Laboratory Workup of Hypereosinophilia

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Abstract

Hypereosinophilia (HE) can be caused by a wide variety of non-hematologic (secondary or reactive) and hematologic (primary, clonal) disorders. Diagnosing hypereosinophilia/hypereosinophilic syndrome (HE/HES) is challenging due to the complex nature of disease manifestations and numerous underlying etiologies. Knowing that only rare cases are clonal, it is wise to rule out reactive conditions and proceed with molecular and other advanced tools. The exclusion of secondary causes needs a detailed clinical evaluation followed by a wide range of serological and imaging investigations. Once reactive eosinophilia has been ruled out, the diagnosis of primary HE/HES is made using a combination of morphologic examination of the blood and bone marrow, conventional cytogenetics, fluorescent in situ hybridization, flow-cytometry, and T-cell clonality evaluation to look for histopathologic or clonal evidence of an underlying hematological disorder. The accurate diagnosis of clonal eosinophilia-causing myeloid and lymphoid neoplasms and the identification of numerous gene rearrangements significantly enhance patient outcomes, because a proportion of these patients (such as *PDGFRA* and *PDGFRB* rearrangements) responds well to tyrosine kinase inhibitors. Considering the complex etiopathologies, the cost of testing, and the time involved, the workup needs to be tailored according to the urgency of the situation and the resources available. In urgent situations with organ damage, it is crucial to initiate appropriate management without waiting for the results of investigations. In contrast, in a resource-limited situation, it is acceptable to employ step-by-step rather than comprehensive testing to rule out the most common causes first. Here, we discuss various laboratory investigations employed in diagnosing HE/HES, highlighting their importance in different situations.

Keywords
► hypereosinophilia
► hypereosinophilic syndrome
► mastocytosis
► tyrosine kinase domain fusions
► myelodysplastic syndromes
► myeloproliferative neoplasms

Introduction

Peripheral blood and tissue eosinophilia can be caused by a heterogeneous group of hematological and non-hematological conditions. The normal peripheral blood eosinophil ranges from 1 to 5%, with absolute eosinophil count (AEC) less than 0.5×10^9/L, and the bone marrow eosinophil percentage ranges between 1 and 6%.[1]
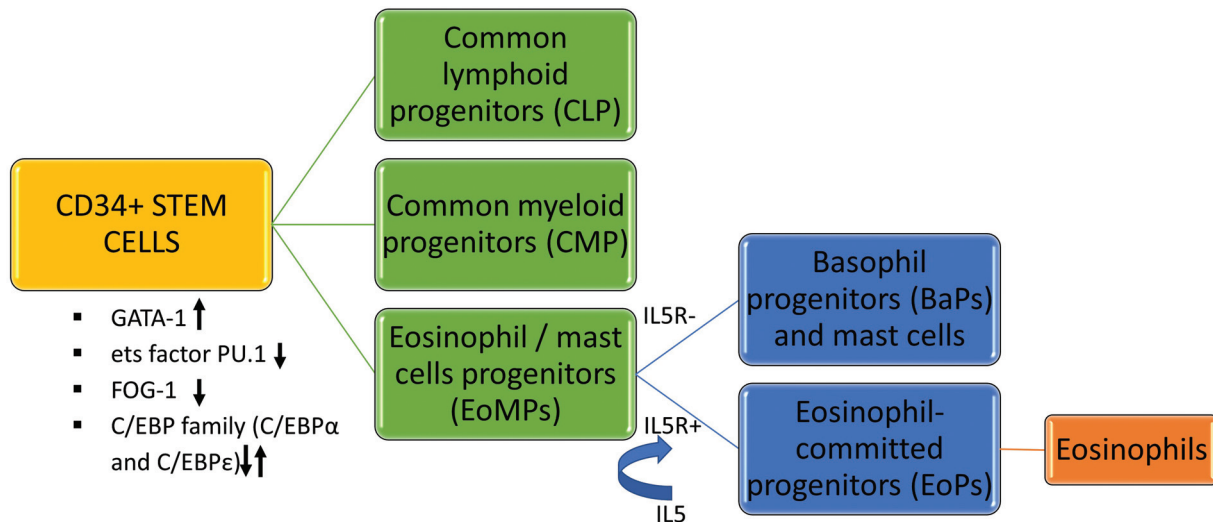
Eosinophilopoiesis and Biology of Eosinophils

Eosinophils are derived from CD34+ multipotent hematopoietic stem cells that are driven by variable levels of transcription factors like GATA binding protein 1 (GATA-1), Ets (erythroblast transformation specific) factor- PU.1, friend of GATA protein 1 (FOG-1), and CCAAT/enhancer binding

Fig. 1 The process of eosinophilopoiesis.

