Eosinophilia/Hypereosinophilia in the Setting of Reactive and Idiopathic Causes, Well-Defined Myeloid or Lymphoid Leukemias, or Germline Disorders

Report of the 2019 Society for Hematopathology/ European Association for Haematopathology Workshop

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ABSTRACT

Objectives: *To report the findings of the 2019 Society for Hematopathology/European Association for Haematopathology Workshop within the categories of reactive eosinophilia, hypereosinophilic syndrome (HES), germline disorders with eosinophilia (GDE), and myeloid and lymphoid neoplasms associated with eosinophilia (excluding entities covered by other studies in this series).*

Methods: *The workshop panel reviewed 109 cases, assigned consensus diagnosis, and created diagnosis-specific sessions.*

Results: *The most frequent diagnosis was reactive eosinophilia (35), followed by acute leukemia (24). Myeloproliferative neoplasms (MPNs) received 17 submissions, including chronic eosinophilic leukemia, not otherwise specified (CEL, NOS). Myelodysplastic syndrome (MDS), MDS/MPN, and therapy-related myeloid neoplasms received 11, while GDE and HES received 12 and 11 submissions, respectively.*

Conclusions: *Hypereosinophilia and HES are defined by specific clinical and laboratory criteria. Eosinophilia is commonly reactive. An acute leukemic onset with eosinophilia may suggest core-binding factor acute myeloid leukemia, blast phase of chronic myeloid leukemia,* BCR-ABL1*–positive leukemia, or t(5;14) B-lymphoblastic leukemia. Eosinophilia is rare in MDS but common in MDS/MPN. CEL, NOS is a clinically aggressive MPN with eosinophilia as the dominant feature. Bone marrow morphology and cytogenetic and/ or molecular clonality may distinguish CEL from HES. Molecular testing helps to better subclassify myeloid neoplasms with eosinophilia and to identify patients for targeted treatments.*

Key Points

- Hypereosinophilia and hypereosinophilic syndrome are defined by specific clinical and laboratory criteria. Eosinophilia is commonly reactive.
- An acute leukemic onset with eosinophilia may suggest core-binding factor acute myeloid leukemia, blast phase of chronic myeloid leukemia, *BCR-ABL1*–positive leukemia, or t(5;14) B-lymphoblastic leukemia.
- Eosinophilia is rare in myelodysplastic syndrome (MDS) but common in MDS/myeloproliferative neoplasm (MPN). Chronic eosinophilic leukemia, not otherwise specified is an aggressive MPN with eosinophilia as the dominant feature and may show cytogenetic and/or molecular clonality.

The hypereosinophilic disorders are a heterogeneous group of proliferations manifesting with elevated peripheral blood (PB) and/or bone marrow (BM) and tissue eosinophils. These disorders exhibit a broad spectrum of clinical presentations ranging from no clinical issues to life-threatening endomyocardial fibrosis and central nervous system (CNS) involvement. Similarly, the etiologies underlying these hypereosinophilic states range from benign nonclonal (secondary) proliferations to neoplastic clonal (primary) proliferations **Table 1**.[1-4] Given this disease diversity and potential therapeutic implications, it is clear that a systematic and often comprehensive approach to these cases is necessary.[1-4] Depending on the patient, clinical, laboratory, microbiologic, radiologic, pathologic, and genetic information may need to be integrated to arrive at the final diagnosis. For example, the testing may be limited if the underlying cause for the eosinophilia is relatively clear-cut (eg, secondary to known drug/medication administration, known asthmatic/allergic disorder). On the other hand, cases of idiopathic hypereosinophilia (HE) may require the full gamut of testing to effectively rule out specific disease entities. As a result, firsthand consideration of the potential disease entities, communication with the primary treating physician, and knowledge of pertinent pathologic results are key components to the successful diagnosis and classification of hypereosinophilic conditions.

Definitions and Terminology

In PB, an absolute eosinophil count (AEC) (determined by multiplying the total WBC count WBC by the percentage of eosinophils) of less than 0.5×10^9/L is typically considered normal. A historical "grading" system exists that arbitrarily assigns the degree of peripheral eosinophilia as follows—mild eosinophilia (AEC 0.5-1.5×10^9/L, moderate eosinophilia (AEC 1.5-5.0×10^9/L), and marked/severe eosinophilia (AEC >5.0×10^9/L). An AEC of 1.5×10^9/L or more is considered HE.

For the purposes of the 2019 Society for Hematopathology/European Association for Haematopathology (SH-EAHP) workshop, the review panel adopted the definitions and classifications proposed by Valent et al[5] **Table 2** and **Table 3**. Hypereosinophilic syndrome (HES) requires sustained PB HE, and there must also be evidence of organ damage/dysfunction that is directly attributable to the eosinophil infiltrate or degranulation. Stated another way, the organ damage/dysfunction should not be due to some other coexisting disease or condition. While skin, lung, and gastrointestinal tract are the most commonly affected organ systems, essentially almost any tissue can be affected **Table 4**. Although less common, cardiac and CNS involvement is more serious and potentially has life-threatening sequelae. Thromboembolic events may also occur in

Table 1
Etiology of Eosinophilic Conditions (Not Exhaustive)

	Secondary (Nonclonal Eosinophils)	
Primary (Clonal Eosinophils)	**Underlying Neoplasm**	**Nonneoplastic Disorder**
CEL, NOS	Lymphoma	Infection
Myeloid and lymphoid neoplasms with eosinophilia and rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM1-JAK2*	T-cell lymphoma	Allergic disorders
	Classic Hodgkin lymphoma	Drug reaction
	Lymphoblastic leukemia	Rheumatologic disorders
	Carcinoma	Immunodeficiency
AML (particularly inv(16))		Familial/germline disorders
CML		Miscellaneous (radiation exposure, hypoadrenalism)
MDS		LV-HES
MPN		
Systemic mastocytosis		

AML, acute myeloid leukemia; CEL, NOS, chronic eosinophilic leukemia, not otherwise specified; CML, chronic myeloid leukemia, *BCR-ABL1* positive; LV-HES, lymphocytic variant of hypereosinophilic syndrome; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm.

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HES and may be fatal and/or associated with significant comorbidity, potentially requiring amputation of extremities that are affected (see case 63 for further discussion).

The Workshop Review Panel also recognized some overlapping eosinophilic disorders that involve single organs that are accompanied by PB eosinophilia or HE but in which the effect of the increased eosinophils is uncertain. As an example, these types of disorders are distinct clinicopathologic entities such as eosinophilic granulomatosis with polyangiitis (previously known as Churg-Strauss syndrome), eosinophilic esophagitis, eosinophilic myocarditis, eosinophilic pneumonia and other pulmonary eosinophilic conditions, and a variety of eosinophilic dermatoses (Table 3).

Diagnostic Evaluation of Eosinophilia

The diagnostic workup of eosinophilia is a complex task that usually requires an extensive multidisciplinary workup Figure 1. An initial clinical evaluation aims to rule out potential causes of secondary/reactive eosinophilia.

Germline disorders can be associated with eosinophilia, such as hyper-IgE syndrome, Wiskott-Aldrich syndrome, and severe congenital neutropenia, among others.[6] A complete family history and appropriate genetic testing are necessary to establish a diagnosis in these cases.

Once secondary causes of eosinophilia are excluded and germline disorders considered, the workup should proceed to evaluate for a primary (clonal) eosinophilia.

Table 2
Definition of Hypereosinophilia and Hypereosinophilic Syndrome

Hypereosinophilia (HE)
Persistent eosinophilia >1.5 × 10^9/L on two separate examinations (>1 month) and/or
Tissue HE defined by >20% eosinophils in bone marrow, and/or extensive tissue infiltration determined by a pathologist, and/or marked deposition of eosinophil granules and proteins in tissue
Hypereosinophilic syndrome
Criteria for HE fulfilled and organ damage/dysfunction due to HE and must exclude other reasons for organ damage
Eosinophil-associated single-organ disease
Criteria of HE fulfilled and single-organ disease

Table 3
Classification of Hypereosinophilia, Conditions Associated With Hypereosinophilia and Hypereosinophilic Syndrome[5]

Classification Terminology	Comments
Hypereosinophilia (HE)	
Hereditary (familial) HE	Unexplained HE among family members and no evidence of an underlying primary or secondary cause or immunodeficiency state that may be associated with HE
HE of undetermined significance	Unexplained HE in which an exhaustive search reveals no primary, secondary, or familial explanation
Primary HE	HE occurring in the setting of an underlying neoplasm in which the eosinophils are considered (or shown) to be neoplastic (clonal)
Secondary HE	HE wherein the eosinophils resulting from an underlying condition/disease are considered nonclonal
Hypereosinophilic syndrome (HES)	
Idiopathic HES	End-organ damage directly attributable to HE and no discernible underlying cause of the HE (ie, secondary [reactive] and primary [clonal] defined disorders are excluded)
Primary HES	End-organ damage directly attributable to HE and in the setting of an underlying neoplasm in which the eosinophils are considered (or shown) to be neoplastic (clonal)
Secondary HES	End-organ damage directly attributable to HE and the eosinophils resulting from an underlying condition/disease are considered nonclonal; in this setting, the HE is often cytokine driven
Other	
Specific syndromes associated with HE	Distinct clinicopathologic entity(ies) accompanied by HE but the effect of eosinophilia is unclear[a]
Other conditions associated with HE	Organ-restricted conditions accompanied by HE but the effect of eosinophilia is unclear[b]

[a]Examples would include Gleich syndrome, eosinophilia granulomatosis with polyangiitis (aka Churg-Strauss syndrome), and eosinophilia-myalgia syndrome (may be related to L-tryptophan exposure; workshop case 269 is an example).
[b]Examples would include but are not limited to eosinophilic gastrointestinal disorders (eg, eosinophilic esophagitis), eosinophilic pulmonary disorders (eg, eosinophilic pneumonia), and eosinophilic dermatologic conditions (broad spectrum).

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Table 4
Clinical Symptoms Associated With Tissue Eosinophilia

System Involved	Potential Symptoms
Gastrointestinal	Diarrhea, abdominal pain, bloating
Pulmonary	Cough, wheezing, rhinitis, dyspnea, pulmonary embolus
Skin	Rash, erythroderma, pruritus
Heart	Endomyocardial fibrosis, intraventricular thrombosis
Central nervous system	Altered mental status, cognitive deficits, gait ataxia, visual loss
Renal	Hematuria, urinary frequency, dysuria
Vascular	Thromboembolic events, including arterial thromboses

Morphologic evaluation of PB smear and BM biopsy specimen may reveal important clues, such as circulating blasts, dysplastic cells, basophilia, mast cells, monocytosis, BM cellularity, and fibrosis, in addition to eosinophilia. The BM may reveal a B- or T-cell lymphoma, carcinoma, or granulomatous inflammation as a cause of the reactive eosinophilia. Flow cytometry and immunohistochemistry may provide valuable information, respectively (eg, lymphocytic variant of HES or aberrant mast cells).

The laboratory evaluation should begin with a routine chromosome and fluorescence in situ hybridization (FISH) analysis for myeloid disorders that are defined by specific cytogenetic abnormalities. *KIT* D816V mutation analysis is necessary in the evaluation of systemic mastocytosis, especially when aberrant mast cell infiltrates and/or aberrant mast cell immunophenotypes are detected. Exclusion of one of the "classical" myeloid/lymphoid (M/L) neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2* require interphase FISH for *FIP1L1-PDGFRA* gene fusion (M/L neoplasm with eosinophilia) ("FISH for the *CHIC2* deletion") or reverse transcription polymerase chain reaction (RT-PCR) since this rearrangement is not visible by a routine chromosome analysis. Translocations of 5q32 (*PDGFRB*) or 8p11.23 (*FGFR1*) are usually accompanied by an abnormal karyotype on cytogenetic evaluation.[7] Rarely, *PDGFRB* rearrangements are cytogenetically cryptic and can be detected by RT-PCR or RNA sequencing analysis.[8] The *PCM1-JAK2* fusion was recently added to this World Health Organization (WHO) category as a provisional entity. Fusion tyrosine kinases involving *FLT3* (most commonly *ETV6-FLT3* fusion), typically present with a myeloproliferative neoplasm (MPN) and/or a T-cell lymphoblastic leukemia/lymphoma, have not been formally included in this category.[4]

A negative screen for the aforementioned abnormalities should prompt a consideration of the diagnosis of chronic eosinophilic leukemia (CEL), not otherwise specified (NOS), when there is cytogenetic, molecular, and morphologic evidence of a myeloid malignancy that has a predominant eosinophilia but cannot be classified as a specific myeloid neoplasm otherwise. CEL, NOS is distinguished from HES by the presence of a clonal cytogenetic or molecular abnormality or increased blast cells (>2% in the PB or >5% in the BM but <20% blasts in either compartment).

