGFRA, PDGFRB, FGFR1 rearrangement</td>
</tr>
<tr>
<td><strong>Marked eosinophilia</strong> ((&gt;5 \times 10^9/L))</td>
<td></td>
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<tr>
<td>Visceral larva migrans</td>
<td>Chronic Eosinophilic Leukemia</td>
</tr>
<tr>
<td>Tissue migration during larval stage (ascaridiasis, trichinosis, Strongyloides sp.)</td>
<td>Hypereosinophilic Syndrome</td>
</tr>
</tbody>
</table>

2. acquired eosinophilia:
   - primary – clonal; idiopathic (HES)
   - secondary eosinophilia – as reactive phenomenon in allergy, infection or malignant diseases

Primary eosinophilia is not reactive. It is clonal when a molecular or cytogenetic abnormality is identified and idiopathic when both clonality and any known cause of hypereosinophilia are excluded.

In 1968, Hardy and Anderson first used the term Hypereosinophilic Syndrome to describe patients with prolonged eosinophilia of unknown cause and organ damage due to eosinophilic infiltration.

In 1975, Chusid et al used three diagnostic criteria for HES (4):
- persistent eosinophilia of \(>1.5 \times 10^9/L\) for more than six months
- lack of evidence for reactive eosinophilia
- organ involvement

In 2005, in Bern, on behalf of Hypereosinophilic Syndromes Working Group, Klion et al proposed a new classification for the multitude of hypereosinophilic pathogenic variants and divided them in subtypes (7-10):
- FIP1L1-PDGFRA (F/P) – associated HES or F/P+ HES: patients with clonal hypereosinophilia due to chromosomal rearrangement resulting in FIP1L1/ PDGFRA – fusion gene on 4q12.
- Chronic eosinophilic leukemia (CEL) – patients with proven eosinophil clonality (including FIP1L1-PDGFRA fusion gene) or with increased marrow blasts; some of these cases may have acute leukemia evolution.
HYPEREOSINOPHILIC SYNDROMES – RECENT ADVANCES IN DEFINITION, CLASSIFICATION AND THERAPEUTIC APPROACH

Lymphocytic-HES (L-HES): patients with chronic reactive eosinophilia in response to IL-5 over-production by T cells.

Myeloproliferative-HES (M-HES): patients with a large array of clinical and biological features (elevated levels of vitamin B12, hepatomegaly, splenomegaly, anemia, thrombocytopenia, circulating myeloid, displastic eosinophils, bone marrow hypercellularity with increased number of myeloid precursors, myelofibrosis, increased serum tryptase and response to imatinib), suggesting an underlying myeloproliferative disorder associated with eosinophilia but having no evidence of molecular defects.

Idiopathic hypereosinophilic syndrome (HES): patients with eosinophilia of unknown cause.

Organ-restricted eosinophilic disease: only one specific tissue or organ involvement (e.g. eosinophilic esophagitis, eosinophilic pneumonia, Kimura disease, eosinophilic dermatitis, eosinophilic fasciitis)

Although this rather complicated classification raised multiple debates regarding its accuracy, it proved to be useful for defining certain pathogenic entities (11).

Lately, new molecular data improved the diagnosis guidelines of the eosinophilic disorders and an entirely new classification emerged (WHO classification of tumors of haematopoietic and lymphoid tissues 2008). The following entities are now acknowledged (12):

Chronic eosinophilic leukemia (CEL) defined as a clonal proliferation of eosinophilic precursors, accumulation of eosinophils in peripheral blood and bone marrow, causing end organ damage due to the tissue eosinophilic infiltration and release of eosinophilic proteins. Patients expressing Philadelphia chromosome, BCR-ABL1, PDGFRα, PDGFRβ and FGFR1 fusion genes are excluded.

**Diagnosis criteria:**
- AEC > 1.5 x 10^9/L in peripheral blood or bone marrow eosinophilia
- exclusion of reactive causes of eosinophilia including hematological malignancies (e.g. aCML, CML, CMML and lymphoproliferative disorders)
- exclusion of a T-cell abnormal population that produces increased eosinophilopoietic cytokines
- peripheral blood blasts >2% and bone marrow blasts 5% – 20% or
- evidence of eosinophil clonality

If the clonality of the eosinophils cannot be demonstrated and bone marrow blasts are less than 5%, the term “Idiopathic HES” must be used.

**Idiopathic HES:** is a diagnosis of exclusion. In time, some of these patients initially diagnosed as Idiopathic HES may develop clinical features resembling CEL or hypereosinophilia induces by cytokines (IL-2, IL-3, IL-5).

