

# Blood and Bone Marrow Evaluation for Eosinophilia

Daniel F. Boyer, MD, PhD

• Evaluation of peripheral blood and bone marrow for an indication of persistent eosinophilia can be a challenging task because there are many causes of eosinophilia and the morphologic differences between reactive and neoplastic causes are often subtle or lack specificity. The purpose of this review is to provide an overview of the differential diagnosis for eosinophilia, to recommend specific steps for the pathologist evaluating blood and bone marrow, and to emphasize 2 important causes of eosinophilia that require specific ancillary tests for diagnosis: myeloproliferative neoplasm with *PDGFRA* rearrangement and lymphocyte-variant hypereosinophilic syndrome.

(Arch Pathol Lab Med. 2016;140:1060–1067; doi: 10.5858/arpa.2016-0223-RA)

Eosinophilia is graded based on absolute eosinophil count: mild (500–1500/ $\mu$ L), moderate (1500–5000/ $\mu$ L), or severe (>5000/ $\mu$ L). A patient can be described as having idiopathic hypereosinophilia if there is persistent moderate or severe eosinophilia for at least 6 months without an identified cause, and the diagnosis of hypereosinophilic syndrome (HES) is applicable when end-organ damage occurs because of persistent moderate or severe eosinophilia.<sup>1</sup>

The most common causes of eosinophilia are often taught with the pneumatic device of 3 W's: wind, worms, and wonder drugs. *Wind* refers to asthma and other allergic causes, *worms* refer to tissue-invasive parasites, and *wonder drugs* refer to a nonspecific reaction to various medications or drug-induced hypersensitivity. Another common cause in the inpatient setting is critical illness, which leads to suppressed adrenal function, resulting in decreased steroid production and disinhibition of eosinophil production.<sup>2</sup> There are several multiorgan autoimmune or idiopathic diseases that can cause significant eosinophilia. The autoimmune/idiopathic disorders most commonly associated with eosinophilia are eosinophilic granulomatosis with polyangiitis (EGPA, also known as Churg-Strauss syndrome), Kimura disease, immunoglobulin (Ig)G4-related disease, diffuse fasciitis with eosinophilia, and eosinophilic myositis.<sup>3</sup> Diagnosis of these rare autoimmune disorders is

challenging and requires assessment of a variety of clinical and laboratory findings, in addition to histopathologic evaluation of affected tissues. However, EGPA is the autoimmune condition that most frequently causes sustained hypereosinophilia. The main features that distinguish EGPA from other causes of HES are prominent asthmatic symptoms, perinuclear antineutrophil cytoplasmic antibodies with antimyeloperoxidase specificity (present in 30%–40% of patients with EGPA), and biopsy-proven eosinophilic vasculitis.<sup>4</sup> Single-organ eosinophilic diseases can also cause significant peripheral blood eosinophilia. The organs most frequently involved are the lungs, gastrointestinal tract, and skin.<sup>5–7</sup>

In addition to the nonneoplastic etiologies described above, eosinophilia can be caused by a variety of myeloid, lymphoid, and epithelial neoplasms. Myeloid neoplasms can result in primary eosinophilia because of overproduction of eosinophils from abnormal stem cells. Carcinomas and lymphoproliferative disorders can trigger cytokine production causing secondary eosinophilia. Rare congenital syndromes can also enter into the differential diagnosis of eosinophilia, especially in children, and include hyper-IgE syndrome, Omenn syndrome, and familial eosinophilia.<sup>8,9</sup> The differential diagnosis for persistent peripheral blood eosinophilia is summarized in Table 1.

## MYELOID NEOPLASMS WITH EOSINOPHILIA

When a pathologist receives a peripheral blood or bone marrow specimen to evaluate for eosinophilia, the main focus of investigation should be myeloid and lymphoid neoplasia because there is little that can be done to identify specific nonneoplastic causes of eosinophilia in these specimens. The myeloid neoplasms associated with eosinophilia (Table 2) include chronic myelogenous leukemia (CML); genetically defined neoplasms with rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1*, *JAK2*, and *FLT3*; chronic eosinophilic leukemia, not otherwise specified (CEL, NOS); and acute myeloid leukemia (AML) with core-binding factor translocations, such as inversion 16 or t(8;21). Although mild eosinophilia is common with core-binding factor AML, hypereosinophilia caused by AML is rare. There are rare cases of eosinophilic myeloproliferative neoplasms associated with *JAK2* V617F.<sup>10–13</sup> The morphologic findings in these cases tend to be nonspecific, such as, hypercellular bone marrow with eosinophilia and mild fibrosis, and testing for *JAK2* V617F is necessary to make the diagnosis. The myeloid neoplasms listed so far are associated with primary eosinophilia, meaning that the eosinophils are produced from abnormal myeloid progenitor cells. In contrast, Langerhans cell histiocytosis and systemic mastocytosis (SM) are myeloid neoplasms associated with

Accepted for publication June 2, 2016.

From the Department of Pathology, University of Michigan, Ann Arbor.

The author has no relevant financial interest in the products or companies described in this article.

Presented in part at the New Frontiers in Pathology; October 22–24, 2015; Ann Arbor, Michigan.

Corresponding author: Daniel F. Boyer, MD, PhD, Department of Pathology, University of Michigan, 5242 Medical Science 1, 1301 Catherine St, Ann Arbor, MI 48109 (email: dfboyer@med.umich.edu).

**Table 1. Differential Diagnosis of Eosinophilia**

Common reactions: medications, parasites, asthma/allergy  
 Adrenal insufficiency due to critical illness  
 Multiorgan autoimmune/idiopathic diseases, especially  
 EGPA/Churg-Strauss syndrome  
 Single-organ eosinophilic diseases, especially lung, GI, skin  
 Myeloid neoplasms with eosinophilia  
 Secondary eosinophilia due to lymphoid or epithelial  
 neoplasms  
 Congenital syndromes: hyper-IgE, Omenn syndrome,  
 familial eosinophilia

Abbreviations: EGPA, eosinophilic granulomatosis with polyangiitis; GI, gastrointestinal disease; IgE, immunoglobulin E.

secondary eosinophilia because the eosinophils appear to be reactive, rather than arising from a neoplastic clone.