If none of the aforementioned diseases are identified and organ damage is present, a diagnosis of idiopathic HES is made. Finally, a diagnosis of idiopathic HE is rendered if organ compromise is not found. With a wider availability of next-generation sequencing (NGS) panels, the identification of additional mutations will likely result in decreased frequency of idiopathic HE/HES, as more cases will be reclassified as CEL, NOS or other myeloid neoplasms. Ideally, a diagnosis of idiopathic HES should have essentially ruled out a hematopoietic neoplasm as the primary cause based on a thorough pathologic, molecular, and clinical evaluation as discussed above.

Reactive and Secondary Eosinophilia

A variety of conditions or disease states may be accompanied by eosinophilia. Probably the most frequent are allergic disorders and reactions or hypersensitivity to drugs. Infections that cause eosinophilia are classically of helminths, ectoparasites, and fungi but can also be seen with protozoal and viral infections. Many neoplasms induce reactive eosinophilia, including T-cell lymphoma and Hodgkin lymphoma, in which eosinophils are often seen in the associated inflammatory milieu. Immunologic disorders associated with eosinophilia include autoimmune diseases such as sarcoidosis and connective tissue disease, as well as vasculitis. Other inherited immunodeficiencies associated with eosinophilia are described in another section. Interestingly, radiation exposure and hypoadrenalism have also been associated with increased eosinophilia.

Most reactive/secondary eosinophilias submitted to the workshop were associated with different drugs **Table 5**. Case 269 (virtual scanned case) described a woman with pancytopenia and hip pain who then developed HE, CNS abnormalities, and multiorgan system

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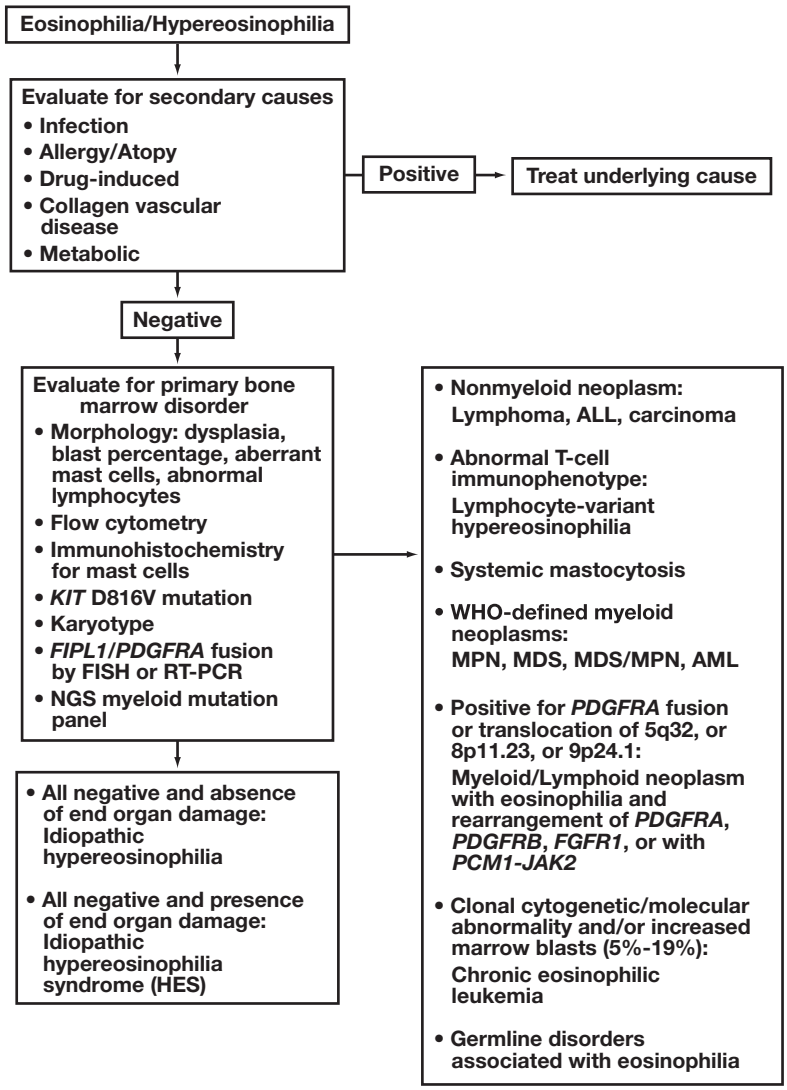

Figure 1 Diagnostic evaluation of eosinophilia. AEC, absolute eosinophil count; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; FISH, fluorescence in situ hybridization; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NGS, next-generation sequencing; RT-PCR, reverse transcription polymerase chain reaction.

failure. She had a history of L-tryptophan ingestion, and this eosinophilia-myalgia syndrome is now known to be induced by ingestion of this supplement, specifically toxins formed as contaminants during its synthesis.[9]

Four cases nicely illustrated the spectrum of drug rash with eosinophilia and systemic symptoms (DRESS) syndrome. While this syndrome was originally described in response to phenytoin, many drugs have since been implicated. Only one case (229) in the workshop was associated with an antiepileptic (carbamazepine). DRESS usually manifests 2 to 6 weeks after drug exposure, which was nicely illustrated by cases 262, 160, and 229. All patients had rash, which is typically morbilliform, in addition to lymphadenopathy and fevers. Case 262 had a lymph node excision, which mimicked lymphoma **Image 1**, a potential pitfall in this diagnosis, while case 229 interestingly showed features similar to Langerhans cell histiocytosis (Image 1). In cases 193 and 262, the patients recovered after the offending drugs were discontinued; however, DRESS has a 10% fatality rate due to complications such as fulminant hepatitis or macrophage activation syndrome. Reactivation of herpesviruses, especially human herpesvirus 6, has been implicated in DRESS pathogenesis.[10]

The remaining drug-related cases further illustrate the diversity of drugs that can lead to eosinophilia. Cases 100, 249, and 199 were all related to therapies for hematolymphoid disorders: fludarabine for chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), IDH2 inhibitor for therapy-related acute myeloid leukemia (AML), and gemcitabine/thioguanine/

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Table 5

Cases of Idiopathic Hypereosinophilic Syndrome and Reactive and Secondary Eosinophilia

Case No.	Panel Diagnosis	Age (y), Sex, Absolute Eosinophil Count ($\times 10^9$/L) (if Known)	Interesting Features of the Case
23	IHES	86, M, 13.4	Intermittent and relapsing pneumonia concurrent with hypereosinophilia—differential diagnosis with a defined lung disorder with associated eosinophilia
41	Hypereosinophilic syndrome, favor idiopathic (exclude drug induced)	46, M, 5.2	Profound eosinophilia with cardiac involvement
62	IHES	50, M, 3.8	Extensive multiorgan involvement, extensive exclusion of reactive causes, importance of tissue biopsy confirmation
63	IHES	19, F, 4.5	Hypercoagulable state with arterial and venous thrombotic events may occur in IHES
85	Hypereosinophilic syndrome of uncertain etiology	16, M, 219	IHES rare in children; extensive multiorgan involvement (brain, skin, hepatosplenomegaly)
95	Hypereosinophilic syndrome, favor reactive	55, F, 7.1	Illustrative of the extensive workup that is needed to exclude reactive etiologies for eosinophilia
139	Eosinophilic pneumonia accompanied by hypereosinophilia, of uncertain etiology	55, M, 27.9	Overlap of primary lung disease with associated eosinophilia and idiopathic hypereosinophilia with secondary lung involvement
169	Eosinophilic myocarditis accompanied by hypereosinophilia, of uncertain etiology, in the setting of CAR-T and salvage therapy	63, F, 10	Eosinophilic myocarditis in the setting of CAR-T therapy
213	Chronic eosinophilic pneumonia accompanied by hypereosinophilia	39, F, 26.3	Classic example of a defined lung disease associated with eosinophilia
239	B-lymphoblastic leukemia arising in the setting of preceding hypereosinophilic syndrome	39, M, 2.0	Rare case of transformation of chronic eosinophilia leukemia to B-lymphoblastic leukemia
285	IHES	24, M, 2.8	Gastric eosinophilia, transient deletion 20q on karyotype
269	Hypereosinophilic syndrome, toxin-induced (presumed HCG weight loss supplement with L-tryptophan)	41, F, 52.8	Hypereosinophilic syndrome with extensive marrow necrosis, multiorgan failure, and brain infarcts and neurologic impairment
262	Hypereosinophilic syndrome, drug-induced (rosuvastatin), manifesting as DRESS with associated lymphadenopathy	54, M, 2.5	DRESS may mimic hematologic malignancy (in this case initial diagnosis of T-cell lymphoma); life-threatening disorder
160	Hypereosinophilic syndrome, drug-induced (minocycline), manifesting as DRESS	17, F, 6.2	DRESS may mimic hematologic malignancy; life-threatening disorder
229	DRESS-associated lymphadenopathy, secondary to carbamazepine	17, F, 1.3	DRESS with extensive Langerhans cell expansion in lymph nodes; may mimic hematologic malignancy; life-threatening disorder
193	Hypereosinophilic syndrome, drug induced, manifesting as DRESS	64, M, 15	DRESS may mimic hematologic malignancy; life-threatening disorder
231	Reactive hypereosinophilia occurring in the setting of post cardiac transplantation, immunosuppressive therapy, and G-CSF	7, M, 5.9	Eosinophilia in the setting of G-CSF administration
100	Reactive hypereosinophilia, drug related (fludarabine)	60, F, 10.4	Drug-associated eosinophilia in setting of treated CLL
249	Therapy-related acute myeloid leukemia associated with eosinophilia attributable to IDH2 inhibitor therapy	68, M, 0.6	Eosinophilia in the setting of IDH2 inhibitor administration
199	Acute myeloid leukemia with myelodysplasia-related changes, persistent, with development of reactive eosinophilia, drug related	65, M, 0.7	Development of eosinophilia after treatment with decitabine/6-thioguanine/allopurinol
170	*Strongyloides stercoralis* hyperinfection syndrome	39, M, 0.9	Clinically unknown chronic strongyloidiasis resulting in hyperinfection syndrome when host immune function is impaired (in this case, patient received steroids for dermatitis)

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Table 5
(cont)

Case No.	Panel Diagnosis	Age (y), Sex, Absolute Eosinophil Count (× 10^9/L) (if Known)	Interesting Features of the Case
54	Hypereosinophilia of undetermined significance (remote history of *Strongyloides* infection)	88, M, 3.0	Benign *JAK2* exon 13 variant detected—do not overdiagnose an MPN
71	Reactive hypereosinophilia, infection related (parvovirus B19)	NA, M, 0.2	Bone marrow eosinophilia (25%) as a reaction to parvovirus B19 infection
165	Eosinophilic granulomatosis with polyangiitis accompanied by hypereosinophilia	30, F, 15.2	Classic example of a defined lung disease associated with eosinophilia
175	Hypereosinophilic syndrome, with single pathogenic mutation by NGS of uncertain significance	57, M, 7.2	Mutation PPM1D (VAF, 15%)
263	Hypereosinophilic syndrome, with low allele frequency mutations by NGS of uncertain significance	25, M, 20.3	ASXL1 (VAF, 4%), ATRX (VAF, 2.4%)

CAR-T, chimeric antigen receptor T-cell therapy; CLL, chronic lymphocytic leukemia; DRESS, drug rash with eosinophilia and systemic symptoms; G-CSF, granulocyte colony stimulating factor; HCG, human chorionic gonadotropin; IHES, idiopathic hypereosinophilic syndrome; MPN, myeloproliferative neoplasm; NGS, next-generation sequencing; VAF, variant allele frequency.

allopurinol for AML with myelodysplasia-related changes, respectively. Case 231 was a young boy who developed eosinophilia while receiving immunosuppressive drugs for a cardiac transplant and later neutropenia despite granulocyte colony-stimulating factor therapy.