**Diagnosis criteria:**
- persistent eosinophilia (AEC > 1.5 x 10^9/L) for more than 6 months
- exclusion of reactive causes of eosinophilia
- exclusion AML, MPD, MDS, MPD/MDS and SM
- exclusion of a T-cell abnormal population with increased production of eosinophilopoietic cytokines
- end organ damage
- no the evidence of eosinophilic clonality (exclusion of Philadelphia chromosome, BCR-ABL1, PDGFRα, PDGFRβ and FGFR1 fusion genes, myeloproliferative disorders as PV, ET, IMF or MPD/MDS are excluded).

**Idiopathic hypereosinophilia** includes all these criteria except for end organ damage.

**Myeloid neoplasms (MPN) with eosinophilia and PDGFRα, PDGFRβ and FGFR1 rearrangements:** three distinct entities with cytogenetic abnormalities resulting in fusion genes with tyrosine-kinase activity; some of them are imatinib sensitive. The hematological features are those of myeloid or lymphoid neoplasms.

**PDGFRα rearrangement:** usually presents as CEL +/- mastocitosis and less often as AML or lymphoblastic lymphoma with eosinophilia. These patients present clinical features of a chronic disease with constitutional symptoms (weakness, weight loss, night sweats, pruritus) and virtually any organ may be involved: heart, lungs (restrictive dysfunction with fibrosis), gastrointestinal tract, skin, spleen, rarely liver. Some patients progress to acute forms. Prognosis depends mainly on the cardiac involvement (endomyocardial fibrosis with restrictive cardi...
omyopathy, heart failure, mitral or tricuspid valves dysfunctions, mural thrombi with high thromboembolic risk). Both chronic and acute forms are associated with imatinib response. The most frequent genetic abnormality is a cryptic interstitial deletion in chromosome 4 (4q12) leading to FIP1L1-PDGFRα fusion gene.

Diagnosis criteria:
- AEC >1,5 x 10⁹/L
- 5-19% bone marrow blasts
- no evidence of Philadelphia chromosome
- no evidence of BCR-ABL fusion gene
- demonstration of del 4q12 (being a cryptic fusion gene FISH exam is required, cytogenetic analysis alone is insufficient) or of FIP1L1-PDGFRα fusion gene (confirmed by RT-PCR or nested RT-PCR).

Other PDGFRα molecular variants are associated with imatinib sensibility. (13,14)

PDGFRβ rearrangement: the hematological features are most often those of CMML with eosinophilia and a variable degree of mastocytosis. Mandatory for the diagnostic of these myeloproliferative neoplasms is the presence of PDGFRβ gene. Patients often have splenomegaly, skin infiltration and cardiac damage but rare liver involvement. Peripheral blood and bone marrow are always involved. Evolution to acute leukemia may occur. Imatinib produces clinical response. Prognosis mainly depends on the cardiac damage. Cytogenetic analysis usually shows t(5;12)(q31~33;p12) with the translocation resulting in formation of an ETV6-PDGFRβ fusion gene.

Diagnosis criteria:
- myeloid neoplasm with prominent eosinophilia +/- neutrophilia or monocytosis
- evidence of t(5;12)(q31~33;p12) or a variant translocation (cytogenetic analysis) or demonstration of an ETV6-PDGFRβ fusion gene or rearrangements of PDGFRβ (PCR or FISH)
- no evidence of Philadelphia chromosome

FGFR1 rearrangement: some patients present as T cell lymphoblastic leukemia/lymphoma, CEL, AML and less often as precursor B cell lymphoblastic leukemia/lymphoma. It represents a heterogeneous group of disorders characterized by lymph nodes involvement, splenomegaly, fever, weight loss and night sweats. A variety of translocations with an 8q11 breakpoint can underlie this syndrome. The prognosis is poor since no TKI has proved effective in FGFR1 rearrangement neoplasms. Interferon has induced a cytogenetic response in several cases. Haematopoietic stem cell transplantation should be considered even in those patients who present in chronic phase.

Diagnosis criteria:
- myeloid neoplasm with increased eosinophilia +/- neutrophilia and/or monocytosis
- acute myeloid leukemia or precursor T or B cell lymphoblastic leukemia/lymphoma precursors AND
- presence of t(8;13)(p11;q12) or a variant translocation leading to FGFR1 rearrangement demonstrated in myeloid cells, lymphoblasts or both.

The above mentioned data represents the new approach of the clonal hypereosinophilias, based mainly on cytogenetic and molecular assays. Several conclusions must be emphasized:

1. According to this classification, older entities as myeloproliferative-HES and lymphocytic-HES are no longer used, being considered as secondary hypereosinophilia.