The 2008 World Health Organization classification of tumors of hematopoietic and lymphoid tissues includes the category of myeloid and lymphoid neoplasms with *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangements.<sup>14</sup> The major disease entities within this category are myeloproliferative neoplasm (MPN) with *PDGFRA* rearrangement, MPN with *PDGFRB* rearrangement, and the so-called 8p11 myeloproliferative syndrome. The MPN with *PDGFRA* rearrangement is a much more common cause of eosinophilia than are neoplasms with *PDGFRB* or *FGFR1* rearrangements, and specific testing for this entity is critically important because *PDGFRA* rearrangements are usually cryptic on routine karyotype. *PDGFRA* rearrangements are identified in 10% to 20% of patients with HES without an identifiable reactive etiology.<sup>15</sup> Myeloproliferative neoplasm with *PDGFRA* rearrangement shows a marked male predominance of at least 30:1, based on reported case series.<sup>13,16–23</sup> It affects a broad range of ages with a median age of around 40 years. Initial presentation is usually with cutaneous or pulmonary symptoms. Splenomegaly, mucosal ulcers, and thromboembolic events are also common. The most serious complication is cardiac dysfunction, which occurs in 20% to 30% of patients if not successfully treated. Typical laboratory findings include markedly elevated serum vitamin B12 levels, often greater than 2000 pg/mL, and elevated serum tryptase, approximately 30 ng/mL on average. In contrast to reactive causes of eosinophilia, serum IgE is only elevated in a few patients. Eosinophils in the peripheral blood smear are often morphologically unremarkable, but, in some cases, the eosinophils show significant cytologic atypia, including hypogranulation, microgranulation, coarse basophilic granules, vacuolization, and nuclear hypersegmentation or hyposegmentation (Figure 1, A through D). Bone marrow findings include hypercellularity, increased eosinophils, increased and atypical mast cells, and reticulin fibrosis (Figure 1, E through I). The extent of reticulin fibrosis is highly variable from case to case. *PDGFRA* rearrangement usually causes a low-grade MPN, but rare cases of AML and T-lymphoblastic lymphoma associated with *PDGFRA* rearrangement have been reported.<sup>24,25</sup> Key features of MPN with *PDGFRA* rearrangement are summarized in Table 3.

The bone marrow and peripheral blood findings of MPN with *PDGFRA* rearrangement share similarities with systemic mastocytosis, and accurate differentiation between these diseases is important because of differences in therapy and the significant risk of cardiac disease associated with MPN with *PDGFRA* rearrangement.<sup>26</sup> A comparison of the

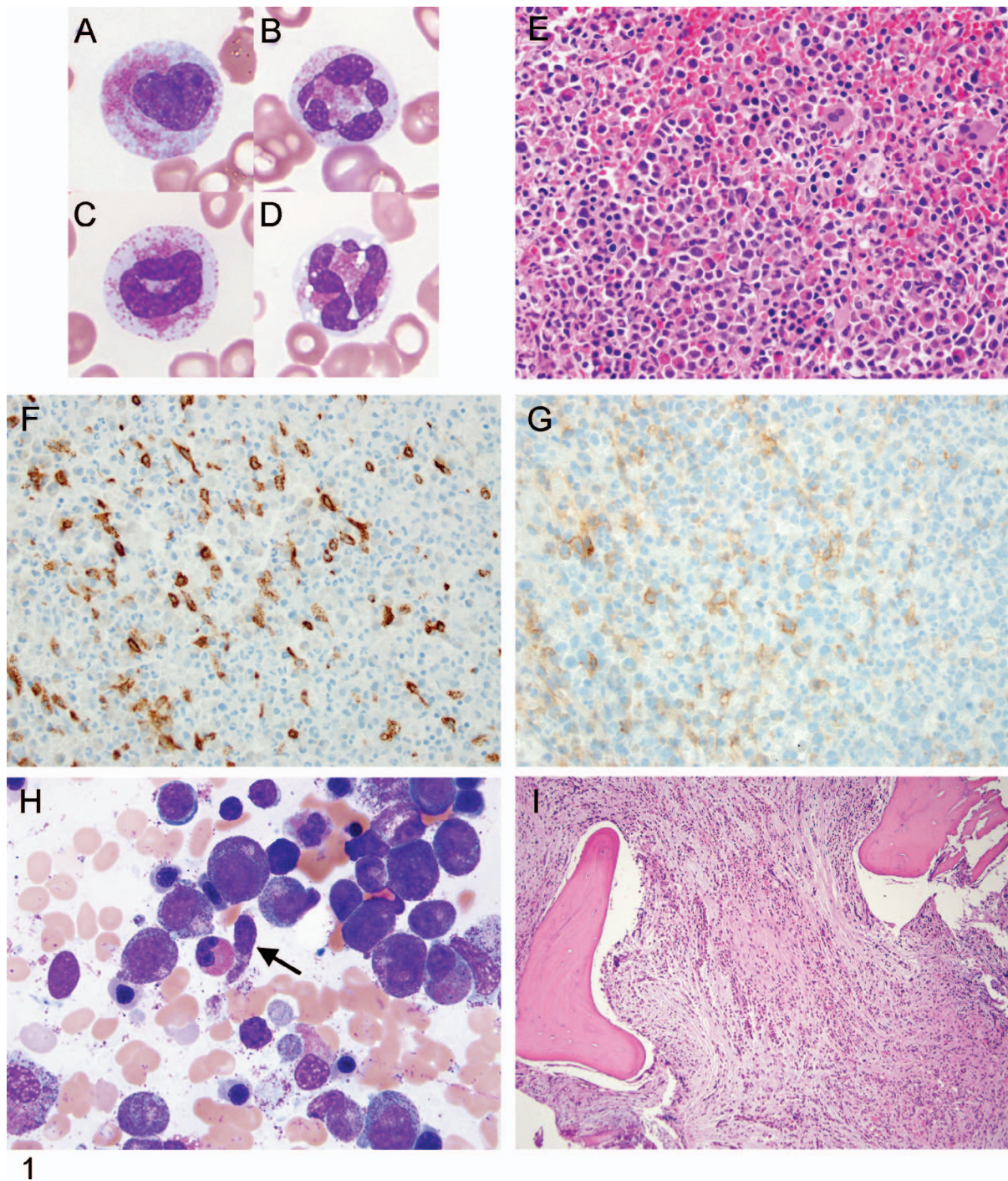
**Table 2. Myeloid Neoplasms With Eosinophilia**

Primary eosinophilia  
 Chronic myelogenous leukemia  
 Myeloid or lymphoid neoplasms with *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangements  
 • del(4)(q12); *FIP1L1-PDGFRB*  
 • t(5;12)(q33;p13); *ETV6-PDGFRB*  
 • t(8;13)(p11;q12); *ZMYM2-FGFR1*  
 Myeloid or lymphoid neoplasms with *JAK2* or *FLT3* rearrangements  
 • t(8;9)(p22;p24); *PCM1-JAK2*  
 • t(12;13)(p13;p12); *ETV6-FLT3*  
 MPN with eosinophilia and *JAK2* V617F  
 • 1%–2% of “idiopathic” hypereosinophilia  
 • Often lacks typical features of PV, ET, or PMF  
 Chronic eosinophilic leukemia, not otherwise specified  
 • AEC >1500/μL, and at least 1 of the following:  
 ◦ >5% blasts in BM  
 ◦ >2% blasts in PB  
 ◦ Clonal genetic abnormality  
 AML with inv(16)(p13.1q22) or t(8;21)(q22;q22)  
 Secondary eosinophilia  
 Systemic mastocytosis  
 Langerhans cell histiocytosis

Abbreviations: AEC, absolute eosinophil count; AML, acute myeloid leukemia; BM, bone marrow; ET, essential thrombocythemia; MPN, myeloproliferative neoplasm; PB, peripheral blood; PMF, primary myelofibrosis; PV, polycythemia vera.