There were three cases of eosinophilia associated with infection. Case 170 (virtual scanned case) was a patient with a history of transient rashes and eosinophilia. Treatment with steroids provoked *Strongyloides* hyperinfection syndrome from his chronic *Strongyloides stercoralis* infection **Image 2**. The patient's nausea, headache, and photophobia were consistent with the fever, while the gastrointestinal and CNS symptoms were caused by autoinfection by filariform larvae and comorbid bacterial meningitis. Enteric bacteria carried by the larvae can cause sepsis, pneumonia, or meningitis, potentially life-threatening complications. Interestingly, the eosinophilia associated with chronic *Strongyloides* infection is absent during the autoinfection cycle.[11] Case 54 also had evidence of previous *Strongyloides* infection (anti-*Strongyloides* IgG positive), but his workup was limited, and a direct link was not established. Case 71 was of a virus-associated HE, specifically parvovirus B19. The patient had eosinophilia and severe anemia after immunochemotherapy for CLL/SLL. BM showed characteristic inclusions in erythroid precursors, and quantitative PCR was confirmatory.

The one case of autoimmune disease or vasculitis was a classic presentation of eosinophilic granulomatosis with polyangiitis (165, EGPA) (virtual scanned case). A young woman with a long history of asthma had eosinophilia and significant respiratory symptoms with lung opacities on imaging. Lung biopsy specimen showed eosinophilic infiltration and vasculitis **Image 3**. She also found had peripheral neuropathy and sinus abnormalities, two other features of EGPA. EGPA is often difficult to distinguish from HES, and asthma and vasculitis are helpful for arriving at a diagnosis of EGPA.[12]

These cases illustrate the wide variety of conditions that can induce eosinophilia. A causative relation, the time course, and the clinical presentations may not be straightforward in some cases, which often lead to an exhaustive workup. BM biopsy was performed in all but two of the cases to evaluate for the possibility of a primary eosinophilia or lymphoma. The remaining two cases had other tissues examined to confirm the underlying diagnoses (pseudolymphoma in DRESS in case 262 and *Strongyloides* larvae in gastrointestinal biopsy specimens in case 170); however, the full eosinophilia workup is usually indicated since reactive/secondary eosinophilia is often a diagnosis of exclusion.

Idiopathic Hypereosinophilia/ Hypereosinophilic Syndrome

Thirteen cases submitted to the workshop illustrated the spectrum of disease manifestations and the challenges in the diagnosis of idiopathic HE/HES. Table 5 summarizes these 13 cases, including the case number and final panel diagnosis. As would be expected, each of these cases was extensively and comprehensively worked up to exclude clear-cut reactive causes, including infection, autoimmune conditions, underlying lymphoma, or

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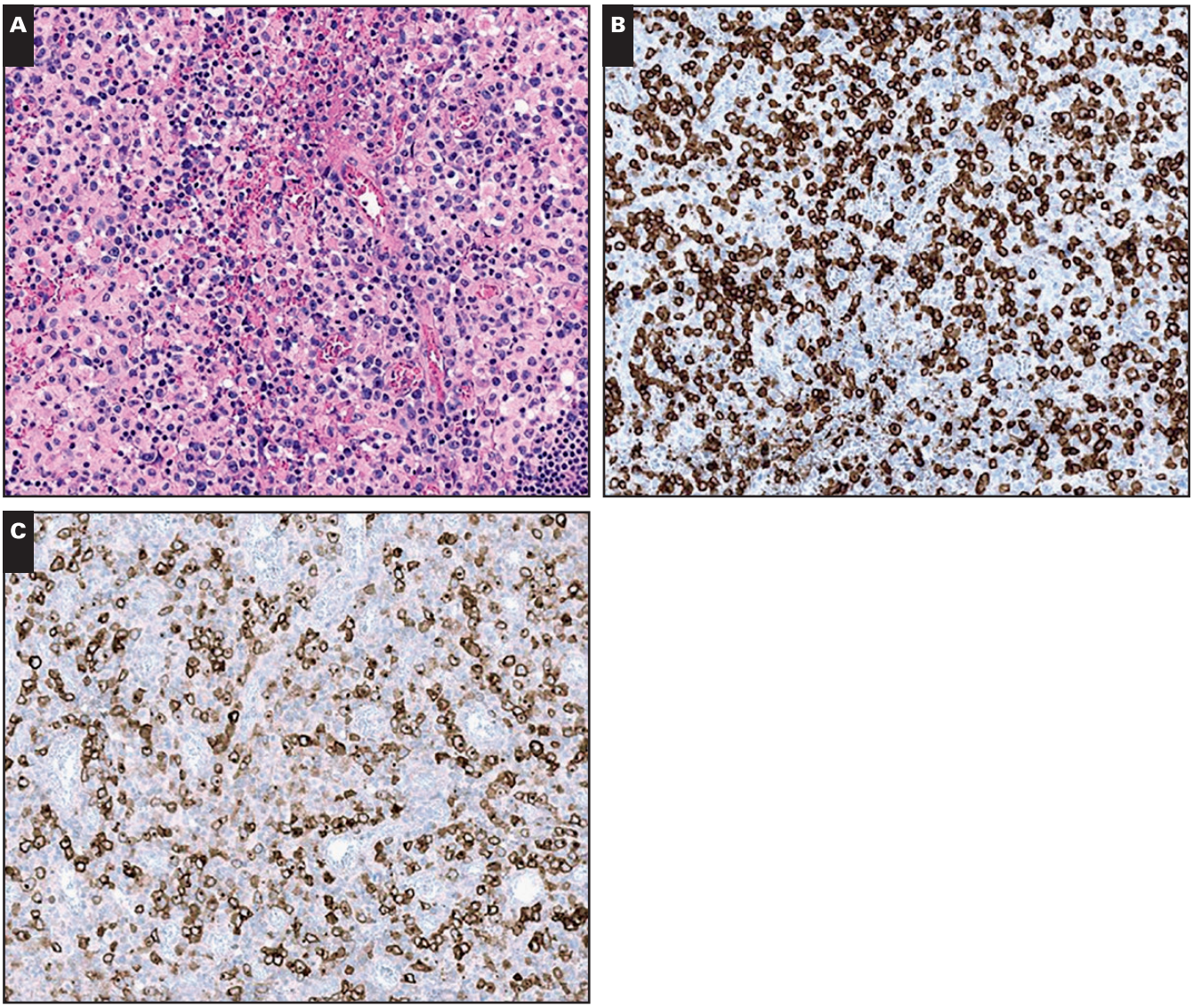

Image 1 Reactive lymphadenopathies in drug reaction with eosinophilia and systemic symptoms. **A-C**, Case 262 showed paracortical expansion of lymphocytes and histiocytes (**A**, ×20), with CD3 showing a predominance of T cells (**B**, ×20) and CD30 highlighting increased immunoblasts (**C**, ×20).

carcinoma and primary (clonal) causes such as WHO-defined entities with eosinophilia. As delineated below, several key themes and concepts emerged from these case presentations.

Case 95 demonstrates the significant and extensive workup and laboratory testing that are often needed to sort out reactive HES vs idiopathic hypereosinophilic syndrome (IHES) vs an underlying primary neoplastic process. In this case, a 55-year-old woman had a complicated clinical history that included elevated IgE, chronic urticaria, asthma, dermatitis, environmental allergies, and chronic diarrhea. All of her laboratory and genetic test results were negative. The BM was normocellular with intact and morphologically unremarkable trilineage hematopoiesis with increased normal-appearing eosinophils (30% of BM nucleated cells). Given her apparent multiorgan involvement by this eosinophilic process, unremarkable BM findings aside from increased eosinophils, and extensive negative laboratory studies, criteria for HES were met, and the HE was favored to be reactive to the underlying atopic and allergic conditions.

HES may involve virtually any organ system, and workshop cases 23, 41, 62, 63, 85, 169, and 285 certainly illustrated this exact point. The spectrum of organ involvement that may occur in cases of IHES included chronic cyclic episodic pneumonia (case 23) (virtual scanned case), eosinophilic myocarditis (case 41 and case 169) **Image 4**, arterial thromboses involving extremities (case 63), gastrointestinal involvement (case 62 and case 285) **Image 5**, and CNS involvement and hepatosplenomegaly (case

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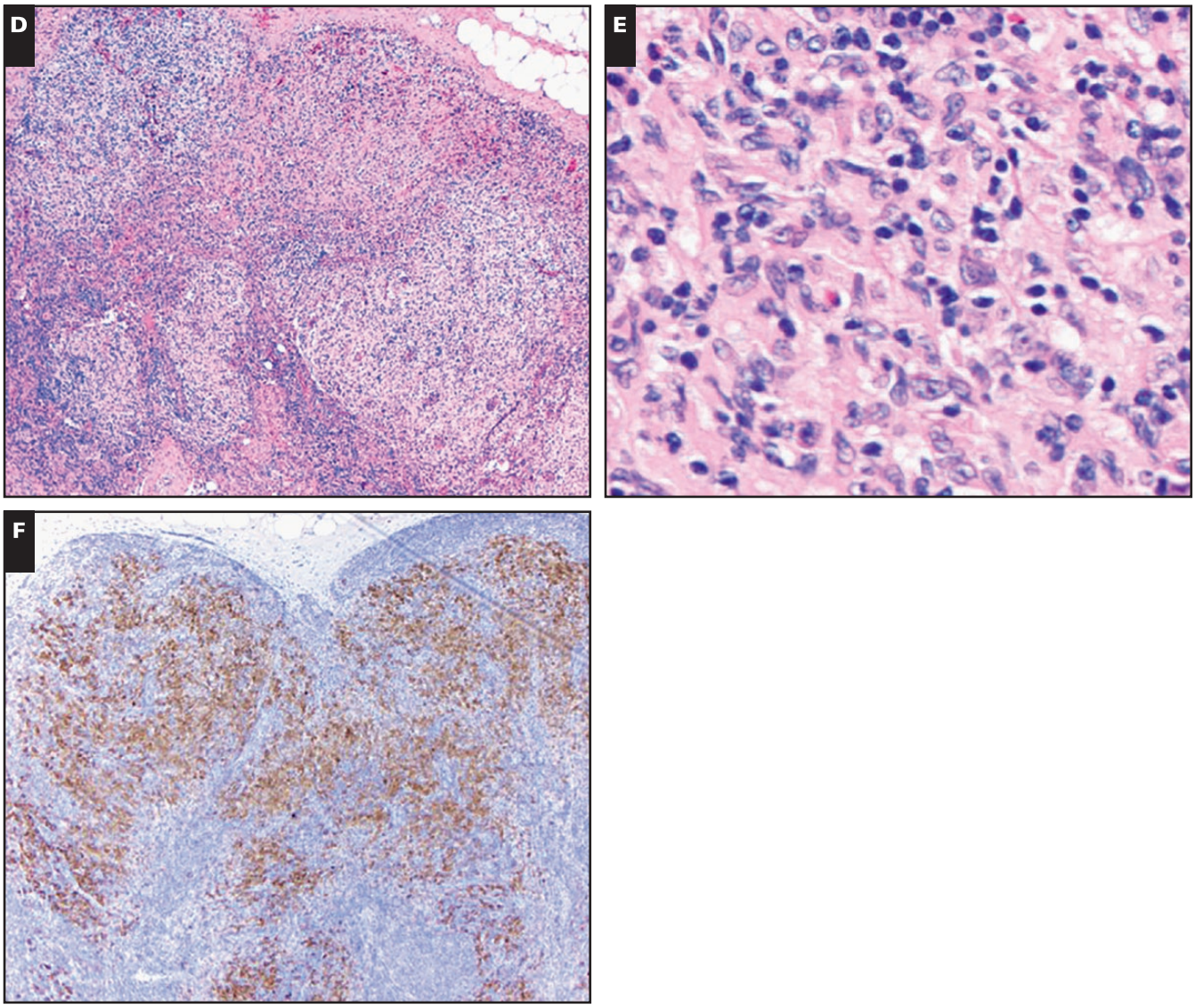

Image 1 (cont) **D**, **E**, Case 229 also showed expansion/effacement of the paracortex (**D**, ×10) by cells with grooved nuclei consistent with Langerhans cells (**E**, ×40) confirmed by CD1a immunohistochemistry (**F**, ×10).

85 in a pediatric patient). Interestingly, in most of these cases, despite the multiorgan system involvement, the BM specimens were overall generally normocellular to slightly hypercellular with progressive trilineage hematopoiesis. The only "abnormal" finding was a variable increase in eosinophils and precursors, as would be expected. Features of dysplasia, monocytosis, increased blasts, mast cell aggregates, and fibrosis were absent.