proteins (C/EBP family)——C/EBPα and C/EBPε. The amalgamation of these transcription factors and their malleable expression ultimately decides the lineage differentiation of stem cells. The combination of GATA-1 expression, low levels of Ets factor PU.1, FOG-1 downregulation, and alternate C/EBP family expression favor the stem cells to differentiate into eosinophil/mast cells progenitors (EoMP) over the others.[2] The interplay of cytokines and chemokines mediates eosinophils' further differentiation, migration, and activation. The EoMPs that contain interleukin-5 receptors (IL-5R) exclusively transform into special eosinophil-committed progenitors (EoPs). These EoPs are further activated by lineage-specific cytokine IL-5 that is the crucial amplifier of proliferation and terminal differentiation of eosinophils. The EoMPs that lack sufficient IL-5R develop into basophil progenitors (BaP) and mast cells. IL-5 is the cardinal cytokine synthesized by mast cells, activated Th2 lymphocytes, macrophages, natural killer cells, endothelial cells, epithelial cells, and fibroblast cells of both the innate and adaptive immune systems. Apart from the IL-5, other cytokines like IL-3, IL-4, granulocyte-macrophage colony-stimulating factor, etc. also contribute in this process (►**Fig. 1**).

Eosinophilia, Hypereosinophilia, Tissue Eosinophilia, and Hypereosinophilic Syndrome

Eosinophilia is defined as blood AEC $>0.5 \times 10^9$/L. Based on the severity, they are graded as[1] mild (0.5–1.5×10^9/L), moderate (1.5–5.0×10^9/L), and severe ($>5.0 \times 10^9$/L). Based on the duration of symptoms, they can be categorized into transient, episodic, or persistent. The term persistent pertains to peripheral blood eosinophilia that is observed or documented on at least two occasions at a minimum of 4 weeks interval. End-organ damage occurs more in persistent eosinophilia due to the protracted release of chemical mediators and their adverse biological effects. However, it does not mean that one can ignore transient and episodic eosinophilia, as they can also be life-threatening and warrant

urgent lifesaving management.[1] Mild eosinophilia is seen in 3 to 10% of the population, and the commonest causes are allergic diseases, drugs, and parasitic infestations. Hypereosinophilia (HE) is defined as persistent moderate-to-severe peripheral blood eosinophilia ($>1.5 \times 10^9$/L) and/or tissue eosinophilia.[3]

Eosinophils are usually not seen in healthy tissues except for their presence in mucosa of the stomach, small and large bowels, uterus, thymus, spleen, and lymph nodes.[4] The criteria for tissue eosinophilia include bone marrow biopsy showing more than 20% eosinophils of all nucleated cells and/or significant amount of tissue infiltration by eosinophils reported by expert pathologist and/or distinct eosinophil granule protein deposition in the tissue with or without extensive tissue infiltration by eosinophils.[3] HE can be diagnosed by hemogram findings and confirmed by peripheral blood film (PBF) examination. Patients with HE and organ dysfunction attributable to HE are diagnosed with hypereosinophilic syndrome (HES). The older criteria of HES by Simon et al[5] date back to 1975 that include blood AEC $>1.5 \times 10^9$/L lasting more than 6 months, no identifiable secondary (reactive) causes of eosinophilia after complete investigations, and evidence of end-organ damage by persistent eosinophilia. The refined definition of HES is based on the changes made in the duration of the first criterion, that, blood AEC $>1.5 \times 10^9$/L lasting more than 1 month.[3] HES with life-threatening organ dysfunction need immediate evaluation and intervention irrespective of the duration of HE.

Classification of HE and HES

They can be categorized into reactive or secondary HE/HES (common), primary or clonal HE/HES (rare), idiopathic HE/HES, and familial HE (very rare).[6] In both reactive and clonal eosinophilia, the key mediator for uncontrolled proliferation of eosinophils is IL-5, however, with different pathogenesis. The stimuli for the increased production of IL-5 in secondary eosinophilia are infections, drugs, allergies,

and various malignancies (paraneoplastic or cytokine induces HE). The eosinophils are not clonal in this condition, though clonal cells like T cells can induce secondary eosinophilia. The most common cause of HE is reactive and allergic disorders. Reactive eosinophilia is predominantly mediated by IL-5 (along with IL-3 and IL-4) and is caused by atopic dermatitis, asthma and seasonal allergic disorders (-rhinitis/hay fever), dermatological disorders like Wells syndrome (nonallergic granulomatous dermatitis), drug-induced (includes drug reaction with eosinophilia and systemic symptoms/DRESS), helminthic and fungal infections, primary gastrointestinal eosinophilic disorders, connective tissue disorders, rheumatological diseases, atheroembolic disease, Gleich syndrome, lymphoproliferative disorders, solid malignancies, and the lymphocytic variant of HE. The major secondary causes[7–20] are summarized in ►**Table 1**.