2. New entities with major therapeutic impact are defined based on cytogenetic and molecular criteria

3. Immunophenotypic analysis is less important in diagnosis as long as the clonal eosinophils have no particular characters but it has its role in identification, characterization and following of the abnormal T-cell population that produces excessively IL-5.

4. The cytogenetic analysis should always be followed by molecular assays, especially in myeloproliferative-like disorders that may have cryptic anomalies.

5. Any MPN or lymphoblastic leukemia/lymphoma with eosinophilia require cytogenetic analysis for PDGFRβ, FGFR1 rearrangements and molecular assays (RT-PCR or FISH) for PDGFRα rearrangements.
Diagnostic algorithm for blood eosinophilia (FIGURE 3)

FIGURE 3. The diagnostic process in a patient with hypereosinophilia [adapted after S Fletcher, B Bain (15)]

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Possible diagnosis yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood smear</td>
<td>Blast cells or lymphoma cells indicating hematologic neoplasm</td>
</tr>
<tr>
<td>Parasitic infection (e.g. stool examination, specific serologic testing)</td>
<td>Parasitic infection</td>
</tr>
<tr>
<td>IgE and tests for allergy</td>
<td>Allergic disease</td>
</tr>
<tr>
<td>Bone marrow aspiration and trephine biopsy</td>
<td>CEL, Hodgkin lymphoma, non-Hodgkin lymphoma, SM</td>
</tr>
<tr>
<td>Cytogenetic analysis on bone marrow aspirate</td>
<td>CEL</td>
</tr>
<tr>
<td>Molecular analysis of peripheral leukocytes for FIP1L1-PDGFRα fusion gene</td>
<td>CEL</td>
</tr>
<tr>
<td>Serum tryptase</td>
<td>CEL or SM</td>
</tr>
<tr>
<td>Immunophenotyping of peripheral blood T cells</td>
<td>Cytokine-driven eosinophilia</td>
</tr>
<tr>
<td>CT-scan of chest and abdomen</td>
<td>Underlying lymphoma or other neoplasm</td>
</tr>
</tbody>
</table>

In addition to looking for the cause of eosinophilia, laboratory tests to assess possible eosinophilic tissue damage may be required and include electrocardiography, echocardiography, pulmonary function tests, CT scanning of the chest, abdomen and pelvis and, in presence of symptoms, tissue biopsy (FIGURE 3 and TABLE 2).

Management of hypereosinophilic syndrome
- **Idiopathic hypereosinophilia** does not require treatment. Such patients are
closely monitored with serum troponin level every 3-6 months and ECHO and pulmonary function tests every 6-12 months.

- **Idiopathic HES** requires urgent treatment in order to prevent cardiac damage. **Gluccorticoids** are the first-line treatment in all patients without FIP1L1/PDGFRA mutation. About one third of patients does not respond to steroids but may benefit from interferon alpha or hydroxyurea. For those who do not respond, a high dose (400mg) of **imatinib** is the treatment of choice. If they still remain non-responsive, other therapeutic agents can be used: chlorambucil, etoposide, vincristine, 2-chlorodeoxyadenosine and cytarabine. **Hematopoietic stem cell transplantation** is an alternative for patients refractory to treatment (16-18).

- **CEL and MPN with PDGFRα and PDGFRB rearrangements** have a very good response to imatinib, even in low doses (100mg/day) but complete remissions usually require 400mg/day (19,20). A lifelong maintenance therapy is required in the majority of cases. Molecular responses are monitored by FISH or RT-PCR at 3-6 months intervals in the first year and at 6-12 months intervals thereafter.

- **Neoplasms with FGFR1 abnormalities** do not respond to TKI. **Hematopoietic stem cell transplantation** should be considered, even in patients presenting in chronic phase. (9)

- **Monoclonal antibodies anti IL-5** (mepolizumab) and **anti CD-52 antibody (alemtuzumab)** have been shown to control symptoms as well as eosinophilia.

- **Leukapheresis** is indicated as an emergency therapy to control symptoms due to hyperleucocytosis and must be accompanied by chemotherapy. (11,17)

- **Splenectomy** may be performed in case of hypersplenism or pain due to splenic infarction.

- **Valve replacement** may be required in patients with severe regurgitant lesions.

- **Anticoagulants and antiplatlets drugs** are not routinely indicated. Despite anticoagulant therapy, evolution is marked by frequent thromboembolic complications.

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**Conclusion**

The last years have brought much progress in understanding hypereosinophilic syndromes, giving new perspective and hope for the management of these patients. It is likely that the ability to precisely diagnose these disorders will improve the prognosis of a large subset of patients which respond to imatinib and that new treatment options will soon become available for the rest of them.

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**REFERENCES**