Cases 139 and 213 highlight the challenges in distinguishing between IHES with pulmonary involvement vs a single-organ (pulmonary) eosinophilic condition accompanied by HE. Case 213 presents a characteristic example of chronic eosinophilic pneumonia. The patient was a 39-year-old woman with asthma and eosinophilia who had bilateral ground-glass opacities on lung imaging Image 6. She had a normocellular BM with prominent eosinophilic infiltrate without significant atypia Image 7. Case 139 shared some features with case 213 in that the 55-year-old male patient had eosinophilia and bilateral ground-glass opacities on pulmonary imaging. Lung biopsy specimen demonstrated eosinophilic pneumonia. In contrast to the former case, however, these symptoms had come on relatively quickly and did not fit with a defined pulmonary disease entity, and an environmental exposure could not be excluded. Importantly, the patient responded well to steroid treatment with resolution of his symptoms. In this case, the panel was reluctant to diagnose IHES outright but rather favored this to be single-organ involvement (pulmonary) in the setting of a probable environmental exposure accompanied by HE. In many of

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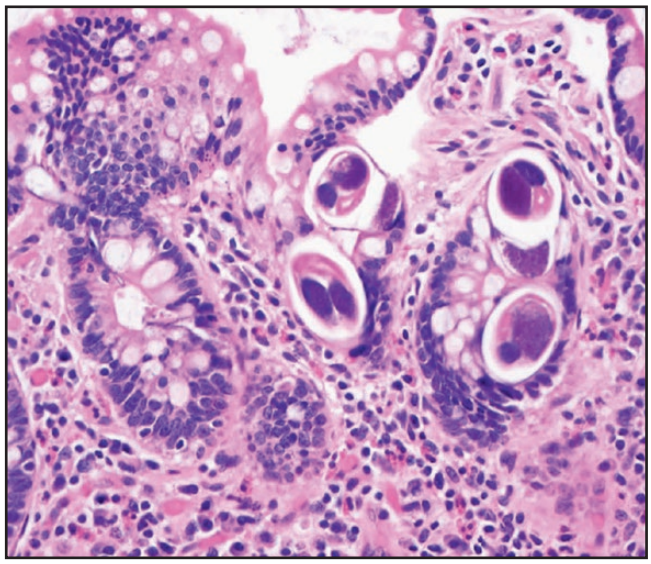

Image 2 Case 170 was of patient with a history of transient rashes and eosinophilia. Treatment with steroids provoked *Strongyloides* hyperinfection syndrome from his chronic *Strongyloides stercoralis* infection.

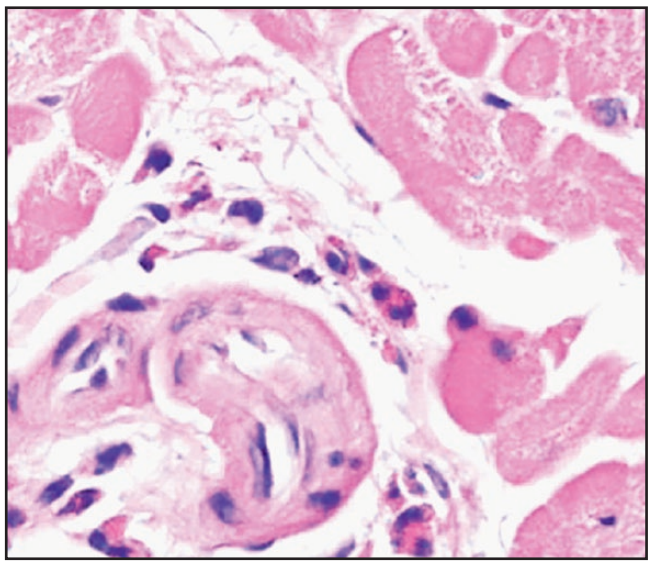

Image 4 Case 41: Idiopathic hypereosinophilic syndrome with involvement of the heart (eosinophilic myocarditis). Scattered eosinophils can be seen percolating through the myocardium (H&E, ×1,000).

these cases, consultation with the treating clinician or radiologist and pertinent laboratory studies are often necessary to discern if a distinct clinicopathologic entity is in fact present (eg, chronic eosinophilic pneumonia) or if the case is better classified as IHES. Importantly, many of these individuals respond to steroid therapy.

From a morphologic perspective, the findings in the workshop IHES cases are quite similar. PB specimens

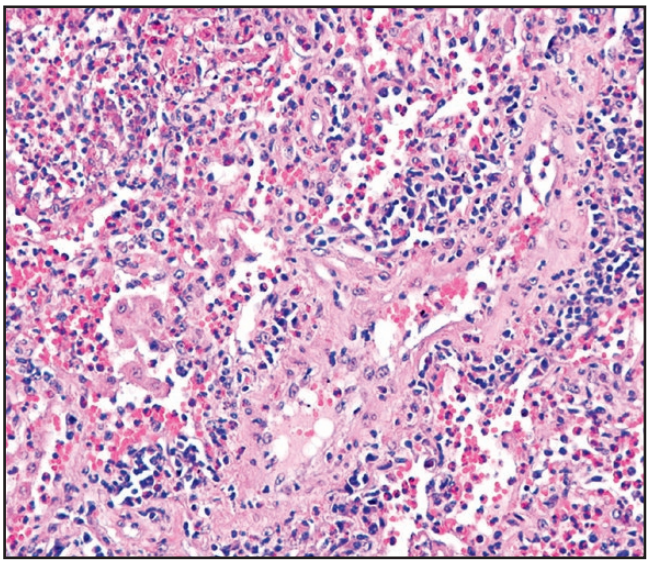

Image 3 The one case of autoimmune disease or vasculitis was a classic presentation of eosinophilic granulomatosis with polyangiitis (165). A young woman with a long history of asthma had eosinophilia and significant respiratory symptoms with lung opacities on imaging. Lung biopsy specimen showed eosinophilic infiltration and vasculitis.

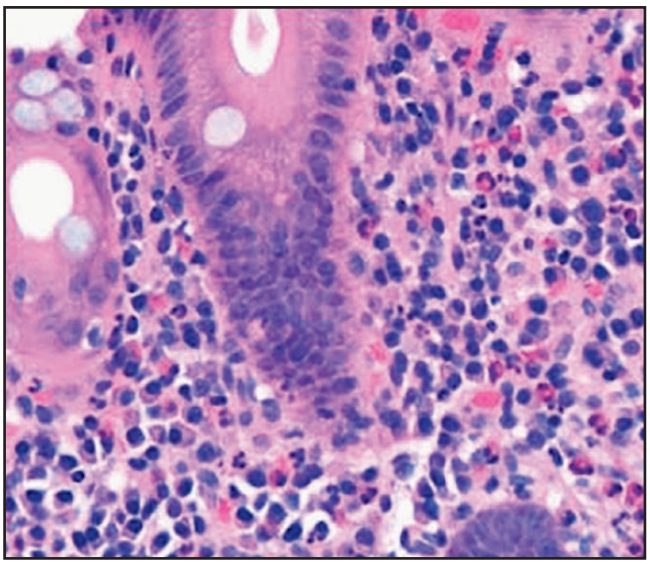

Image 5 Case 62: Idiopathic hypereosinophilic syndrome with involvement of the gastrointestinal tract. Eosinophils are increased in this colon biopsy specimen (50/high-power field) with features of acute colitis and crypt abscesses (H&E, ×600).

show, by definition, HE, and in most of the workshop cases, the eosinophils were reportedly morphologically unremarkable. Given these findings and studies in the literature, it is likely reasonable to infer that the presence of

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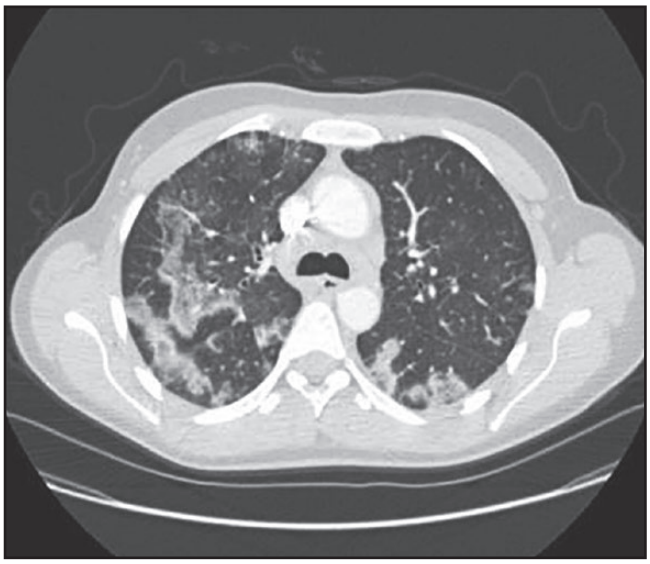

Image 6 Case 213: Chronic eosinophilic pneumonia (CEP). This computed tomography image demonstrates the classic bilateral ground-glass opacities that are seen in CEP.

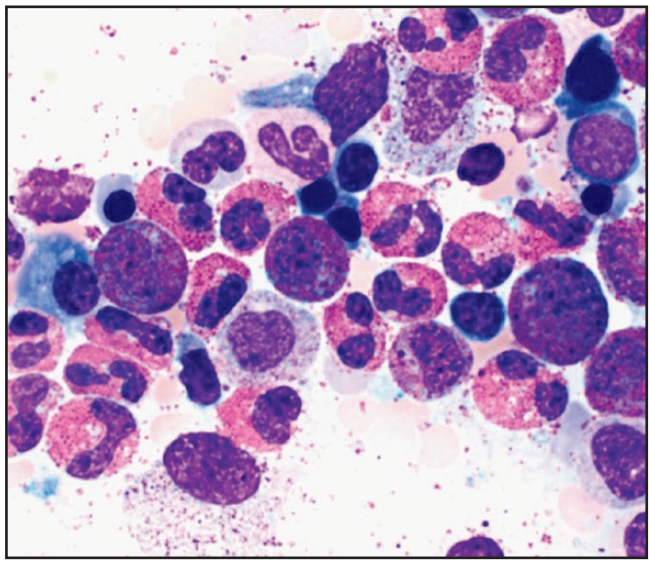

Image 7 Case 213: Chronic eosinophilic pneumonia. The bone marrow aspirate smear is cellular with increased eosinophils and precursors (eosinophilic myelocytes). Occasional eosinophilic myelocytes show darker, basophilic granules (Wright-Giemsa, ×600). The bone marrow biopsy was normocellular without features of dysplasia, fibrosis, mast cell aggregates, or lymphoma.

significant eosinophil cytologic atypia (hyposegmentation, hypogranulation, abnormal consolidation of cytoplasmic granules, vacuolization) would be unusual in IHES and should prompt careful evaluation of other BM characteristics for additional features of a myeloid/stem cell/mast cell malignancy. The BM biopsy specimens in IHES were normocellular (predominantly) to slightly hypercellular BM with intact trilineage hematopoiesis and various percentages of increased eosinophils. In some instances, the eosinophils were left-shifted in the BM and showed the presence of basophil-type granules. Several morphologic "pertinent negatives" are worth mentioning as they tend to argue against the presence of a myeloid malignancy. These negative findings include the lack of the following: significant hypercellularity, significant dysplastic features (particularly in megakaryocytes, which may be an initial subtle clue), an increase in abnormal-appearing or immunophenotypically aberrant blasts, atypical monocytes/monocytosis, dysplastic eosinophils, basophilia, increased or atypical mast cells (eg, hypogranular, spindled forms), and significant myelofibrosis.

Distinction of single-organ disease accompanied by HE from IHES with extramedullary single-organ involvement can be quite challenging and in almost all cases requires a multimodality approach, including clinical impression, pertinent laboratory findings (eg, features of rheumatologic disease), and radiology. As pathologists, we confirm the presence of eosinophilia and exclude alternative explanations for the eosinophilia. If the constellation of features is not clear-cut in their support for a well-defined distinct single-organ clinicopathologic entity (eg, chronic eosinophilic pneumonia [case 213] or eosinophilic granulomatosis with polyangiitis [case 165]; see next section for discussion), then it is preferred to use the terminology of IHES. However, as discussed earlier for case 139, the distinction between the spectrum of single-organ involvement cases can be tricky, and a definitive diagnosis cannot always be rendered. In case 139, the panel agreed that this case did not strictly meet criteria for a well-defined pulmonary disease yet was reluctant to outright diagnose IHES given that there may have been an environmental exposure. Indeed, at presentation in some cases, definitive classification may not be possible, and tincture of time is needed to realize the true nature of the disease.