In clonal eosinophilia, the eosinophils are clonal and result from a defect (mutation or translocation) in the pluripotent stem cells or myeloid progenitors altering tyrosine kinase and/or myeloid differentiation pathways leading to HE.[3] These

patients can present with HE/HES alone or in combination with other myeloid/lymphoid neoplasms like myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS), MDS/MPN overlap, systemic mastocytosis with HE, acute myeloid leukemia ,and B or T lymphoblastic leukemia/lymphomas. Regardless of the nature of stimuli, persistent eosinophilia and its activation release various potent biological mediators present in their granules. These chemical mediators are the sole ones responsible for end-organ damage resulting in fibrosis, thrombosis, or both. Some of them are eosinophil peroxidase, eosinophil cationic protein, major basic protein, and transforming growth factor-β .[3,21]

Organ Involvement in HES

HE can affect any organ and cause damage. It can be gastrointestinal (esophagitis, gastritis, colitis, pancreatitis,) pulmonary (fibrosis, asthma, eosinophilic vasculitis, eosinophilic pneumonia, pleural effusion), upper airways, cardiac (endocardial fibrosis, necrosis, thrombosis), neurological

Table 1 Etiology of secondary/reactive HE/HES[7,8]

Atopy	Asthma, allergic rhinitis, eczema
Fungal infection	Cryptococcus neoformans, Aspergillus spp, *Coccidioides immitis, Paracoccidioides brasiliensis* and *Histoplasma capsulatum.*
Parasitic infection	Anisakis simplex, Taenia solium, Schistosoma mansoni, Strongyloides stercoralis, toxoplasma,[9] Strongyloidiasis (Strongyloides stercoralis), Hookworm (Ancylostoma duodenale and Necator americanus), Filariasis (Loa loa, Wuchereria bancrofti, Mansonella perstans, Brugia malayi, onchocerca spp.), Ascariasis (Ascaris lumbricoides), Toxocariasis (Toxocara canis), Trichinosis (Trichinella spp.), Scabies (Sarcoptes scabiei), Fascioliasis (Fasciola hepatica)
Viral	HIV
Immunological disorders	Hyper-IgE syndrome, DOCK8 deficiency, PGM3 deficiency, STAT3 deficiency, CD40 deficiency, ADA deficiency, ZAP70 deficiency, CD3γ deficiency, MHC II deficiency, TCR-α deficiency, MALT1 deficiency, Kostmann disease, cyclic neutropenia, Omenn syndrome, Wiskott-Aldrich syndrome, autoimmune lymphoproliferative syndrome, immunodysregulation polyendocrinopathy enteropathy X-linked, Papillon-Lefevre syndrome, and CVID[10]
Dermatological disorders	Chronic spontaneous urticaria, atopic dermatitis, eosinophilic dermatoses, eosinophilic cellulitis (Wells syndrome), eosinophilic pustular folliculitis, eosinophilic fasciitis (Shulman disease), granuloma faciale; recurrent cutaneous eosinophilic vasculitis (RCEV)[11]
Pulmonary diseases	Idiopathic acute or chronic eosinophilic pneumonia, Loffler syndrome, allergic bronchopulmonary aspergillosis (ABPA), sarcoidosis
Drugs	NSAIDs, anticonvulsant and DRESS syndrome. Drugs implicated in DRESS syndromes are aromatic antiepileptic drugs like phenytoin, lamotrigine and carbamazepine, allopurinol, antimicrobial sulfonamides (sulfasalazine), and dapsone, other antibiotics such as vancomycin and minocycline[12–14]
Connective tissue and rheumatological disorders	Churg-Strauss syndrome, Wegener's granulomatosis, systemic lupus erythematosus, polyarteritis nodosa (PAN), eosinophilic fasciitis, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), dermatomyositis and Sjogren syndrome
Allergic gastroenteritis and esophagitis, inflammatory bowel disease (ulcerative colitis and Crohn's disease)	
Nonmyeloid malignancies:	Hodgkin disease, non-Hodgkin lymphomas, acute lymphoblastic leukemia, T cell lymphomas
Nonhematological malignancies	Solid tumors like carcinomas arising from lung, GIT, hepatobiliary system, thyroid, and genitourinary system[15]
Other rare causes	Graft versus host disease,[17] adrenal insufficiency,[18] atheroembolic disease,[19] Gleich syndrome (episodic angioedema with eosinophilia (EAE)[20]