features of MPN with *PDGFRA* rearrangement and systemic mastocytosis with eosinophilia (SM-EO) is presented in Table 4. Usually, MPN with *PDGFRA* rearrangement can be differentiated from SM-EO based on the presence of *PDGFRA* rearrangement and the absence of urticaria, dense mast cell aggregates, and *KIT* D816V mutation. However, microdissection experiments have shown that the atypical mast cells in some cases of MPN with *PDGFRA* rearrangement harbor *KIT* D816V mutations, raising the possibility that the 2 diseases share more genetic similarities than previously understood.<sup>27</sup> In addition, a case has been reported that met diagnostic criteria for both MPN with *PDGFRA* rearrangement and SM because of the presence of multiple dense mast cell aggregates in the bone marrow.<sup>28</sup> Cases that fully meet the diagnostic criteria for both diseases are probably best classified as SM with an associated hematologic neoplasm, but it is still essential to document the presence of *PDGFRA* rearrangement because of the implications for therapy (ie, *PDGFRA* rearrangements are associated with excellent response to imatinib).

*PDGFRA* rearrangements can be detected by fluorescence in situ hybridization (FISH) or reverse transcription-polymerase chain reaction. Only a few *PDGFRA* rearrangements can be detected by routine karyotype. The most common *PDGFRA* rearrangement is an 800-kb internal deletion in the q12 region of chromosome 4, which causes fusion of the *FIP1L1* and *PDGFRA* genes. Because *FIP1L1* and *PDGFRA* are relatively close together in the wild-type configuration, FISH probes to evaluate *FIP1L1-PDGFRB* fusions usually include a probe to detect loss of intervening genetic material, typically either the *CHIC2* or *LNX* genes. All the *PDGFRA* rearrangements associated with MPNs have a breakpoint in exon 12 of *PDGFRA*, which disrupts an inhibitory domain.<sup>15</sup> Regardless of the fusion partner, the *PDGFRA* rearrangements seem to manifest the same phenotype and show excellent response to imatinib. There



1

**Figure 1.** Myeloproliferative neoplasm with FIP1L1-PDGFRB fusion. A through D, Atypical eosinophils in peripheral blood. Atypical features include hypogranulation, microgranulation, vacuolization (D), nuclear hyposegmentation (A and C), and hypersegmentation (B and D). E, Hypercellular bone marrow core biopsy with increased eosinophils. F, Anti-tryptase immunohistochemical stain highlights increased mast cells, including frequent spindled forms. G, Anti-CD25 immunohistochemical stain shows aberrant staining of mast cells. H, Bone marrow aspirate shows myeloid hyperplasia and a spindled mast cell (arrow). I, Bone marrow core biopsy of a fibrotic myeloproliferative neoplasm with FIP1L1-PDGFRB (Wright-Giemsa, original magnifications  $\times 2000$  [A through D] and  $\times 1000$  [H]; hematoxylin-eosin, original magnifications  $\times 400$  [E] and  $\times 100$  [I]; original magnification  $\times 400$  [F and G]).

are also rare cases of MPN with *PDGFRB* point mutations without gene rearrangement.<sup>29</sup>

The most common *PDGFRB* rearrangement associated with myeloid neoplasia is t(5;12)(q33;p13); *ETV6-PDGFRB*, but more than 20 different *PDGFRB* translocations have been described.<sup>25,30,31</sup> Myeloproliferative neoplasm with

*PDGFRB* rearrangement most often presents with a combination of monocytosis and eosinophilia but can also present as AML or with features resembling atypical CML, SM, or primary myelofibrosis. Rare cases of composite MPN and T-lymphoblastic lymphoma with *PDGFRB* rearrangements have also been described.<sup>25</sup> Myeloproliferative neoplasms

**Table 3. Myeloproliferative Neoplasm With PDGFRA Rearrangement**

Clinical features	
10%–20% of patients with HES	
Marked male predominance (>30:1)	
Broad age range (average, 40 y)	
Usually presents with cutaneous or pulmonary symptoms	
Splenomegaly, mucosal ulcers, and thromboembolic events also common	
20%–30% develop cardiac dysfunction if not treated	
>90% have elevated serum vitamin B12 at diagnosis (often >2000 pg/mL)	
80% have elevated serum tryptase (median, 30 ng/mL)	
Bone marrow findings	
Hypercellular with increased eosinophils	
Increased and atypical mast cells	
• Frequent spindled forms	
• Granulation may be decreased	
• Usually CD25 <sup>+</sup> but CD2 <sup>-</sup>	
• Dense aggregates are very rare	
Reticulin usually increased	

Abbreviation: HES, hypereosinophilic syndrome.

with *PDGFRB* rearrangement show excellent response to imatinib.<sup>31</sup> The 8p11 myeloproliferative syndrome is caused by *FGFR1* translocations, usually t(8;13)(p11;q12); *ZMYM2-FGFR1*, and typically presents with a mixture of myeloid and lymphoid neoplasia.<sup>25,32</sup> The classic presentation is the triad of nodal T-lymphoblastic lymphoma, peripheral blood eosinophilia, and myeloid hyperplasia in the bone marrow. These patients show frequent progression to AML, but acute lymphoblastic leukemia (ALL) or mixed-lineage leukemia can also occur. The neoplasms with *FGFR1* rearrangement do not respond to imatinib.

In addition to *PDGFRA*, *PDGFRB*, and *FGFR1* gene rearrangements, rearrangements of the *JAK2* and *FLT3* genes have been associated with eosinophilic myeloid neoplasms. The t(8;9)(p22;p24); *PCM1-JAK2* fusion causes an MPN that can resemble atypical CML, chronic eosinophilic leukemia, or primary myelofibrosis.<sup>33–35</sup> It shows male predominance with a broad age range and has a high risk of transformation to AML or Philadelphia-like B-cell ALL (B ALL). Treatment and outcome data for MPN with *PCM1-JAK2* fusion are limited, but some patients have responded to treatment with JAK2 inhibitors. The t(12;13)(p13;q12);

**Table 4. Comparison of Myeloproliferative Neoplasm (MPN) With PDGFRA Rearrangement and Systemic Mastocytosis With Eosinophilia (SM-EO)<sup>a</sup>**

	MPN With PDGFRA	SM-EO
Sex ratio (M:F)	>30:1	1:1
GI symptoms	Rare	Common
Heart/lung symptoms	Common	Rare
Urticaria pigmentosa, %	0	60
Multifocal dense MC aggregates, %	Rare	>90
>25% spindled MC, %	>90	>90
CD25 <sup>+</sup> MC, %	>95	>95
CD2 <sup>+</sup> MC	Rare	Occasional
<i>KIT</i> D816V, %	Rare	>90
Serum AEC/tryptase, (cells per μL/ng per mL)	>100	<100

Abbreviations: AEC, absolute eosinophil count; GI, gastrointestinal; MC, mast cells.