CEL, NOS

CEL, NOS, is a rare clinically aggressive MPN, characterized by autonomous, clonal proliferation of eosinophil precursors, resulting in persistent PB eosinophilia ($\geq 1.5 \times 10^9$/L), increased BM eosinophils, and often eosinophilic infiltration of peripheral tissues.[13] CEL, NOS is a diagnosis of exclusion, with the WHO-defined diagnostic criteria requiring consideration of other types of MPNs; MDS/MPNs; myeloid and lymphoid neoplasms

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Table 6

Clinical, Morphologic, Cytogenetic, and Molecular Features of Chronic Eosinophilic Leukemia, Not Otherwise Specified Cases

Case No.	Clinical Data	Laboratory Results	Morphologic Findings	Cytogenetic and Molecular Findings	Comment
11	43 y/M Persistent cough Normal chest x-ray Splenomegaly	WBC 17 × 10^9/L AEC 8 × 10^9/L Hb 9.5 g/dL PLT 109 × 10^9/L	BM 100% cellularity, few (small) megakaryocytes Histiocytic infiltrates Charcot-Leyden crystals No blast increase	46,XY,t(5;12)(q31;q13) FISH: *ETV6* probe break in 50% of cells	Translocation involving 5q31-33 (*PDGFRB* gene) does not always result in *ETV6-PDGFRB* fusion
38	51 y/M Short of breath	WBC 191 × 10^9/L AEC 186 × 10^9/L Hb 5.9 g/dL PLT 88 × 10^9/L	BM 100% cellularity Charcot-Leyden crystals Dysgranulopoiesis	46,XY,t(5;12)(q33;p13) No fusion of *PDGFRB* and *ETV* (*TEL*), possible *ETV6* rearrangement *PDGFRB* break-apart probe within normal limits	t(5;12) not involving *PDGFRB*; died 10 months after initial diagnosis No response to tyrosine kinase inhibitor treatment
42	89 y/F Chronic heart failure Pneumonia	WBC 16 × 10^9/L AEC 6 × 10^9/L ABS MONO 1.76 × 10^9/L Hb 9.5 g/dL MCV 79 fL PLT 26 × 10^9/L	BM 90% cellularity Increased number of MDS-like megakaryocytes clustered No blast increase MF grade 2 Dysgranulopoiesis No phenotypic aberrations	47≈49,XX, complex including monosomy 5 FISH: no *PDGFRA*, *PDGFRB*, *FGFR1*, *CBFB* *BCR-ABL1* negative No *JAK2* V617F mutation NGS testing: not reported	CMML (with eosinophilia) considered in differential diagnosis No clinical follow-up
89	61 y/M Abdominal pain, weight loss	WBC 28 × 10^9/L AEC 20 × 10^9/L Hb 11.5 g/dL PLT 346 × 10^9/L	BM hypercellular, increased number of atypical megakaryocytes, clustered	46,XY[20] Follow-up: 46,XY,del(3) (q21)[19] NGS: mutations in *ASXL1* (46%), *SRSF2* (47%) FISH: normal (*JAK2*, *BCR-ABL1*, *CBFB*, *MYH11*, *CHIC2*, *FIP1L*, *PDGFRA*), no *KIT* D816V mutation No clonal TCR rearrangement	Clonal evolution in a patient who had abnormal BM morphology already at initial presentation
173	79 y/F Unexplained eosinophilia Anemia	WBC 13.6 × 10^9/L AEC 6.3 × 10^9/L Hb 11.1 g/dL PLT 222 × 10^9/L	BM 60% cellularity, no blast increase 15% RSs Normal findings by flow cytometry	46,XX Normal FISH (*FIP1/CHI2/PDGFRA*, *PDGFRB*, *FGFR1*, *CBFB*) NGS: gene mutations in *ASXL1*, *SRFS2*, *TET2*, *TP53* (VAF 39%-44%)	Follow-up: biopsy-proven eosinophilic cryptitis
180	66 y/M Constitutional symptoms Splenomegaly Pulmonary infiltrates	WBC 7.6 × 10^9/L AEC 3.8 × 10^9/L Hb 10.8 g/dL MCV 109 fL PLT 70 × 10^9/L	BM cellularity 60% Dysmegakaryopoiesis, increased megakaryocytes Loose histiocytic interstitial infiltrates	46,XY,+1, der(1;7)(q10;p10);*NACC2-NOTCH1* fusion, *STAT5B* N642H (subclonal: *U2AF1*, *SETBP1*) No *PDGFRA*, *PDGFRB*, *FGR1*, *PCM1/JAK2*, *BCR-ABL1* No MPN-related mutations	Demonstrated mutations include *STAT5B*
183	75 y/F Skin rash PB eosinophilia for 5 years	WBC 20 × 10^9/L AEC 6.6 × 10^9/L Hb 11.9 g/dL MCV 108 fL PLT 464 × 10^9/L	BM hypercellular, dysmegakaryopoiesis, dysgranulopoiesis, and dyserythropoiesis with >15% RSs No blast increase	46,XX,del(20)(q11.2)[6]/46,XX[14], FISH studies (*PDGFRA/B*, *FGR*, *CBFB*) negative PCR (*FIP1L1-PDGFRa*, *BCR-ABL1*, *JAK2*, *KIT* D816V) negative NGS: *STAT5B*, *SFRB1*, *TP53* (5% VAF) mutations, PCR: TCR gene rearrangement	Demonstrated mutations include *STAT5B*
227	76 y/F Pneumonia	WBC 58 × 10^9/L AEC 43 × 10^9/L Hb 8.9 g/dL PLT 149 × 10^9/L	BM 70%-90% cellularity, increased number of dysplastic megakaryocytes, blasts <5%	70-72, X, complex karyotype PCR: no clonal lymphoid population FISH: negative for *PDGFRA*, *PDGRB*, *FGFR1*, *CBFB*, *ETV6*, *BCR-ABL1*; *JAK2* negative	Rapid clinical deterioration; died within days
282	57 y/M Ischemic stroke	WBC 658 × 10^9/L AEC 460 × 10^9/L Hb 8.2 g/dL PLT 14 × 10^9/L	BM 100% cellularity, reduced number of megakaryocytes Small, hypolobated megakaryocytes present	46,XY FISH: partial *CHIC2* deletion No *FIP1L1-PDGFRA* fusion	Partial *CHIC2* deletion Patient lost to follow-up

ABS MONO, absolute monocyte count; AEC, absolute eosinophil count; BM, bone marrow; CMML, chronic myelomonocytic leukemia; FISH, fluorescence in situ hybridization; Hb, hemoglobin; MCV, mean corpuscular volume; MDS, myelodysplastic syndrome; MF, myelofibrosis; NGS, next-generation sequencing; PB, peripheral blood; PCR, polymerase chain reaction; PLT, platelet count; RS, ring sideroblast; TCR, T-cell receptor; VAF, variant allele frequency.

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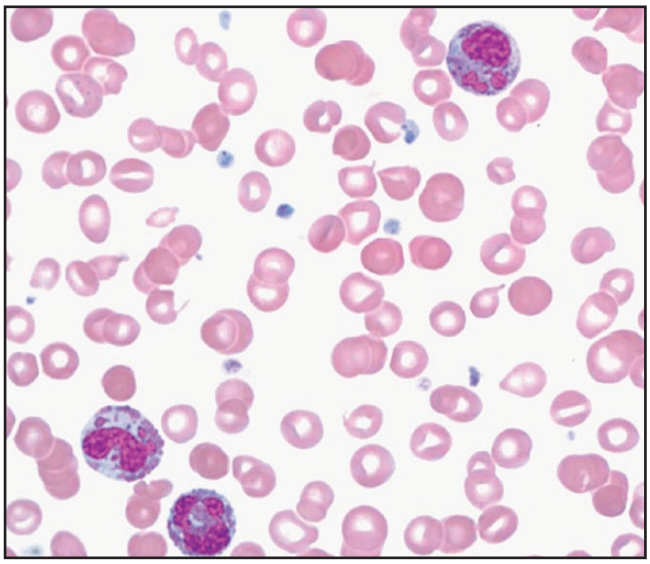

Image 8 Eosinophil abnormalities, including sparse granulation with clear areas of cytoplasm and abnormal nuclear segmentation, were seen in five of nine chronic eosinophilic leukemia workshop cases (case 89).

with eosinophilia associated with recognized recurrent genetic abnormalities, such as *PDGFRA*, *PDGFRB*, *FGFR1* rearrangement or fusions of *PCM1-JAK2*, *ETV6-JAK2*, or *BCR-JAK2*; and acute leukemia, specifically core-binding factor acute myeloid leukemia. In addition, CEL, NOS must be distinguished from HES, with the WHO criteria focusing on increased blasts in the PB (≥2%; <20%) or BM (≥5%; <20%) or presence of a clonal cytogenetic or molecular abnormality. However, no single or specific cytogenetic or molecular genetic abnormality has been identified in CEL, NOS, and it usually lacks the mutations associated with other well-defined MPNs, such as *JAK2* V617F, *CALR*, and *MPL* mutations.[14]

The cases discussed in session 3 of the workshop illustrate the spectrum of findings in CEL, NOS and its diagnostic challenges: nine patients with CEL, NOS summarized in **Table 6**, two other WHO-defined myeloid neoplasms with associated eosinophilia (cases 133, 243), and two patients with HES (cases 175, 263). CEL, NOS patients (five male, four female) ranged in age from 43 to 89 years (mean, 66 years). Eight of nine patients had leukocytosis, and all had persistent eosinophilia in association with mild to moderate, normo- or macrocytic anemia. In addition, four of the nine patients (11, 38, 42, and 180) had a low platelet count, and one case (183) had a mild thrombocytosis. Eosinophil abnormalities, including sparse granulation and nuclear hyper- or hyposegmentation (as illustrated in **Image 8**, case 89), were reported in five of nine CEL cases (38, 89, 183, 180, and 282) and in one case of HES (263). Dysgranulopoiesis, dyserythropoiesis, and dysmegakaryopoiesis were described in most cases (38, 42, 173, 180, 183, 227, and 282) with 15% or more ring sideroblasts seen in three cases (173, 183, and 243), while none had an increase of blasts cells by either cytomorphology (PB or BM smears) or histomorphology using CD34 immunohistochemistry. Significant marrow fibrosis (grade ≥2) was reported in only one case (42).

In some cases, the clinical and morphologic findings in CEL, NOS may overlap those of other MPNs and MDS/MPNs, such as chronic myelomonocytic leukemia (CMML). The possibility of CMML was raised for case 42 because of the absolute monocyte count of 1×10^9/L or more, accounting for 11% of the leukocytes. However, it is uncertain for this case if the monocytosis was sustained or related to coexisting pneumonia, highlighting the importance of supporting clinical information and whether NGS testing for the mutational profile typical for CMML was performed.

The distinction between CEL, NOS and MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN–RS-T) was illustrated by case 183, which describes a 75-year-old-woman with diffuse skin rash, leukocytosis, and absolute eosinophilia. In addition, the patient had macrocytic anemia and mild thrombocytosis. By cytomorphology, there were dysplastic features in all three cell lineages, ring sideroblasts (>15% of erythroid precursors), and no increase in blasts. The BM biopsy specimen showed morphologic features of MDS/MPN with hypercellularity and increased number of atypical megakaryocytes of varying size with clustering, including small hypolobated/monolobated, MDS-like megakaryocytes (**Image 9**, case 183).

Cytogenetic analysis showed a del(20)(q11.2) and NGS testing revealed *STAT5B* (see below for further discussion of *STAT5B* mutation), *SF3B1*, and *TP53* mutations at low frequency (variant allele frequency [VAF], 5%), while MPN-related mutations were not detected. On the basis of laboratory, molecular, and morphologic findings, MDS/MPN–RS-T was raised as a possible differential diagnosis with CEL, NOS. However, the lack of *JAK2* V617F mutation and presence of significant PB and BM eosinophilia were considered to favor CEL, NOS.

Two workshop cases with an abnormal karyotype with t(5;12) (cases 11 and 38) illustrate that translocations involving 5q31-33 (*PDGFRB* gene region) do not always result in *ETV6-PDGFRB* fusion; therefore, further FISH and/or molecular studies are needed to rule out *PDGFRB* rearrangements. By morphology, the BM in both cases showed 100% cellularity with a high myeloid/erythroid (M:E) ratio and significant eosinophilia with the presence of Charcot-Leyden crystals and no increase in blasts. The FISH findings were considered to indicate clonality, without evidence of *PDGFRB* rearrangement.