Abbreviations: ADA, adenosine deaminase; CVID, common variable immunodeficiency; GIT, gastrointestinal tract; HE/HES, hypereosinophilia/hypereosinophilic syndrome; HIV, HIV, human immunodeficiency virus; MHC II, major histocompatibility complex II; NSAIDs, nonsteroidal anti-inflammatory drugs.
Lymphocyte variant HES.[16]
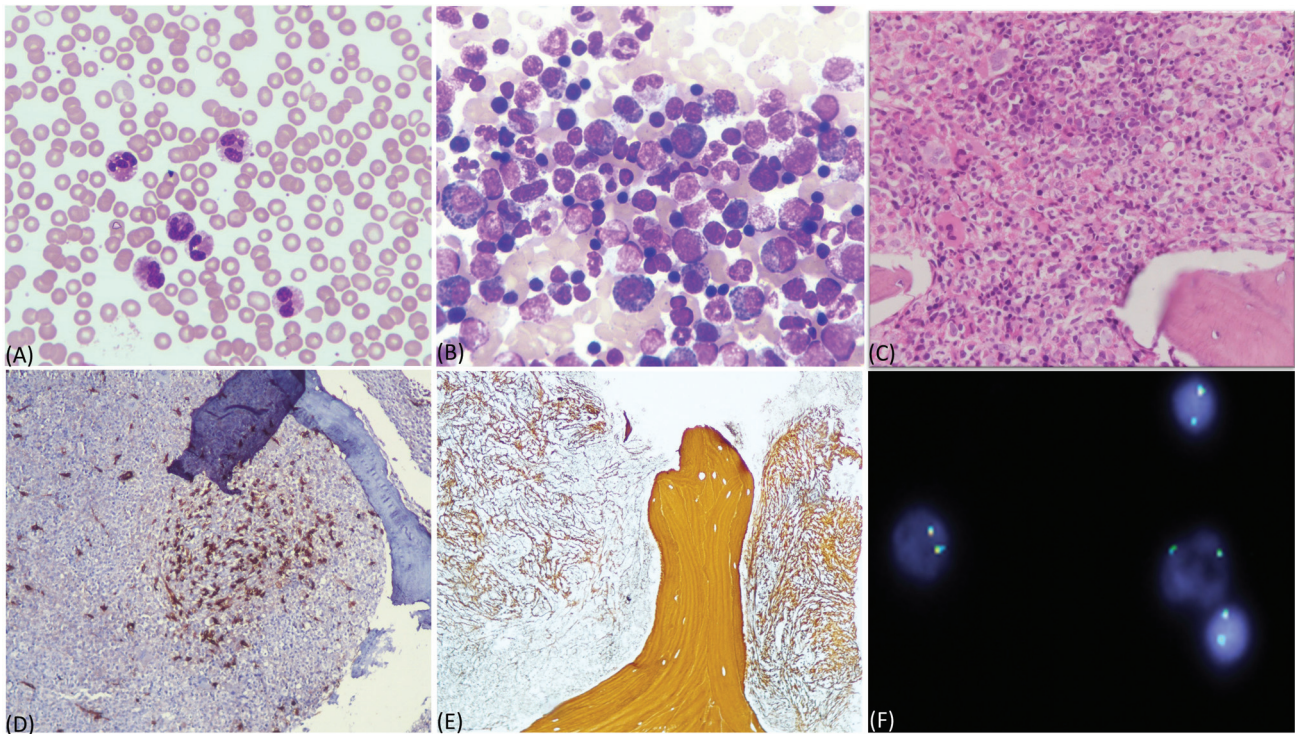
Fig. 2 The peripheral blood and bone marrow findings from a 56-year-old male patient who presented with paraparesis. Imaging showed a paraspinal mass; and subsequent fine-needle aspiration cytology confirmed a diagnosis of myeloid sarcoma. Hemogram showed leukocytosis (32 × 10^9/L) with eosinophilia (23%), mild anemia (hemoglobin 11 g/dL), and normal platelet counts (240 × 10^9/L). Peripheral blood film (**A**) showed eosinophils with bilobed and trilobed nuclei and cytoplasmic vacuoles (40x, May Grunwald Giemsa stain); Bone marrow aspirate (**B**) was hypercellular and showed excess of eosinophil precursors and eosinophils (40x, May Grunwald Giemsa stain); Bone marrow biopsy (**C**) showed interstitial excess of eosinophils (20x, hematoxylin and eosin stain) and reticulin stain (**D**) for mast cell tryptase highlighted the scattered as well as loose aggregates of mast cells (20x). Fluorescent *in-situ* hybridization (Vysis *PDGFRA, CHIC2, FIP1L1* tricolor rearrangement probe, Abbott molecular, Illinois, United States) showed *CHIC2* deletion (orange) (**E**) showed 3+ paratrabecular as well as interstitial fibrosis (20x). Immunohistochemistry (**F**) in 70% cells consistent with *FIP1L1::PDGFRA* translocation. A diagnosis of myeloid neoplasm with eosinophilia and *FIP1L1::PDGFRA* translocation was made; and the patient responded to 100mg imatinib.

(neuropathies), skin (angioedema, eczema, cellulitis, panniculitis), and bone marrow fibrosis.