<sup>a</sup> Derived from data in Maric et al,<sup>26</sup> 2007.

**Table 5. Lymphoid Neoplasms With Secondary Eosinophilia**

Lymphocyte-variant hypereosinophilia
Cutaneous T-cell lymphoma, especially advanced-stage MF and SS
Lymphoblastic leukemia/lymphoma, especially B ALL with t(5;14)(q31;q32)
Classical Hodgkin lymphoma
Adult T-cell leukemia/lymphoma
Angioimmunoblastic T-cell lymphoma

Abbreviations: B ALL, B lymphoblastic leukemia; MF, mycosis fungoides; SS, Sézary syndrome.

*ETV6-FLT3* fusion also causes an MPN with eosinophilia and high-risk of AML. Only 5 cases have been reported in the literature; of which, 3 presented with concurrent T-cell lymphoma.<sup>36–39</sup>

If the genetically defined eosinophilic neoplasms have been ruled out, the next consideration is CEL, NOS. The diagnostic criteria are persistent eosinophilia greater than 1500/μL, no definitive features of other myeloid neoplasms, and the presence of either increased blasts (>2% in blood or >5% in bone marrow) or a nonspecific clonal genetic abnormality, such as trisomy 8 or isochromosome band 17q.<sup>40</sup> Chronic eosinophilic leukemia, NOS, shows frequent transformation to AML and carries a poor prognosis.<sup>41</sup>

#### LYMPHOID NEOPLASMS WITH SECONDARY EOSINOPHILIA

In the myeloid neoplasms described above, eosinophilia results from overproduction of eosinophils from abnormal myeloid progenitor cells. In contrast, lymphoid neoplasms can cause significant eosinophilia because of cytokine production (Table 5). T-cell neoplasms are most commonly associated with eosinophilia, and significant eosinophilia is most frequently seen in the context of cutaneous T-cell lymphoma, adult T-cell leukemia/lymphoma, and angioimmunoblastic T-cell lymphoma.<sup>42</sup> The B-cell neoplasms most significantly associated with eosinophilia are classical Hodgkin lymphoma and B-lymphoblastic leukemia/lymphoma, especially B ALL with t(5;14)(q31;q32); *IGH-IL3*. This uncommon translocation causes eosinophilia from overproduction of interleukin (IL) 3 by the neoplastic B cells.<sup>43</sup> The t(5;14)(q31;q32) translocation associated with B ALL and eosinophilia should not be confused with t(5;14)(q35;q32); *TLX3-BCL11*, which is associated with T-cell ALL.

Another important lymphocytic cause of eosinophilia is proliferation of abnormal T helper 2-type T cells, which is known as lymphocyte-variant hypereosinophilic syndrome (Table 6). The name of this disease has caused some confusion because the term *lymphocytic HES* was used for many years to refer to any etiology of HES associated with increased cytokine production by lymphocytes.<sup>44</sup> The current usage of the term *lymphocyte-variant hypereosinophilic syndrome* (L-HES) refers to an eosinophilic condition caused by a T-cell lymphoproliferative disease characterized by immunophenotypically aberrant and/or clonal T cells. Lymphocyte-variant HES accounts for 10% to 20% of HES without another definitive reactive or neoplastic cause.<sup>22,45–47</sup> The diagnosis is often challenging because symptoms usually develop gradually and resemble an allergic reaction, and most patients do not have significant lymphadenopathy or lymphocytosis. Patients usually present with an erythem-

**Table 6. Lymphocyte-Variant Hypereosinophilic Syndrome (L-HES)**

Patients with "idiopathic" HES (10%–20%) Usually presents with pruritic rash <ul style="list-style-type: none"><li>• Erythematous dermatitis or papular/nodular rash</li><li>• Episodic angioedema, urticaria, and poikiloderma also occur</li></ul> Abnormal T cells detected in peripheral blood Flow cytometric findings: <ul style="list-style-type: none"><li>• Classic phenotype: CD3<sup>-</sup>, CD4<sup>+</sup>, CD7<sup>-</sup>, CD5<sup>++</sup></li><li>• Also common: CD3<sup>+</sup>, CD4<sup>+</sup>, CD7<sup>-</sup>, or CD3<sup>+</sup>, CD4<sup>-</sup>, CD8<sup>-</sup>, and TCR-AB<sup>+</sup></li></ul> Corticosteroids are first-line therapy 10%–25% progress to T-cell lymphoma <ul style="list-style-type: none"><li>• Usually AITL; CTCL; or PTCL, NOS</li><li>• Rare cases of ALK-ALCL</li></ul>
--

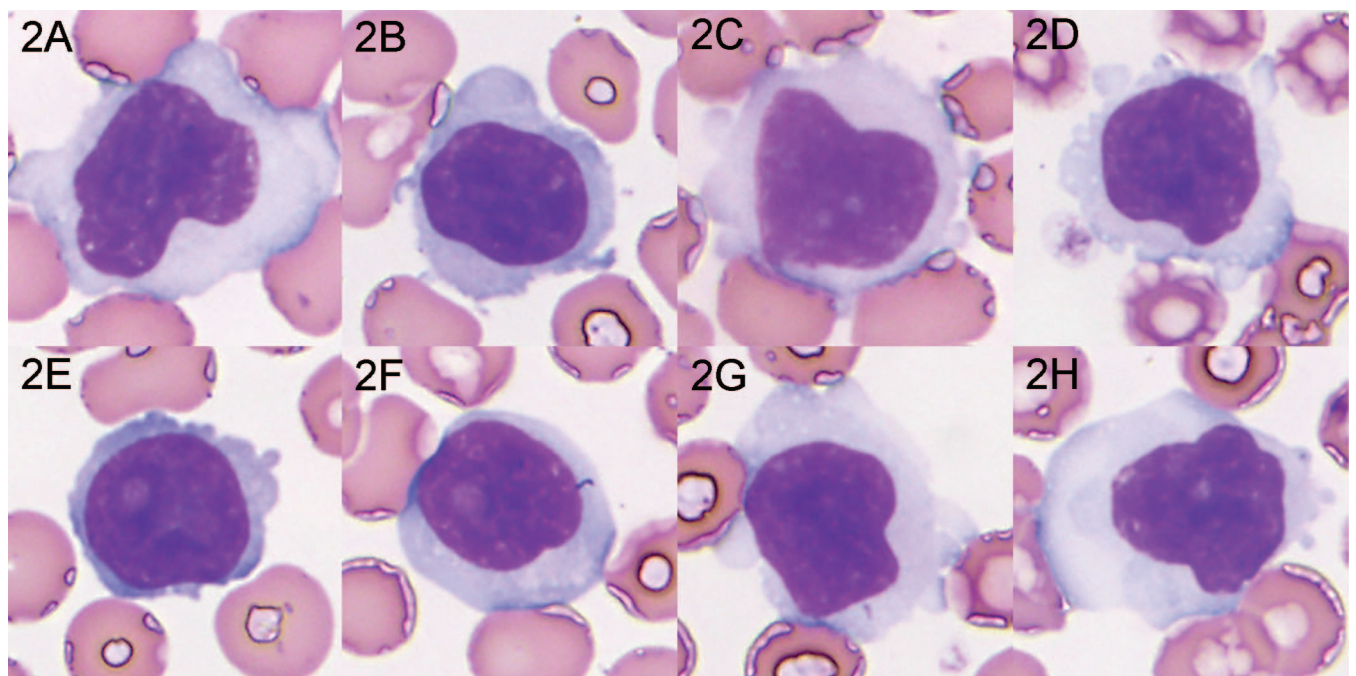
Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; ALK-ALCL, ALK<sup>-</sup> anaplastic large cell lymphoma; CTCL, cutaneous T-cell lymphoma; NOS, not otherwise specified; PTCL, peripheral T-cell lymphoma.