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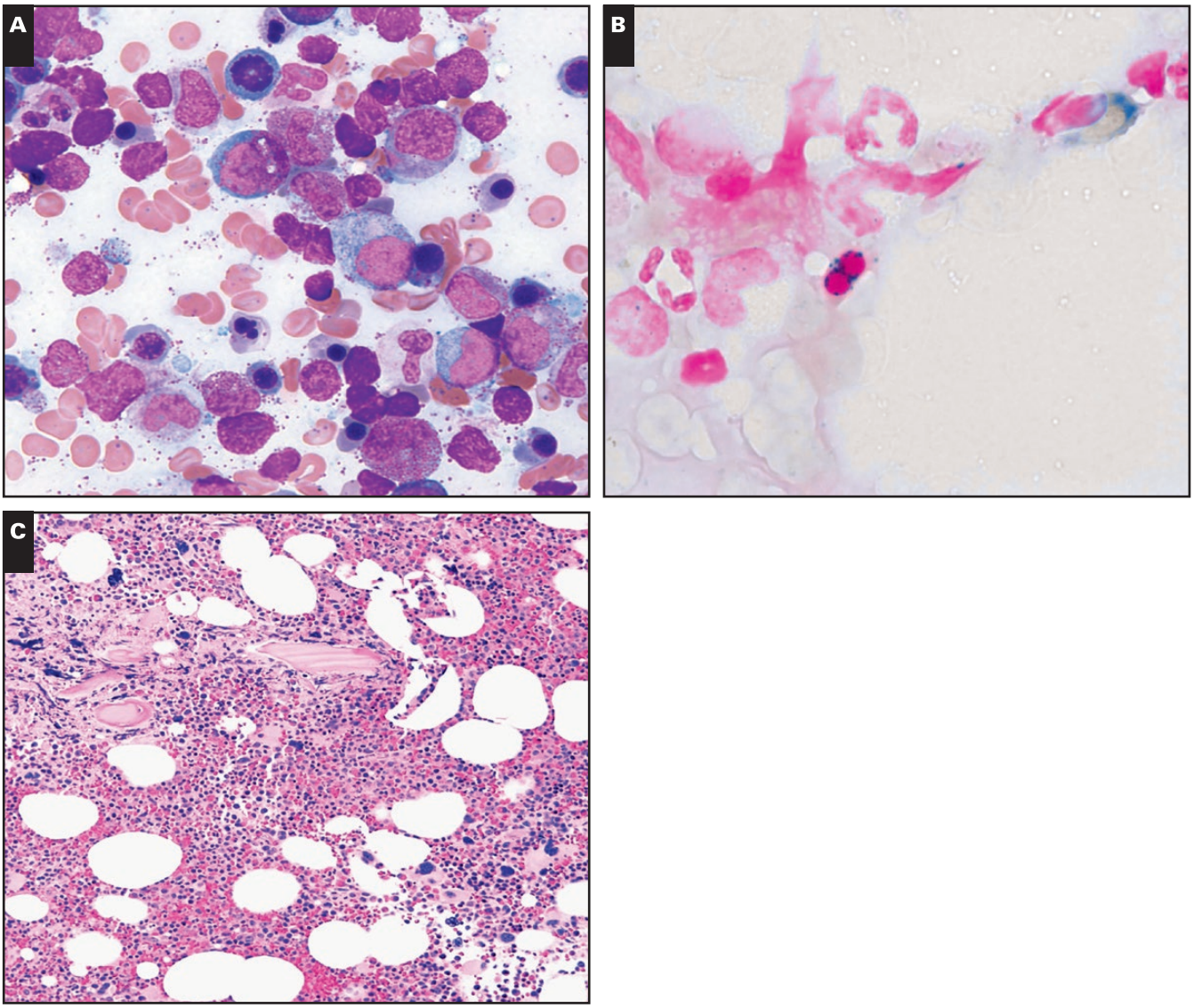

Image 9 **A-C**, Case 183 describes a 75-year-old-woman with diffuse skin rash, leukocytosis with absolute eosinophilia, macrocytic anemia, and mild thrombocytosis. The bone marrow (BM) aspirate (**A**) revealed dysplastic features in all three cell lineages with more than 15% ring sideroblasts (**B**). BM histology (**C**) showed features of myelodysplastic syndrome/myeloproliferative neoplasm with increased number of megakaryocytes with clustering.

Therefore, these cases were considered compatible with CEL, NOS.

Three of the cases of CEL, NOS had a normal karyotype at initial presentation, but molecular abnormalities were found by either FISH or NGS (cases 89, 173, and 282). Case 173 describes a 79-year old woman with anemia, leukocytosis, and eosinophilia. BM examination showed a hypercellular marrow with significant eosinophilia and abnormal megakaryopoiesis, with a mixture of normal-size and atypical, small monolobated MDS-like megakaryocytes (Image 10, case 173). There was no evidence of increased blasts by either cytomorphology or flow cytometry, but 15% ring sideroblasts were present. Cytogenetic studies were normal, while NGS testing revealed pathogenetic mutations in *ASXL1*, *SRF2*, *TET2*, and *TP53* at VAF between 39% and 44%, which can be seen in both MDS and CEL. At follow-up, the patient was also found to have a symptomatic, biopsy-proven eosinophilic colitis, which in the context of the overall clinical picture was suggestive of tissue damage secondary to clonal HE. Another case (282) with normal karyotype at diagnosis demonstrated features of an MPN with CML-like morphologic findings in the PB and BM associated with significant eosinophilia. The only molecular abnormality found was a partial *CHIC2* deletion by FISH, while *BCR-ABL1* and *FIP1L1-PDGFRA* studies were repeatedly negative. The patient appeared to respond to tyrosine kinase inhibitors but was lost for further follow-up. While BM

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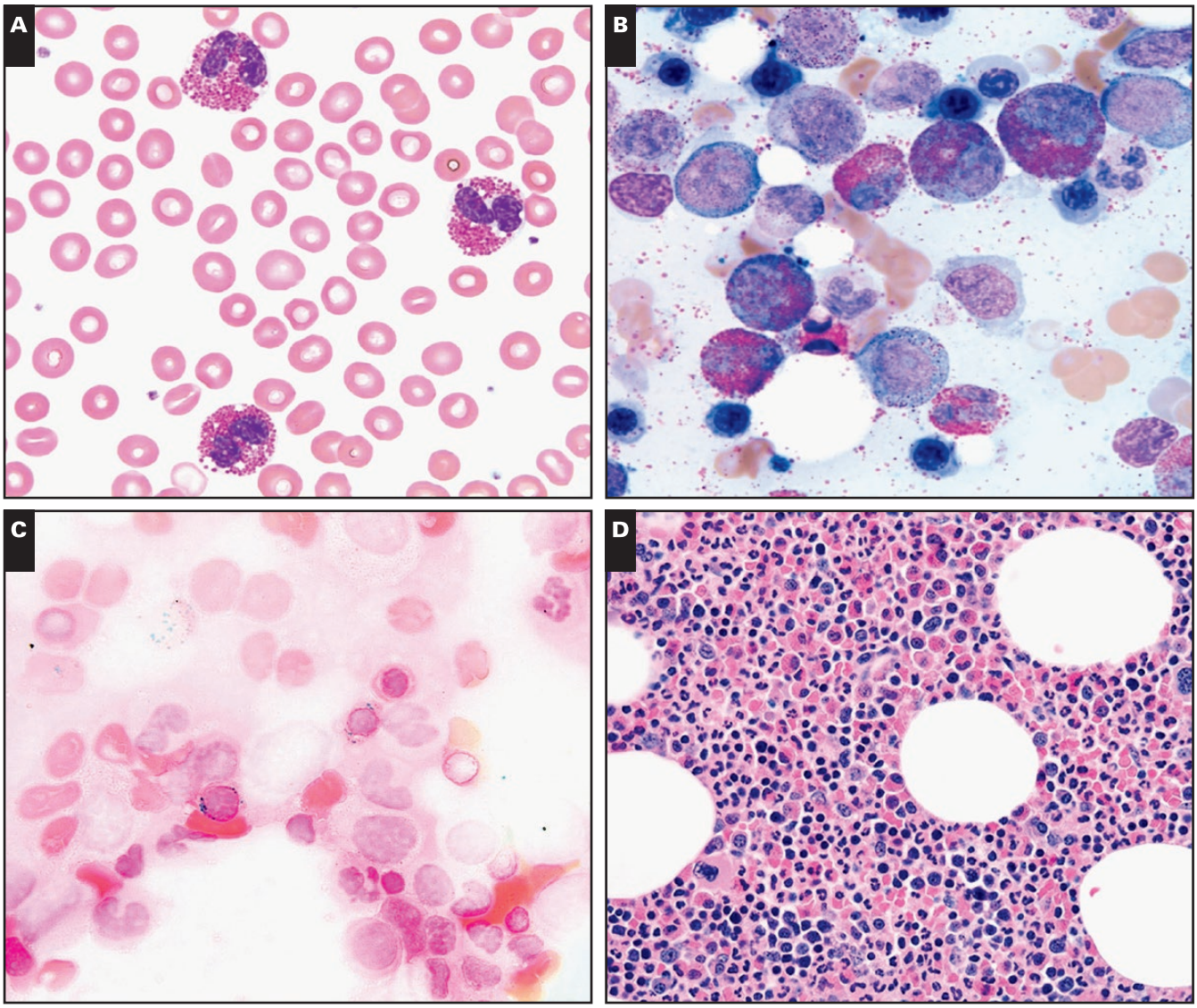

Image 10 **A-D**, Case 173 describes a 79-year-old woman with anemia, leukocytosis, and peripheral eosinophilia (**A**). The bone marrow (BM) aspirate (**B**) showed increased numbers of eosinophils and eosinophil precursors and 15% ring sideroblasts (**C**) and the BM trephine (**D**) increased cellularity with high myeloid/erythroid ratio and presence of atypical megakaryocytes.

morphologic findings were suggestive of an MPN already at initial presentation in case 89, a clonal abnormality became evident first by repeated testing, including karyotypic abnormalities and the detection of two pathogenic mutations in *ASXL1* and *SRSF2*. Thus, morphologic findings may precede cytogenetic and molecular abnormalities in CEL, NOS and warrant clinical follow-up.

An interesting, previously reported observation is the association of *STAT5B* mutation by NGS and eosinophilia in myeloid neoplasms.[15] *STAT5B* mutations are acquired oncogenic mutations that have been described in various lymphoid neoplasms[16-19] but are rare in myeloid neoplasms.[15] *STAT5B* mutations were reported in four workshop cases: two cases of CEL, NOS (180, 183); one case of CMML with eosinophilia (133); and one case of MDS with eosinophilia (243), respectively. Although the presence of a *STAT5B* N642H mutation warrants further investigation as a possible marker of chronic eosinophilic neoplasms, similar mutations have also been described in nonclonal HE and atopic dermatitis–like skin lesions[20] and are not considered sufficient on their own to establish a diagnosis of CEL, NOS.