Diagnostic Approach to Eosinophilic Disorders[7,22–29]

Diagnosing HE/HES is challenging due to the complex nature of disease manifestations and numerous underlying etiologies. Eventually, the patients are referred to multiple specialists and are investigated to find a needle in a haystack. However, proper evaluation, step-wise appropriate investigation, and precise treatment can save the precious life of the patients and avoid mismanagement. It also requires knowledge about the disease's etiology, pathogenesis, and the utility of various investigations in a sagacious manner to narrow down the intricate cause. Knowing that only rare cases are clonal, it is wise to foremost rule out reactive conditions and proceed with molecular and other exorbitant workup. Here we discuss a practical approach to patients with HE/HES. While it is essential to have a detailed medical history and physical examination, from a laboratory point of view, the first and most important tool is a good PBF examination. In patients with life-threatening organ damage, samples for all necessary investigations should be collected before starting therapy, especially steroids (►**Fig. 2**).

The diagnostic evaluation includes detailed medical history and physical examination, PBF examination, investigations to exclude common reactive/secondary causes of HE, and imaging to assess organ involvement. The importance of these is highlighted in ►**Table 2**. Because of the wide spectrum of infections associated with HE/HES and the nonavailability of complete infectious disease workup in most situations, it may not be possible to demonstrate the exact infectious agent, especially helminths. In such a situation, an empirical antihelminthic medication may be given; and further workup done in patients without a response.[7,8] In view of the high incidence of parasitic infections, it is essential to understand that a positive serology may indicate exposure in the past and not necessarily the cause of HE.

The significance of PBF and bone marrow examination and specialized hematological investigations are highlighted here.

Peripheral Blood Film Examination

PBF examination helps us to confirm whether HE is isolated or part of another clonal process. There are no morphological features that can distinguish clonal from reactive eosinophils. Monolobated eosinophils, tri or quadrilobed eosinophils, degranulated eosinophils, and eosinophil vacuoles can be seen in both clonal and reactive processes. Similarly, thrombocytosis cannot distinguish between two processes.[28] A

Table 2 Steps in the diagnostic evaluation of hypereosinophilia and their importance

Medical history	To identify the cause and recognize any organ damage
− Symptoms related to allergic disorders and family history of allergy	HES secondary to allergy
− Poor socioeconomic situation, poor nutrition and hygiene − Recent travel to tropical or endemic areas, exposure to pets, intake of undercooked meat or exotic dishes − Accidental ingestion/exposure to excreta of pets/insects − Drug intake (including indigenous drugs) − Recurrent infections with or without skin manifestations in children − Rheumatological/connective tissue disorders − Cough, breathlessness, pleuritic pain, abdominal pain, diarrhea, and neurological symptoms	Increased risk of parasitic infections and infestations Increased risk of parasitic infections and infestations Increased risk of parasitic infections and infestations HES secondary to drugs including drug reaction with eosinophilia and systemic symptoms (DRESS) Possibility of primary immunodeficiency disorders with HE HE/HES secondary to rheumatological disorders May indicate organ damage due to HES
Physical examination	**To identify the cause and recognize any organ damage**
− Pallor	May indicate gastrointestinal helminthic infection
− Moderate-to-massive splenomegaly	May indicate clonal HE/myeloproliferative neoplasm with HE
− Significant lymphadenopathy	May indicate lymphoproliferative neoplasm or leukemia
− Eczema	Possibility of HE/HES secondary to allergy
− Skin plaques/nodules	Possibility of HE secondary to lymphoma
− Pulmonary findings	May indicate allergic bronchopulmonary aspergillosis or tropical pulmonary eosinophilia or HE-induced organ damage
Investigations to exclude common reactive/secondary causes of HE	
− Fresh stool microscopy for ova, cysts, and parasites − Serological tests for parasites − Serological tests for viruses − ANA, C-ANCA, P-ANCA, anti-Rho/La, anti-smith, and other autoimmune serological markers − IgE levels − Aspergillus specific IgE − Serum LDH − Serum vitamin B12 − Day/night blood smears − Muscle biopsy − Microscopy of sputum or bronchoalveolar lavage fluid and fungal culture of respiratory samples	Exclude parasitic infections Exclude microfilaria, trichinella, toxoplasma, Toxocara, cysticercus, hydatid, amoeba Exclude HIV, HBV, HCV Exclude autoimmune disorders and vasculitis High IgE in children may suggest reactive HE but may point towards hyper-IgE syndrome Exclude/diagnose aspergillosis High LDH may indicate lymphoproliferative disorder High vitamin B12 may indicate myeloproliferative disorder Exclude/diagnose filariasis Exclude/diagnosis trichinellosis Exclude/diagnosis fungal infections
Other investigations − Chest X-ray − Computed tomography, − Electrocardiography (lungs and abdomen), − Pulmonary function test − Serum troponin T	To assess organ involvement especially cardiac and lung Imaging gives us a clue about the cause of HE (e.g., lymphomas, solid malignancies, parasitic infections—cysticercosis, hydatid cyst)

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HE, hypereosinophilia; HES, hypereosinophilic syndrome; HIV, human immunodeficiency virus; IgE, immunoglobulin E; LDH, lactate dehydrogenase.

microcytic hypochromic anemia with HE with/without thrombocytosis may indicate gastrointestinal helminth infection. However, the presence of blasts, basophilia, erythrocytosis, leucoerythroblastic picture, chronic myeloid leukemia like picture, and significant granulocytic dysplasia should raise strong suspicion of underlying hematological malignancies leading to bone marrow examination and other flow cytometric/cytogenetic/molecular investigations directly

without delay. The PBF examination also gives an opportunity to exclude the presence of parasites, especially microfilaria.