atous or papular/nodular pruritic rash. Urticaria, poikiloderma, and episodic angioedema can also occur. Episodic angioedema with eosinophilia is also known as *Gleich syndrome*, and identification of clonal CD3<sup>-</sup>, CD4<sup>+</sup> T cells in most patients with Gleich syndrome suggests that it is probably a subtype of L-HES.<sup>48</sup> The abnormal T-cell populations in L-HES produce T helper 2 cytokines, including IL4, IL5, and IL13, and stimulate production of CCL17/TARC (thymus and activation-regulated cytokine); however, testing for production of these cytokines is currently performed using research assays and is not readily available as a clinically validated test.

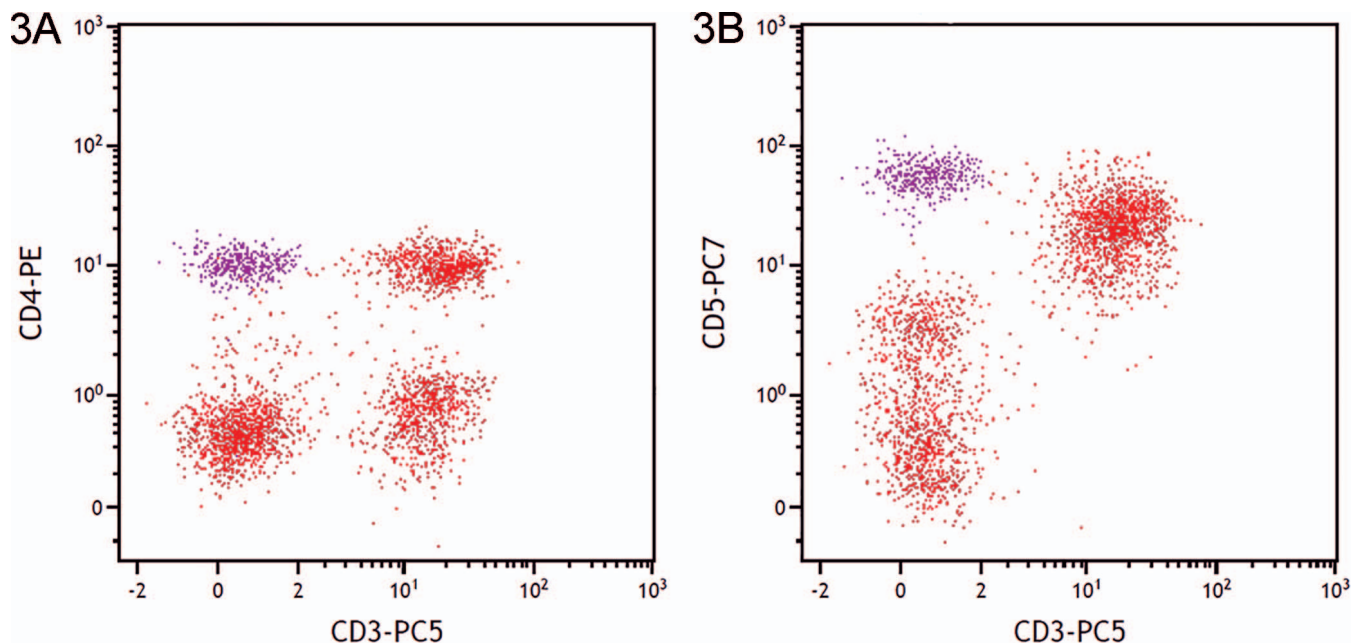
Peripheral blood lymphocytes are cytologically unremarkable in most cases of L-HES; however, atypical lymphocytes can be identified on the peripheral blood smear in some

cases.<sup>49</sup> These cells have moderately abundant cytoplasm, which is lightly basophilic and agranular (Figure 2, A through H). Mild nuclear-contour irregularities may occur, but the classic cerebriform nuclei of Sézary cells are not present. Flow cytometry is the most definitive method for diagnosing L-HES. The classic immunophenotype associated with L-HES is CD4<sup>+</sup> T cells with dim-to-negative surface CD3 and CD7 and an abnormally bright CD5 signal (Figure 3, A and B). However, the classic pattern is present in less than half of cases. Other abnormal T-cell immunophenotypes commonly occurring in L-HES include CD3<sup>+</sup> and CD4<sup>+</sup> T cells with loss of CD7 and/or CD2; and CD3<sup>+</sup>, CD4<sup>-</sup>, CD8<sup>-</sup>, and T-cell antigen receptor (TCR)-AB<sup>+</sup> T cells.<sup>46</sup> When flow cytometry results are equivocal, identification of a T-cell clone by polymerase chain reaction (PCR) can help support the diagnosis of L-HES. It is controversial whether L-HES can be diagnosed when PCR identifies a T-cell clone, but flow cytometry does not reveal an abnormal population. A recent study identified clonal T-cell populations by PCR in 23% of otherwise typical cases of MPN with *PDGFRA* rearrangement.<sup>23</sup> This finding emphasizes the importance of carefully evaluating all potential causes of eosinophilia rather than assuming that detection of a clonal T-cell population is diagnostic of L-HES.

Usually, L-HES is a chronic disease and is indolent. It is not considered to be a malignancy, even though immunophenotypic aberrancy and genetic clonality are present in most cases. The symptoms caused by L-HES can be managed with corticosteroids and immunomodulatory agents, and cytotoxic chemotherapy has not proven effective in eliminating the abnormal T-cell clone.<sup>46</sup> In reported case series, 10% to 25% of patients eventually developed an overt T-cell lymphoma.<sup>45,47,49–51</sup> Secondary lymphomas reported in patients with L-HES include angioimmunoblastic T-cell lymphoma; peripheral T-cell lymphoma, not otherwise specified; cutaneous T-cell lymphoma, and ALK<sup>-</sup> anaplastic



**Figure 2.** Atypical lymphocytes in lymphocyte-variant hypereosinophilic syndrome (L-HES). A through H, Selected lymphocytes from the peripheral blood smear of a patient with L-HES show atypical features, including irregular nuclear contours, occasionally prominent nucleoli, and moderately abundant agranular cytoplasm (Wright-Giemsa, original magnification  $\times 3000$ ).