CEL, NOS vs Reactive Hypereosinophilia vs IHES

The differential diagnosis between CEL, NOS and IHES or reactive HE can be problematic. The high frequency of abnormal BM morphology in the workshop cases of CEL, NOS with dysmegakaryopoiesis present

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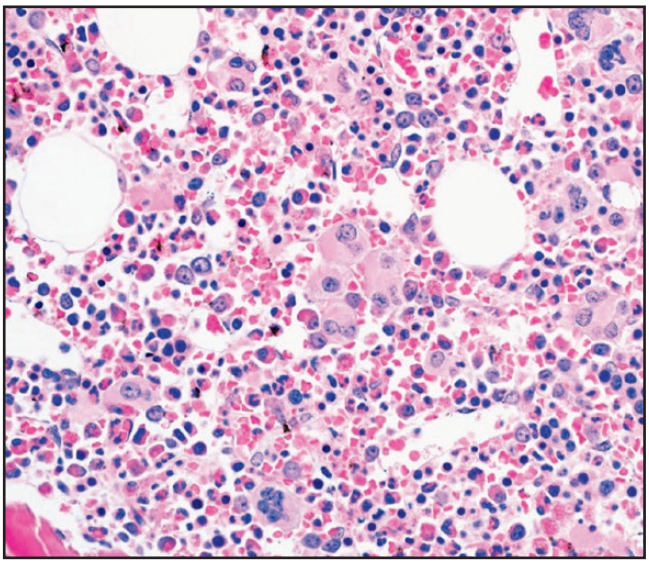

Image 11 Case 89 describes a 61-year-old male patient with leukocytosis and elevated absolute eosinophil count. The bone marrow histology demonstrated many of the features of chronic eosinophilic leukemia, not otherwise specified (CEL, NOS) at presentation, including hypercellularity with significant eosinophilia and abnormal megakaryocytes, but did not meet World Health Organization diagnostic criteria due to the lack of evidence of clonality or blast increase. However, the subsequent acquisition of a clonal cytogenetic abnormality confirmed the diagnosis of CEL, NOS.

in all nine cases supports the use of histomorphology in the distinction of CEL, NOS and reactive causes of eosinophilia. Wang et al[21] have reported frequent dysmegakaryopoiesis and other dysplastic features in CEL, NOS and suggested that, similar to other myeloid neoplasms, BM morphology should be one of the major criteria to distinguish CEL, NOS from IHES. The importance of dysmegakaryopoiesis is well illustrated by case 89, which demonstrated many of the features of CEL, NOS at presentation, including increased eosinophils, BM hypercellularity, and abnormal megakaryocytes (**Image 11**, case 89), but did not meet WHO diagnostic criteria due to the lack of evidence of clonality. However, the subsequent acquisition of a clonal cytogenetic abnormality confirmed the diagnosis of CEL, NOS.

Another key concept that has emerged from both the literature and cases submitted to the workshop regarding the diagnostic challenges in distinguishing IHES from CEL is using sequencing techniques (eg, NGS to assess for clonality, beyond the standard cytogenetic and FISH evaluation).[14,21-24] Two cases with a panel diagnosis of HES and mutations detected by NGS were included in the session with cases of CEL, NOS for the purposes of discussion (cases 175 and 263) (Table 5). Both presented with elevated WBC and significant eosinophilia in PB and BM, constitutional symptoms, and evidence of organ damage, which would meet criteria for HES if clonality could be excluded. Cytogenetic analysis was normal in both cases, and although molecular abnormalities previously described in myeloid neoplasms were detected, they affected only a single gene or were at a low VAF (case 175, PPMD1D mutation with VAF 15%; case 263, ASXL1 and ATRX mutations with VAF <5%). Importantly, the BM showed no clear morphologic features suggestive of a myeloid neoplasm, although cellularity was increased in one of the cases. In addition, case 263 had a long history (>3 year) of eosinophilia without treatment, whereas CEL, NOS typically has an aggressive clinical course. Therefore, after considering the clinical information, morphologic findings, genes involved, number of mutations, and allele frequency, the panel felt the information was insufficient to establish a diagnosis of CEL, NOS.[21]

While the number of publications on NGS in IHES is limited, the study by Wang et al[14,24] reported that patients with IHES who had clonality detected by NGS showed clinical features more akin to CEL, NOS, in contrast to patients with IHES who lacked evidence of molecular clonality. It is, however, also important to consider whether mutations might represent clonal hematopoiesis of indeterminate significance (CHIP). As in consideration of myelodysplastic syndromes,[22] there is no easy way to distinguish whether a mutation is CHIP or pathologic. However, a single mutation, especially if involving the genes *DNMT3A*, *TET2*, and *ASXL1* in a morphologically normal BM, most likely represents CHIP, whereas mutations involving *TP53*, *EZH2*, *SEPBP1*, *STAT5B*, *CSF3R*, *NRAS*, *KRAS*, and more than one mutation, particularly if of higher allele frequency (VAF >10%), in association with an abnormal finding in BM and in the correct clinical context, could be used to provide clonality confirmation in establishing a diagnosis of CEL, NOS.[21]

In summary, CEL, NOS is a MPN that is characterized by persistent PB eosinophilia, BM hypercellularity with increased eosinophils, and frequent dysmegakaryopoiesis. Dysplastic features in the erythroid or granulocytic lineages may also be present, often in association with one or more cytopenias (usually anemia). These findings have raised the question if CEL, NOS would be more appropriately considered as a form of MDS/MPN rather than its current assignment under myeloproliferative neoplasms, but this remains a subject of debate. Regardless, comprehensive molecular studies are often needed to establish the diagnosis, since cytogenetic abnormalities are not always present, and exclusion

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of other myeloid neoplasms with overlapping features is essential.

MPN, MDS, MDS/MPN, and Therapy-Related Myeloid Neoplasm

Eosinophilia and/or marrow proliferation of eosinophils can be encountered in many different types of chronic myeloid diseases Table 7. Eosinophilia is frequently seen in classical MPN.[6,7] Typically, in chronic myeloid leukemia (CML), *BCR-ABL1* positive, eosinophilia and basophilia are frequently present at diagnosis and often also when the disease progresses. Eosinophilia is usually mild in this setting; however, cases of CML with HE have been proposed as "eosinophilic variant CML." In patients with typical *JAK2* V617F–positive MPN, eosinophilia (eo) is less frequently detected but may occur.[23] This also holds true for myeloid neoplasms classified as MDS/MPN, such as CMML (CMML-eo). Although not specifically mentioned in the WHO classification, eosinophilia is not a feature of atypical CML, *BCR-ABL1* negative. In MDS, eosinophilia is rare and usually mild. These cases have been referred to as MDS-eo and must be distinguished from CEL, NOS.

In all of these instances, it is important first to exclude a myeloid/lymphoid neoplasm with eosinophilia associated with *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2* or other gene fusions. In addition, it is important to exclude the possibility of an evolving core-binding factor (CBF) AML, which can present with low blast count simulating MDS.[24] More complicated is sometimes the separation of CEL, NOS from cases of MDS/MPN with eosinophilia when the latter carry mutations most typically seen in MPN.

In patients with MPN, MDS, or MDS/MPN, eosinophilia can also develop during the course of disease. In particular, 2.1% of all patients with MDS develop massive eosinophilia in the follow-up.[25] In some of these cases, the occurrence of eosinophilia is associated with disease progression. Moreover, in MDS, eosinophilia (in particular, BM eosinophilia) at diagnosis is of prognostic significance at least in some studies.[26-28] These patients apparently have an increased risk to develop secondary AML and a reduced survival.[26,27] Eosinophilia has been reported in rare case of MDS in association with der(1;7)(q10;p10).[29,30]

Clinically, it is important to note that patients with CML, MDS/MPN, or MDS who have persistent eosinophilia develop neither endomyocardial fibrosis nor other HES-like end-organ damage even when eosinophilia persists for years, contrasting with the course seen in *FIP1L1*/*PDGFRA*-positive disease.[6]

Two cases of *BCR-ABL1*–positive CML with eosinophilia were submitted to the workshop (cases 159 and 275). Case 159 illustrated an acute lymphoblastic crisis of CML in a pediatric patient (case 159). In case 275, a case of CML in chronic phase, the proliferation of eosinophils was limited to the BM.

Two cases of *BCR-ABL1*–negative MPNs were both advanced stage *JAK2*-mutated MPNs. The first case (83) was a post–essential thrombocythemia myelofibrosis showing leukocytosis, monocytosis, basophilia (5.5×10^9/L), and eosinophilia (3.08×10^9/L), all features that have been associated with disease progression in MPNs.[31,32] The case had a *PHF6* mutation, which has been previously associated with CML blast crisis.[33] The second case (98) was a primary myelofibrosis (PMF) with eosinophilia. The associated del(12)(q21.2q23.1) abnormality is of unclear clinical significance.

Case 293 was considered MPN unclassifiable (MPN-U) in accelerated phase (AP). This case appears morphologically to be most consistent with a triple-negative PMF in AP. However, the lack of a molecular-defined driver mutation makes a precise characterization impossible.

Case 192 highlights the difficulties in separating CEL, NOS from MDS/MPN-U in the presence of ring sideroblasts, *SF3B1* mutation, and normal karyotype. Despite the comutation of *SF3B1* and *JAK2*, the lack of thrombocytosis (confirmed at follow-up) and the increased eosinophilia were felt to be consistent with CEL, NOS. Ring sideroblasts occur in variable proportions in case of MPNs (eg, in PMF),[34] and their presence is not necessarily inconsistent with a diagnosis of MPN.

The cases of MDS/MPN included two examples of CMML with eosinophilia (cases 288 and 241). Case 288 is discussed below. Case 241 fits well with a diagnosis of CMML with eosinophilia per the WHO 2017 classification (ie, this can be rendered if diagnostic criteria for CMML are met and peripheral blood eosinophils are $\geq 1.5 \times 10^9$/L).

Two cases of juvenile myelomonocytic leukemia (JMML) were submitted (cases 18 and 32). Both were typical examples of JMML associated with *PTPN11* mutation. Case 32 showed atypical eosinophils. Both cases broaden the differential diagnosis of myeloid neoplasms presenting with eosinophilia to include rare cases of JMML.

The rarity of eosinophilia in properly diagnosed cases of MDS is well known. Only two patients were submitted to the workshop as MDS with eosinophilia. Case 289 is an MDS with excess blasts 1, in whom the patient, following treatment, developed increased eosinophils only in the BM. The second case (51) was an MDS with excess blasts 2 with fibrosis associated with inv(3)

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Table 7

Clinical, Laboratory, Cytogenetic, and Molecular Findings of Myeloproliferative Neoplasm, Myelodysplastic Syndrome, Myelodysplastic/Myeloproliferative Neoplasm, and Therapy-Related Myeloid Neoplasm Cases

Case No.	Age/Sex	Diagnosis	Key Laboratory Results and Morphology	Cytogenetic and Molecular Findings	Interesting Features
293	92 y/M	MPN-U in accelerated phase (with eosinophilia)	WBC 25.8 × 10^9/L ANC 18.5 × 10^9/L AEC 3.31 × 10^9/L ABS MONO 0.65 × 10^9/L ABS BASO 0.18 × 10^9/L Hb 11.9 g/dL PLT 1,868 × 10^9/L	46,XY[20] FISH shows no rearrangements of 4q12 (*FIP1L1/CHIC2/PDGFRA*), 5q33.1 (*PDGFRB*), 8p12 (*FGFR1*), or t(9;22)(q34;q11.2) No evidence of *BCR-ABL1*, *JAK2* V617F, *CALR* exon 9, or *MPL* codon 515 mutations in PB	Morphologically an MPN-U in AP most compatible with a triple-negative primary myelofibrosis in AP; despite extensive cytogenetic, FISH, and molecular workup, no molecular alteration or clonal abnormality found; genetic testing such as RNA sequencing could be considered to exclude the possibility of a cryptic gene fusion
275	55 y/F	CML, *BCR-ABL1* positive in AP, with increased eosinophils in the BM	BM: M:E ratio of 11.9 with marked eosinophilia (25.7%) and basophilia (21%) and no increase in blasts (0.3%); basophilia but no increased eosinophils in PB	PCR for *BCR-ABL1*: p210 transcript *BCR-ABL1* transcript at a level >10% IS *BCR-ABL1* mutational analysis on PB detected two mutations reported to confer TKI resistance: p.T315I (c.944C>T) (53.0%) and p.E255V (c.764A>T) (47.8%)	CML-AP showed an unusual eosinophilia that developed over 2 months; TKI-resistance detected and TKI therapy was changed to ponatinib
159	13 y/M	CML, *BCR-ABL1* positive, in B-lymphoid blast crisis (with eosinophilia)	WBC 270 × 10^9/L, 89% blasts Flow cytometry: CD45 (dim), TdT, CD34, CD38 (variable), CD58, HLA-DR, CD19 (dim), sCD22 (dim), CD10 (bright), and CD9 (variable)	Positive for *BCR/ABL* rearrangement (88.0% of cells) with a second Philadelphia chromosome (6.0% of cells)	The diagnosis of CML presenting in acute lymphoblastic crisis is unusual in pediatric patients
83	63 y/M	Post essential thrombocythemia myelofibrosis with marked basophilia and mild eosinophilia	Leukoerythroblastosis WBC 22 × 10^9/L Hb 10 g/dL PLT 35 × 10^9/L NRBC 78/100 Neutrophils 28%, bands 4%, myelocytes 2%, metamyelocytes 4%, blasts 2%, lymphocytes 12%, monocytes 9%, eosinophils 14%, basophils 25%	*JAK2* c.1849G>T; p.V617F, VAF 95% PHF6 c.1096_1098delTAGinsA; p.*3666Sfs*22, VAF 93%	Advanced stage MPN showing basophilia (5.5 × 10^9/L) and eosinophilia (3.08 × 10^9/L); most likely representing disease progression
98	62 y/M	PMF with eosinophilia	WBC 27.3 × 10^9/L Hb 6.8 g/dL PLT 156 × 10^9/L BM: M:E ratio 6:1 6% blast, 20% eosinophils	46,XY,del(12)(q21.2q23.1)[20] *JAK2* V617F detected (VAF unavailable)	PMF with *JAK2* V617F and del(12)(q21.2q23.1) with eosinophilia
192	70 y/M	CEL, NOS (with ring sideroblasts)	WBC 17.5 × 10^9/L AEC 7.0 × 10^9/L Lung biopsy consistent with eosinophilic lung disease	*DNMT3A* p.Arg882His—49.6% *SF3B1* p.Lys700Glu—44.9% *TET2* p.Glu808*—23.6% *JAK2* p.Val617Phe—13.5% Karyotype: 46,XY[19]	Never developed thrombocytosis; favor CEL, NOS with RS over MDS/MPN-U
241	58 y/F	CMML-1 (with eosinophilia)	WBC 21.36 × 10^9/L Hb 13.8 g/dL PLT 59 × 10^9/L ANC 5.12 × 10^9/L ABS MONO 10.25 × 10^9/L AEC 1.92 × 10^9/L	Karyotype: 45,XX,–7[20] FISH for *BCR/ABL1*, *CBFB/MYH11*, and rearrangements of *PDGFRA*, *PDGFRB*, and *FGFR1* were negative NGS: *ASXL1* p.Q780*, c.2338C>T (VAF 47.6%) *NRAS* p.G13R, c.37G>C) (VAF 47%) *SETBP1* p.I871T, c.2612T>C (VAF 42.6%) *FLT3* ITD negative	Example of CMML with eosinophilia; the WHO specifies that a diagnosis of CMML with eosinophilia can be rendered if diagnostic criteria for CMML are met and PB eosinophils are ≥1.5 × 10^9/L