Bone Marrow Examination
A bone marrow examination should be performed in patients without a definite secondary cause identified by the above investigations. It helps to exclude acute leukemia (especially acute lymphoblastic leukemia/ALL) masked by HE.[30] Patients

with excess blasts need exclusion of myeloid and lymphoid neoplasms with rearrangement of *PDGFRA, PDGFRB, FGFR1, JAK2,* or other tyrosine kinase domain fusions.[6,31,32] Patients with B lineage ALL also need exclusion of *IL3::IGH* translocation, where reactive HE occurs as a secondary phenomenon.[33]

Other findings which support the diagnosis of neoplastic HE/HES includes spindle shaped mast cell more than 25% (systemic mastocytosis with HE), loose (*PDGFRA* associated HE) or tight clusters of mast cells (systemic mastocytosis with HE), myelofibrosis and myeloid or megakaryocytic dysplasia[34] (►**Fig. 2**). Occasionally, HE may be secondary to bone marrow infiltration by a lymphoproliferative process, including T-cell non-Hodgkin lymphoma or Hodgkin Lymphoma.

Other Ancillary Hematological Investigations on Blood or Bone Marrow

The following ancillary investigations should supplement the bone marrow examination to exclude a clonal disorder.

(a) Conventional karyotyping: The identification of karyotypic abnormalities helps in the confirmation of primary/clonal eosinophilia. However, we should be cautious while interpreting age-related abnormalities such as –Y, –X, +15, and +Y.[35,36]

(b) Fluorescent *in-situ* hybridization/FISH: It is strongly advised to perform molecular cytogenetic testing even in patients with normal cytogenetics, as cryptic cytogenetic abnormalities (especially *FIP1L1::PDGFRA*) may be missed. FISH testing using a panel of tricolor re-arrangement probe for *FIP1L1::PDGFRA* and break-apart probes for *PDGFRB, FGFR1, JAK2,* and *ETV6* is a cost-effective strategy that

can identify a large majority of myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (MLN-TK).[22,26,37,38]

(c) Basic molecular investigations, including reverse transcriptase PCR (RT-PCR): Occasional reports showed that FISH (depending on the probe) might not detect all cases of *FIP1L1::PDGFRA*.[39] In such cases, RT-PCR is more sensitive. It is preferable to exclude common markers like *BCR::ABL1, JAK2V617F, CALR, RUNX1::RUNX1T1, and CBFB::MYH11* before ordering higher investigations.[8,22]

(d) Next-generation sequencing (NGS) for somatic variants and other rare fusions: In unsolved cases, NGS should be advised.[40] A list of markers identifiable by cytogenetic/molecular testing and their significance is summarized in ►**Table 3**.

(e) Immunohistochemistry on bone marrow biopsy sections: Highlight the mast cells in the bone marrow trephine biopsy by utilizing the antibodies like CD25, CD117, and tryptase. The distribution of mast cells (scattered or clusters) and the nature of the infiltration (benign or clonal) can be highlighted. The presence of mastocytosis favors a diagnosis of MPN, especially *FIP1L1::PDGFRA* or systemic mastocytosis with HE. It will also help to exclude abnormal lymphoid infiltration.[26,34]

(f) Flow cytometry and immunophenotyping: Immunophenotyping is a useful investigation to confirm the underlying clonal disorders like acute leukemia (B/T/MPAL), lymphoproliferative disorders and mastocytosis. High sensitivity flow cytometry also identifies abnormal T cell clones, most commonly CD3-CD4+ T cells. They are shown to secrete IL-5 leading to HE/HES (lymphocytic variant of HE/HES).[8,16,37]

Table 3 Cytogenetic and molecular markers of clonal eosinophilia and associated morphology[8,22,24,26,27,37]