**Figure 3.** Classic immunophenotype of lymphocyte-variant hypereosinophilic syndrome. *A*, Flow cytometry identifies a population of atypical CD4<sup>+</sup> T cells (purple), which are negative for surface CD3. *B*, The atypical T cells also have abnormally bright anti-CD5 staining. Normal lymphocytes are colored red.

large cell lymphoma; and these lymphomas mostly arose 3 to 10 years after initial diagnosis of L-HES.<sup>45,47,49–53</sup> The diagnosis of angioimmunoblastic T-cell lymphoma or anaplastic large cell lymphoma after L-HES is straightforward because these lymphomas have highly specific morphologic and immunohistochemical features. In some cases, the differential diagnosis between L-HES and cutaneous T-cell lymphoma may be difficult because both can present with erythematous skin, eosinophilia, and abnormal T cells in the peripheral blood. However, L-HES presents with a gradual onset of cutaneous symptoms and causes hypereosinophilia much earlier in the course of the disease compared with Sézary syndrome. Widespread erythroderma and classic Sézary cells are not typical of L-HES and should raise suspicion for Sézary syndrome even if the patient has a history of L-HES. Although abnormal T cells can be detected in skin biopsies of patients with L-HES, they usually show a perivascular and dermal pattern of infiltration and do not show significant epidermotropism or other features of mycosis fungoides, such as Pautrier microabscesses or formation of patches, plaques, and tumors.<sup>49</sup> A recent series of L-HES cases from the French Eosinophil Network (Lille, France) found that some patients had numerous abnormal, circulating T cells and infiltration of lymph nodes by atypical T cells but remained stable for many years, with only 2 patients progressing to overt lymphoma.<sup>49</sup> The published literature does not offer firm conclusions on what degree of lymphadenopathy, cytologic atypia, or peripheral blood involvement defines the border between L-HES and peripheral T-cell lymphoma, not otherwise specified; however, a rapid increase in peripheral blood involvement or lymphadenopathy is strongly suggestive of progression to malignancy. Cytogenetic abnormalities have been detected in a few cases of L-HES.<sup>51,52,54</sup> One case with del(6q) and one with t(6;11) subsequently developed peripheral T-cell lymphoma, not otherwise

specified<sup>51,52</sup>; however, the predictive value of genetic abnormalities in L-HES has not been established.

#### DIAGNOSTIC WORKUP OF PERIPHERAL BLOOD AND BONE MARROW

To test for the neoplastic causes of eosinophilia described above, a thorough and consistent approach to the evaluation of peripheral blood and bone marrow is recommended. Peripheral blood testing should include assessment of

**Table 7. Peripheral Blood Evaluation for Eosinophilia**

Morphology
Evaluate for evidence of AML, ALL, or CML
Assess eosinophils for cytologic atypia
<ul style="list-style-type: none"> <li>• Hypogranulation</li> <li>• Microgranulation</li> <li>• Vacuolization</li> <li>• Nuclear hypersegmentation or hyposegmentation</li> </ul>
Look for Sézary cells or other atypical lymphocytes
Flow cytometry
Aberrant T-cell phenotypes associated with L-HES
<ul style="list-style-type: none"> <li>• CD3<sup>-</sup>, CD4<sup>+</sup>, CD7<sup>-</sup>, CD5<sup>++</sup></li> <li>• CD3<sup>+</sup>, CD4<sup>+</sup>, CD7<sup>-</sup></li> <li>• CD3<sup>+</sup>, CD4<sup>-</sup>, CD8<sup>-</sup>, TCR-AB<sup>+</sup></li> </ul>
No flow tests for abnormal eosinophils in clinical use
Molecular diagnostics
FISH for <i>FIP1L1-PDGFR</i> A
PCR for clonal TCR rearrangements
FISH for <i>BCR-ABL1</i>
PCR for <i>JAK2 V617F</i>
Consider PCR for <i>KIT D816V</i>
Consider FISH for <i>PDGFRB</i> , <i>JAK2</i> , and <i>FGFR1</i> break-apart
Chemistry
Serum tryptase, B12, and IgE may be helpful

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; FISH, fluorescence in situ hybridization; Ig, immunoglobulin; L-HES, lymphocyte-variant hypereosinophilic syndrome; PCR, polymerase chain reaction.

**Table 8. Bone Marrow Evaluation For Eosinophilia**

Assess for increased or aberrant blasts
• AML or ALL
• MPN in accelerated phase
• CEL, NOS
Reticulin fibrosis favors MPN over reactive eosinophilia
Assess for increased or aberrant mast cells
• Morphology and IHC (CD117, tryptase, CD25)
• Spindled, CD25 <sup>+</sup> mast cells are present in SM and MPN with <i>PDGFRA</i> rearrangement
• Dense mast cell clusters favor SM
FISH for <i>FIP1L1-PDGFR</i> A (if not done on blood)
Karyotype
• <i>PDGFRB</i> , <i>JAK2</i> , <i>FGFR1</i> , or <i>FLT3</i> rearrangements
• Other clonal abnormalities (CEL, NOS)
Assess for infiltration by an occult metastatic malignancy

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CEL, chronic eosinophilic leukemia; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; MPN, myeloproliferative neoplasm; NOS, not otherwise specified; SM, systemic mastocytosis.

morphology, flow cytometry, molecular diagnostics, and serum chemistry (Table 7). The morphologic assessment should include looking for blasts, basophilia, and granulocytic left shift, which could indicate AML, ALL, or CML. The eosinophils should be evaluated for cytologic atypia, even though myeloid neoplasms with primary eosinophilia often lack significant atypia. The peripheral blood smear should also be scanned for Sézary cells or other atypical lymphocytes because of the frequent association of secondary eosinophilia with T-cell neoplasms. The main utility of flow cytometry for eosinophilia is detecting abnormal T-cell populations, which is critical for the diagnosis of L-HES. There are currently no clinically validated flow cytometric assays that differentiate reactive and neoplastic eosinophils.

The most useful molecular diagnostic tests are FISH or PCR for *PDGFRA* gene rearrangement (including *FIP1L1-PDGFR*A fusion), PCR for assessment of T-cell clonality, FISH or PCR for *BCR-ABL1* fusion, and PCR for *JAK2* V617F. Testing for *PDGFRA* gene rearrangement is especially important because it has critical diagnostic and therapeutic implications and is usually not detected on routine karyotype. Polymerase chain reaction for *KIT* D816V can be helpful to identify SM with eosinophilia; however, consideration of patient history and clinical findings before testing for *KIT* D816V is suggested to promote efficient use of this test. Serum chemistry for levels of IgE, tryptase, and vitamin B12 can help narrow the differential diagnosis of eosinophilia.<sup>22</sup> Immunoglobulin E is usually elevated in patients with secondary eosinophilia but only rarely in patients who have myeloid neoplasms with primary eosinophilia. Tryptase is significantly elevated in systemic mastocytosis and MPN with *PDGFRA* rearrangement. Vitamin B12 is usually markedly elevated in patients with MPN with *PDGFRA* rearrangement and is often elevated in association with other myeloid neoplasms.

Even if the peripheral blood findings are diagnostic of a neoplastic process, bone marrow biopsy is still recommended to assess for findings that could change the diagnosis or prognosis. The key elements are blast count, aberrant mast cells, reticulin fibrosis, and karyotype (Table 8). In rare cases, bone marrow biopsy may reveal an occult metastatic malignancy causing secondary eosinophilia. Cultured bone marrow is usually superior to peripheral blood for cytogenetic analysis, and the bone marrow karyotype is helpful to

identify translocations of *PDGFRB*, *JAK2*, *FGFR1*, and *FLT3*, as well as clonal abnormalities that could establish a diagnosis of CEL, NOS, in the appropriate context.

#### References

1. Gotlib J. World Health Organization–defined eosinophilic disorders: 2015 update on diagnosis, risk stratification, and management. *Am J Hematol*. 2015;90(11):1077–1089.
2. Angelis M, Yu M, Takamishi D, Hasaniya NW, Brown MR. Eosinophilia as a marker of adrenal insufficiency in the surgical intensive care unit. *J Am Coll Surg*. 1996;183(6):589–596.
3. Tamaki H, Chatterjee S, Langford CA. Eosinophilia in rheumatologic/vascular disorders. *Immunol Allergy Clin North Am*. 2015;35(3):453–476.
4. Greco A, Rizzo MI, De Virgilio A, et al. Churg-Strauss syndrome. *Autoimmun Rev*. 2015;14(4):341–348.
5. Woolnough K, Wardlaw AJ. Eosinophilia in pulmonary disorders. *Immunol Allergy Clin North Am*. 2015;35(3):477–492.
6. Mehta P, Furuta GT. Eosinophils in gastrointestinal disorders: eosinophilic gastrointestinal diseases, celiac disease, inflammatory bowel diseases, and parasitic infections. *Immunol Allergy Clin North Am*. 2015;35(3):413–437.
7. de Graauw E, Beltraminelli H, Simon HU, Simon D. Eosinophilia in dermatologic disorders. *Immunol Allergy Clin North Am*. 2015;35(3):545–560.
8. Valent P, Klion AD, Horny HP, et al. Contemporary consensus proposal on criteria and classification of eosinophilic disorders and related syndromes. *J Allergy Clin Immunol*. 2012;130(3):607–612.
9. Curtis C, Ogbogu PU. Evaluation and differential diagnosis of persistent marked eosinophilia. *Immunol Allergy Clin North Am*. 2015;35(3):387–402.
10. Jones AV, Kreil S, Zoi K, et al. Widespread occurrence of the *JAK2* V617F mutation in chronic myeloproliferative disorders. *Blood*. 2005;106(6):2162–2168.
11. Dahabreh IJ, Giannouli S, Zoi C, Zoi K, Loukopoulou D, Voulgarelis M. Hypereosinophilic syndrome: another face of Janus? *Leuk Res*. 2008;32(9):1483–1485.
12. Helbig G, Majewski M, Wiczorkiewicz A, et al. Screening for *JAK2* V617F point mutation in patients with hypereosinophilic syndrome in response to “Hypereosinophilic syndrome: another face of Janus?” by Dahabreh et al published in *Leukemia Research Leuk Res*. 2009;33(3):e1–e2.
13. Schwaab J, Umbach R, Metzgeroth G, et al. *KIT* D816V and *JAK2* V617F mutations are seen recurrently in hypereosinophilia of unknown significance. *Am J Hematol*. 2015;90(9):774–777.
14. Bain BJ, Gilliland DG, Horny H-P, Vardiman JW. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1*. In: Swerdlow SH, Campo E, Harris NL, et al, eds. *WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:68–73. *World Health Organization Classification of Tumours*; vol 2.
15. Gotlib J, Cools J. Five years since the discovery of *FIP1L1-PDGFR*A: what we have learned about the fusion and other molecularly defined eosinophilias. *Leukemia*. 2008;22(11):1999–2010.
16. Cools J, DeAngelo DJ, Gotlib J, et al. A tyrosine kinase created by fusion of the *PDGFRA* and *FIP1L1* genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N Engl J Med*. 2003;348(13):1201–1214.
17. Pardanani A, Brockman SR, Paternoster SF, et al. *FIP1L1-PDGFR*A fusion: prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe eosinophilia. *Blood*. 2004;104(10):3038–3045.
18. Vandenberghe P, Wlodarska I, Michaux L, et al. Clinical and molecular features of *FIP1L1-PDFGRA* (+) chronic eosinophilic leukemias. *Leukemia*. 2004;18(4):734–742.
19. Roche-Lestienne C, Lepers S, Soenen-Cornu V, et al. Molecular characterization of the idiopathic hypereosinophilic syndrome (HES) in 35 French patients with normal conventional cytogenetics. *Leukemia*. 2005;19(5):792–798.
20. La Starza R, Specchia G, Cuneo A, et al. The hypereosinophilic syndrome: fluorescence in situ hybridization detects the del(4)(q12)-*FIP1L1/PDGFR*A but not genomic rearrangements of other tyrosine kinases. *Haematologica*. 2005;90(5):596–601.
21. Bacarani M, Cilloni D, Rondoni M, et al. The efficacy of imatinib mesylate in patients with *FIP1L1-PDGFR*A-positive hypereosinophilic syndrome: results of a multicenter prospective study. *Haematologica*. 2007;92(9):1173–1179.
22. Ogbogu PU, Bochner BS, Butterfield JH, et al. Hypereosinophilic syndrome: a multicenter, retrospective analysis of clinical characteristics and response to therapy. *J Allergy Clin Immunol*. 2009;124(6):1319–1325.e3.
23. Legrand F, Renneville A, Macintyre E, et al; for the French Eosinophil Network. The spectrum of *FIP1L1-PDGFR*A-associated chronic eosinophilic leukemia: new insights based on a survey of 44 cases [published online ahead of print August 26, 2013]. *Medicine (Baltimore)*. 2013;92(5):e1–e9. doi:10.1097/MD.0b013e3182a71eba
24. Metzgeroth G, Walz C, Score J, et al. Recurrent finding of the *FIP1L1-PDGFR*A fusion gene in eosinophilia-associated acute myeloid leukemia and lymphoblastic T-cell lymphoma. *Leukemia*. 2007;21(6):1183–1188.
25. Vega F, Medeiros LJ, Bueso-Ramos CE, Arboleda P, Miranda RN. Hematolymphoid neoplasms associated with rearrangements of *PDGFRA*, *PDGFRB*, and *FGFR1*. *Am J Clin Pathol*. 2015;144(3):377–392.
26. Maric I, Robyn J, Metcalfe DD, et al. *KIT* D816V-associated systemic mastocytosis with eosinophilia and *FIP1L1/PDGFR*A-associated chronic eosino-

philic leukemia are distinct entities. *J Allergy Clin Immunol*. 2007;120(3):680–687.