Abnormality	Morphology
RUNX1::RUNX1T1, CBFB: MYH11	AML
BCR::ABL1	CML, AML, MPAL, B-ALL
PDGFRA rearrangement (partners: FIP1L1, ETV6, BCR, FOXP1, STRN, TNKS2, KIF5B, SPECC1L, etc.)	Isolated HE, MPN, MDS/MPN, blast phase of myeloid or lymphoid lineage (with eosinophilia ± neutrophilia, basophilia)
PDGFRB rearrangement (partners: ETV6, PDE4DIP, WDR48, TPM3, SPTBN1, GOLGA4/B1, PRKG2, TNIP1, CEP85L, HIP1, BIN2, etc.)	Isolated HE, MDS/MPN, MPN, blast phase of myeloid or lymphoid lineage (with eosinophilia)
FGFR1 rearrangement (partners: ZMYM2, BCR, TPR, CEP43, CNTRL, CEP43G, etc.)	B-ALL, T-ALL, MPAL, MPN, MPN/MDS (with eosinophilia ± neutrophilia, basophilia, monocytosis, erythrocytosis depending on the partner gene)
JAK2 rearrangement (partners: PCM1, ETV6, BCR, etc.)	MPN, MPN/MDS with eosinophilia and/or monocytosis
FLT3 rearrangement (partners: ETV6, BCR, ZMYM2, TRIP11, SPTBN1, GOLGB1, CCDC88C, ZBTB44, and MYO18A)	MDS, MPNs, MDS/MPNs, AML, B-ALL, T-ALL with eosinophilia. Myeloid sarcoma
ETV6: ABL1 fusion	CML like picture
KIT p.D816V, JAK2 p.V617F, JAK2 exon 13 indels, STAT5B p. N642H,[43] *DNMT3A, ASXL1, TET2, EZH2, SRSF2, SETBP1, CBL*[a]	CEL, SM associated with eosinophilia, PV with eosinophilia
+8, -7, isochromosome 17, complex karyotype, 13q, 20q del, 1q abnormalities	CEL

Abbreviations: AML, acute myeloid leukemia; B-ALL, B lineage acute lymphoblastic leukemia; CML, chronic myeloid leukemia; HE, hypereosinophilia; MDS, myelodysplastic syndrome; MPAL, mixed phenotypic acute leukemia; MPN, myeloproliferative neoplasm; PV, polycythemia vera; SM, systemic mastocytosis; T-ALL, T lineage acute lymphoblastic leukemia.
[a]Variant allele frequency and age-related clonal hematopoiesis should be considered while interpretation.
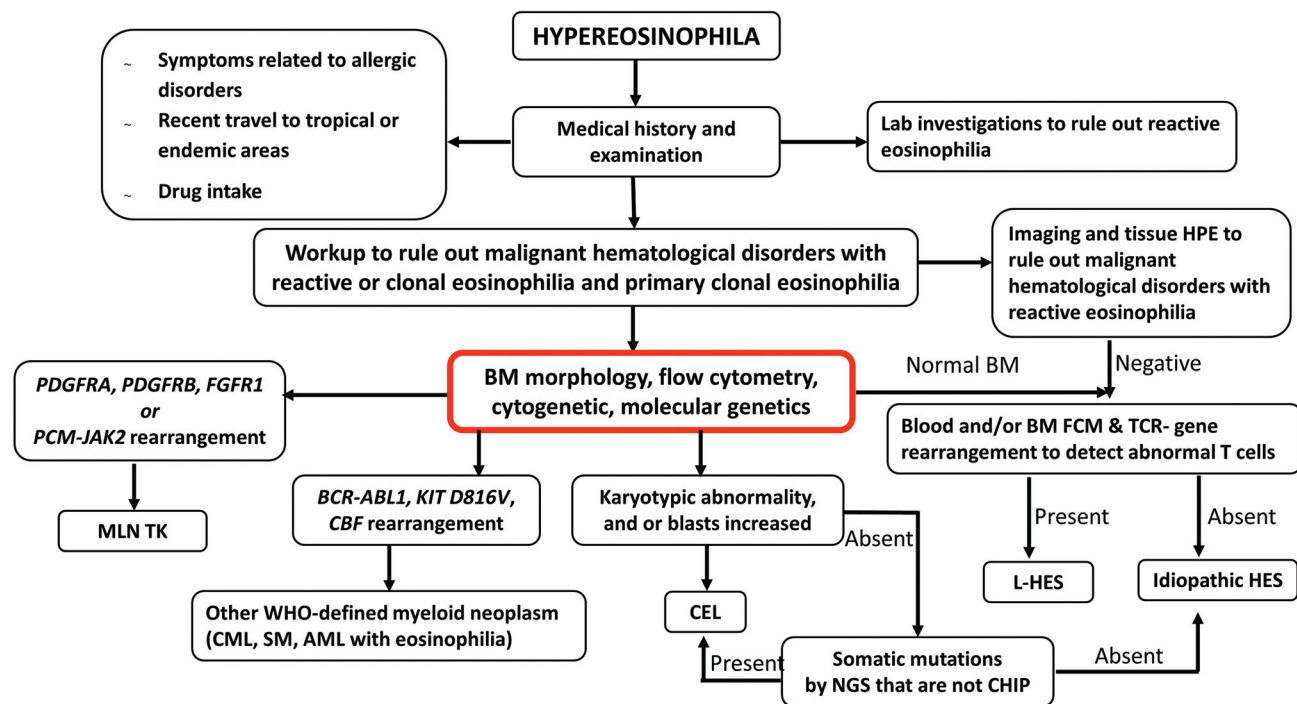
Fig. 3 Algorithmic approach in the diagnosis of hypereosinophilia/hypereosinophilic syndrome. AML, acute myeloid leukemia; BM, bone marrow; CBF, core binding factor; CEL, chronic eosinophilic leukemia; CHIP, clonal hematopoiesis of indeterminate potential; CML, chronic myeloid leukemia; FCM, flow cytometry; L-HES, lymphocytic variant of hypereosinophilia; MLN TK, myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions; NGS, next-generation sequencing; SM, systemic mastocytosis.