27. Schmitt-Graeff AH, Erben P, Schwaab J, et al. The *FIP1L1-PDGFRα* fusion gene and the *KIT* D816V mutation are coexisting in a small subset of myeloid/lymphoid neoplasms with eosinophilia. *Blood*. 2014;123(4):595–597.

28. Florian S, Esterbauer H, Binder T, et al. Systemic mastocytosis (SM) associated with chronic eosinophilic leukemia (SM-CEL): detection of *FIP1L1/PDGFRα*, classification by WHO criteria, and response to therapy with imatinib. *Leuk Res*. 2006;30(9):1201–1205.

29. Elling C, Erben P, Walz C, et al. Novel imatinib-sensitive *PDGFRα*-activating point mutations in hypereosinophilic syndrome induce growth factor independence and leukemia-like disease. *Blood*. 2011;117(10):2935–2943.

30. Arefi M, Garcia JL, Peñarrubia MJ, et al. Incidence and clinical characteristics of myeloproliferative neoplasms displaying a *PDGFRB* rearrangement. *Eur J Haematol*. 2012;89(1):37–41.

31. Cheah CY, Burbury K, Apperley JF, et al. Patients with myeloid malignancies bearing *PDGFRB* fusion genes achieve durable long-term remissions with imatinib. *Blood*. 2014;123(23):3574–3577.

32. Jackson CC, Medeiros LJ, Miranda RN. 8p11 myeloproliferative syndrome: a review. *Hum Pathol*. 2010;41(4):461–476.

33. Reiter A, Walz C, Watmore A, et al. The t(8;9)(p22;p24) is a recurrent abnormality in chronic and acute leukemia that fuses *PCM1* to *JAK2*. *Cancer Res*. 2005;65(7):2662–2667.

34. Patterer V, Schnittger S, Kern W, Haferlach T, Haferlach C. Hematologic malignancies with *PCM1-JAK2* gene fusion share characteristics with myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRα*, *PDGFRB*, and *FGFR1*. *Ann Hematol*. 2013;92(6):759–769.

35. Bain BJ, Ahmad S. Should myeloid and lymphoid neoplasms with *PCM1-JAK2* and other rearrangements of *JAK2* be recognized as specific entities? *Br J Haematol*. 2014;166(6):809–817.

36. Vu HA, Xinh PT, Masuda M, et al. *FLT3* is fused to *ETV6* in a myeloproliferative disorder with hypereosinophilia and a t(12;13)(p13;q12) translocation. *Leukemia*. 2006;20(8):1414–1421.

37. Walz C, Erben P, Ritter M, et al. Response of *ETV6-FLT3*-positive myeloid/lymphoid neoplasm with eosinophilia to inhibitors of *FMS*-like tyrosine kinase 3. *Blood*. 2011;118(8):2239–2242.

38. Chonabayashi K, Hishizawa M, Matsui M, et al. Successful allogeneic stem cell transplantation with long-term remission of *ETV6/FLT3*-positive myeloid/lymphoid neoplasm with eosinophilia. *Ann Hematol*. 2014;93(3):535–537.

39. Falchi L, Mehrotra M, Newberry KJ, et al. *ETV6-FLT3* fusion gene-positive, eosinophilia-associated myeloproliferative neoplasm successfully treated with sorafenib and allogeneic stem cell transplant. *Leukemia*. 2014;28(10):2090–2092.

40. Bain BJ, Gilliland DG, Horny H-P, Vardiman JW. Chronic eosinophilic leukaemia, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, et al, eds. *WHO Classification of Tumours of the Haematopoietic and Lymphoid*

*Tissues*. 4th ed. Lyon, France: IARC Press; 2008:51–53. *World Health Organization Classification of Tumours*; vol 2.

41. Helbig G, Soja A, Bartkowska-Chrobok A, Kyrzcz-Krzemien S. Chronic eosinophilic leukemia-not otherwise specified has a poor prognosis with unresponsiveness to conventional treatment and high risk of acute transformation. *Am J Hematol*. 2012;87(6):643–645.

42. Roufousse F, Garaud S, de Leval L. Lymphoproliferative disorders associated with hypereosinophilia. *Semin Hematol*. 2012;49(2):138–148.

43. Meeker TC, Hardy D, Willman C, Hogan T, Abrams J. Activation of the interleukin-3 gene by chromosome translocation in acute lymphocytic leukemia with eosinophilia. *Blood*. 1990;76(2):285–289.

44. Simon HU, Rothenberg ME, Bochner BS, et al. Refining the definition of hypereosinophilic syndrome. *J Allergy Clin Immunol*. 2010;126(1):45–49.

45. Simon HU, Plotz SG, Dummer R, Blaser K. Abnormal clones of T cells producing interleukin-5 in idiopathic eosinophilia. *N Engl J Med*. 1999;341(15):1112–1120.

46. Roufousse F, Cogan E, Goldman M. Lymphocytic variant hypereosinophilic syndromes. *Immunol Allergy Clin North Am*. 2007;27(3):389–413.

47. Vaklavas C, Tefferi A, Butterfield J, et al. 'Idiopathic' eosinophilia with an occult T-cell clone: prevalence and clinical course. *Leuk Res*. 2007;31(5):691–694.

48. Khoury P, Herold J, Alpaugh A, et al. Episodic angioedema with eosinophilia (Gleich syndrome) is a multilineage cell cycling disorder. *Haematologica*. 2015;100(3):300–307.

49. Lefevre G, Copin MC, Roumier C, et al; for the French Eosinophil Network. CD3<sup>+</sup>CD4<sup>+</sup> lymphoid variant of hypereosinophilic syndrome: nodal and extranodal histopathological and immunophenotypic features of a peripheral indolent clonal T-cell lymphoproliferative disorder. *Haematologica*. 2015;100(8):1086–1095.

50. Roufousse F, Schandené L, Sibille C, et al. Clonal Th2 lymphocytes in patients with the idiopathic hypereosinophilic syndrome. *Br J Haematol*. 2000;109(3):540–548.

51. Helbig G, Wiczorkiewicz A, Dziaczkowska-Suszek J, Majewski M, Kyrzcz-Krzemien S. T-cell abnormalities are present at high frequencies in patients with hypereosinophilic syndrome. *Haematologica*. 2009;94(9):1236–1241.

52. Ravoet M, Sibille C, Roufousse F, et al. 6q– is an early and persistent chromosomal aberration in CD3<sup>+</sup>CD4<sup>+</sup> T-cell clones associated with the lymphocytic variant of hypereosinophilic syndrome. *Haematologica*. 2005;90(6):753–765.

53. Roufousse F, de Leval L, van Krieken H, van Deuren M. Lymphocytic variant hypereosinophilic syndrome progressing to angioimmunoblastic T-cell lymphoma. *Leuk Lymphoma*. 2015;56(6):1891–1894.

54. Roumier AS, Gardel N, Laï JL, et al. Hypereosinophilia with abnormal T cells, trisomy 7 and elevated TARC serum level. *Haematologica*. 2003;88(7):e104–e107.