(g) TCR-gene re-arrangement: Helps to confirm the clonality of T-cells in L-HES and lymphoproliferative disorders. BIOMED-2-based multiplex polymerase chain reaction (PCR) is the standard procedure to detect clonal TCR re-arrangements in *TCRβ, TCRγ, and TCRδ* genes.[41] However, the isolated presence of clonal TCR gene re-arrangement without any aberrant T-cells does not support the diagnosis of L-HES as TCR clonal gene re-arrangement is also seen in elderly persons and patients with severe viral infections.

How to Diagnose Chronic Eosinophilic Leukemia

There are no morphological features that are specific to chronic eosinophilic leukemia (CEL). A recent report showed dysplastic eosinophils (47%), dysplastic and increased mega-karyocytes (41%, 18%), myelofibrosis (18%), and myeloid hyperplasia (47%) in these patients.[42] The latest World Health Organization (WHO) classification (5th edition) has laid down essential features for diagnosing CEL. The essential criteria include [1] HE (AEC > 1.5×10^9/L on at least two occasions over at least four weeks, [2] evidence of clonality, [3] abnormal bone marrow morphology, [4] not meeting criteria for other myeloid or lymphoid neoplasms with HE. While considering clonal markers, the possibility of age-related clonal hematopoiesis of indeterminate potential should always be considered.[31,43]

When to Diagnose Idiopathic HE/HES?

Rarely, even after a detailed history, proper examination, and utilizing algorithmic approach commencing from baseline

investigations to molecular genetic studies, a few patients cannot be categorized under any classifications mentioned above. Such patients are classified under the idiopathic HE and idiopathic HES category, which is a diagnosis of exclusion. It is associated with tissue damage; the most common organ systems involved are the heart, lungs, skin, peripheral and central nervous systems, and gastrointestinal tract. These patients frequently present with thromboembolic complications. A few studies have shown that sporadic mutations diagnosed by NGS are associated with idiopathic HES. However, their significance in disease pathogenesis should be further explored in the future. In any instance, it is vital to carefully rule out all other diagnoses before determining that a patient has idiopathic HES.[7,22,26]

Importance of Identifying the Exact Etiology

Eosinophilia should be treated at the root of the problem. It is crucial to pinpoint the precise etiology because different etiologies have distinct treatment requirements and responses. Ivermectin or other antihelminthic therapy is widely used for parasitic infestation. For underlying immu-nological problems, corticosteroids and immunosuppressive treatment are used. Corticosteroids, hydroxyurea, and IL-5/IL-5 receptor antibodies such as mepolizumab, benralizu-mab, reslizumab (anti-IL-5 IgG4 monoclonal antibody), and pegylated-interferon are used to treat the lymphocytic type of HES. Imatinib at a low dose of 100 mg/day for HE associat-ed with *PDGFRA*-rearrangement and at the usual full dose for HE with *PDGFRB*-rearrangement are preferred. Leukocytosis can be reduced by hydroxyurea. Patients with idiopathic HES

can be treated similarly to those with the lymphocytic form of HES. Individuals with severe idiopathic HES who are refractory to previous treatments should consider alemtuzumab, an anti-CD52 monoclonal antibody. It may also be helpful for patients with idiopathic HES-related cardiac and cerebral dysfunction. When accessible, hematopoietic stem-cell transplantation should be considered for patients with CEL, clonal eosinophilia with *FGFR1* re-arrangement, and HES who are resistant to or intolerant of both standard TKI therapy and experimental medical therapy or who exhibit progressive end-organ damage.[7,44] The diagnostic algorithm[26] in the approach of HE is shown in ►**Fig. 3**.

Conclusion

Considering the complex etiopathologies, the cost of testing, and the time involved, the workup needs to be tailored according to the urgency of the situation and the resources available. In urgent situations with organ damage, it is crucial to initiate appropriate management without waiting for the results of investigations. In contrast, in a resource-limited situation, it is acceptable to employ step-by-step rather than comprehensive testing to rule out the most common causes first.

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