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Table 7
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Case No.	Age/Sex	Diagnosis	Key Laboratory Results and Morphology	Cytogenetic and Molecular Findings	Interesting Features
288	69 y/F	CMML-1 (with eosinophilia)	WBC 13 × 10^9/L Hb 11.9 g/dL PLT 73 × 10^9/L Differential: 3% blasts, 2% myelocytes, 10% bands, 24% neutrophils, 19% lymphocytes, 21% monocytes (2.7 × 10^9/L), 16% eosinophils (2.1 × 10^9/L), 5% basophils	Karyotype: 45,XX,–7[20] FISH: Positive for loss of 7q11.23 and 7q31.2 Negative for *PDGFRA*, *PDGFRB*, *FGFR1* rearrangements and abnormalities of 5p/5q, 8cen, 20q, *BCR/ABL1*, *KMT2A* NGS: *NRAS* G13R (VAF 45.5%) and *RUNX1* R107C (4.2%) mutations	Interestingly, BM and/or PB demonstrated expansion of several myeloid lineages, including monocytes, eosinophils, basophils, and mast cells Genetic testing such as RNA sequencing should be considered in this case to exclude the possibility of a cryptic fusion
18	11 mo/M	JMML, Noonan syndrome associated (with eosinophilia)	WBC 34.68 × 10^9/L AEC 0.31 × 10^9/L ABS MONO 4.68 × 10^9/L Hb 11.6 g/dL PLT 713 × 10^9/L	Karyotype: 46,XY[20] NGS: *PTPN11* NM_002834 c.184T>G p.Y62D- in 55.6% of 720 reads Variants of unknown significance (VUS): *JAK3* NM_000215 c.2164G>A p.V722I (49.1% of 116 reads)	Typical JMML with *PTPN11* mutation
32	3 y/M	JMML (with eosinophilia)	WBC 50.5 × 10^9/L AEC 3.03 × 10^9/L ABS MONO 4.04 × 10^9/L Hb 11.1 g/dL PLT 15 × 10^9/L Fetal Hb was 65%	Karyotype: 46,XY[20] Positive for *PTPN11*, *NF1*, and *SH2B3* mutations No mutations were detected in *CBL*, *KRAS*, and *NRAS*	The case shows HE with atypical eosinophils present; it broadens the differential diagnosis of myeloid neoplasms presenting with eosinophilia; JMML should be included in the differential of childhood eosinophilic bone marrow disorders
289	65 y/M	MDS with excess of blasts 1, following treatment (increased eosinophils in the BM)	WBC 1.97 × 10^9/L Hb 6.1 g/dL PLT 17 × 10^9/L No eosinophilia	Karyotype: The BM and PB showed deletion of 12p13 and 13q14 by G-band analysis FISH using *ETV6* (TEL) break-apart probe and D13S319/13q34 probe showed deletion of one copy of *ETV6* and deletion 13q14	The exact relationship between the presumptive newly acquired eosinophilia and the longstanding MDS is unknown Eosinophilia has been associated with poor prognosis in de novo MDS; may represent evidence of disease progression
51	62 y/M	MDS with excess blasts 2 with fibrosis associated with inv(3); progression to acute myeloid leukemia with inv(3) (mild eosinophilia)	WBC 12.34 × 10^9/L AEC 0.62 × 10^9/L ABS MONO 0.49 × 10^9/L Hb 12.5 g/dL PLT 49 × 10^9/L	FISH: +8 (18% of cells) No *PDGFRA*, *PDGFRB*, or *FGFR1* Karyotype analysis subsequently performed on the leukemic peripheral blood 4 months later showed an abnormal clone with inv(3) with breakpoints at 3q21 and 3q26.2, +21 and additional material with unknown origin on 15q24 NGS: *DNMT3A*, *TET2*, *SF3B1*	AML with inv(3) is often preceded by a brief MDS; eosinophilia noted
110	72 y/M	Therapy-related myeloid neoplasm not further classifiable (cytopenic patient who developed eosinophilia and basophila)	WBC 6.6 × 10^9/L ANC 2.55 × 10^9/L ABS MONO 0.34 × 10^9/L AEC 2.68 × 10^9/L ABS BASO 0.47 × 10^9/L Hb 15.3 g/dL PLT 382 × 10^9/L	Karyotype: 46,XY[15] FISH negative for *PDGFRA*, *PDGFRB*, *FGFR1*, and *JAK2* rearrangements (BM) NGS: *GNAS*, p.R844H, c.2531G>A (VAF 39%); SRSF2, p.P95_R102delc.284_307delCCCCGGACTCACACCACAGCCGCC (VAF 54%)	Cytopenias preceded the development of eosinophilia, suggesting disease progression from a TR-MDS; the presence of concurrent basophilia can also be seen in this type of disease progression
181	77 y/M	Therapy-related myeloid neoplasm, (favor TR-MDS/MPN) with progression to therapy-related AML	WBC 24.3 × 10^9/L Hb 9.3 g/dL PLT 110 × 10^9/L Differential: polys 3%, bands 2%, lymphocytes 19%, monocytes 3%, eosinophils 45%, basophils 23%, blasts 5%	Karyotype: 47,XY,+8,t(9;12)(q34;p13)[20] FISH positive for trisomy 8, one additional *FGFR1* signal (consistent with trisomy 8), one additional *ABL1* (9q34) signal, and loss of one copy of *ETV6* without breakage of the remaining allele; *BCR-ABL1* negative NGS: *RUNX1* (c.762_763dupCG, p.D255Afs*30, VAF: 43.8%), *SRSF2* (c.284C>A, p.P95H, VAF: 45.9%), and *TET2* (c.3622A>T, p.K1208*, VAF: 46.2%)	The case showed a t(9;12); the possibility of *ETV6-ABL1* fusion was considered; this could cause myeloid neoplasia with eosinophilia Subsequent FISH studies demonstrated gain of *ABL1* gene and loss of one copy of *ETV6* without breakage of the second allele, which did not fully refute this possibility; the product of the translocation remains undetermined Genetic testing such as RNA sequencing should be considered in this case to exclude the possibility of a cryptic fusion

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Table 7
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Case No.	Age/Sex	Diagnosis	Key Laboratory Results and Morphology	Cytogenetic and Molecular Findings	Interesting Features
253	61 y/M	Therapy-related myeloid neoplasm consistent with TR-CMML with eosinophilia and relapsed T-ALL	WBC 42.6 × 10^9/L Hb 12.6 g/dL PLT 78 × 10^9/L ANC 15.3 × 10^9/L ABS MONO 3.41 × 10^9/L AEC 19.61 × 10^9/L ABS BASO 0.01 × 10^9/L	Chromosome analysis on BM revealed a translocation between the long arms of chromosomes 1 and 16, resulting in a derivative chromosome 16; previous cytogenetic studies did not identify this cytogenetic abnormality; negative FISH for *PDGFRA*, *PDGFRB*, and *FGFR1*	Therapy-related myeloid neoplasm consistent with TR-CMML with eosinophilia after treatment of T-ALL at the time of T-ALL relapse

ABS BASO, absolute basophil count; ABS MONO, absolute monocyte count; AEC, absolute eosinophil count; AML, acute myeloid leukemia; ANC, absolute neutrophil count; AP, accelerated phase; BM, bone marrow; CEL, NOS, chronic eosinophilic leukemia, not otherwise specified; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukemia; FISH, fluorescence in situ hybridization; Hb, hemoglobin; HE, hypereosinophilia; IS, international scale; JMML, juvenile myelomonocytic leukemia; MDS, myelodysplastic syndrome; M:E, myeloid/erythroid; MPN, myeloproliferative neoplasm; MPN-U, myeloproliferative neoplasm unclassifiable; NRBC, nucleated RBCs; PB, peripheral blood; PCR, polymerase chain reaction; PLT, platelet count; PMF, primary myelofibrosis; RS, ring sideroblast; T-ALL, T-lymphoblastic leukemia/lymphoma; TKI, tyrosine kinase inhibitor; TR, therapy related; VAF, variant allele frequency; WHO, World Health Organization.

that progressed rapidly to acute myeloid leukemia with inv(3) and had only mild eosinophilia (0.62 × 10^9/L) at the time of diagnosis. In most cases, the development of eosinophilia in the course of MDS is most likely a manifestation of disease progression and has been associated with adverse outcome.[25]

Last, three cases of therapy-related myeloid neoplasms (TR-MN) were included (110, 181, and 253). Two of these cases were examples of therapy-related MDS/MPN (TR-MDS/MPN); the third case (110) is a TR-MN that is hard to classify in view of the history of intermittent cytopenias with spontaneous normalization antedating the development of the eosinophilia and basophilia, better left as a TR-MN not further classifiable. Case 181, which showed a t(9;12)(q34;p13) translocation that raised the possibility of *ETV6-ABL1* fusion, is discussed below.

Several cases in this session, which were classified in their respective WHO categories, showed the need for appropriate molecular genetic studies. They raised the possibility of one of the less frequent variants of M/L neoplasm with eosinophilia. In these cases, often cryptic by conventional karyotype and FISH studies, genetic testing such as RNA sequencing is recommended to exclude the possibility of a specific gene fusion. These cases included case 293, morphologically an MPN-U in AP that was not further classifiable due to the lack of a molecular-defined driver mutation. In this case, exclusion of an underlying gene fusion could be indicated to differentiate a myeloid/lymphoid neoplasm with eosinophilia and gene rearrangements from a triple-negative PMF in AP. Case 288 was classified as CMML-1. BM and PB demonstrated a proliferation of several myeloid lineages, including monocytes, eosinophils, basophils, and mast cells. FISH studies for the classical M/L neoplasms with eosinophilia were negative. However, the possibility of a cryptic fusion could still be entertained. Case 181 was classified as a TR-MDS/MPN. The case showed, in addition to a +8, a t(9;12)(q34;p13), a finding that raised the possibility of an *ETV6-ABL1* fusion. Subsequent FISH studies that demonstrated gain of *ABL1* gene and loss of one copy of *ETV6* without breakage of the second allele did not fully refute this possibility. In the end, the product of the translocation in this case remains undetermined.

Acute Leukemia and Eosinophilia

Eosinophilia is a common finding in acute leukemia. The SH/EAHP workshop received 24 acute leukemia cases, including 12 AMLs, 11 B-lymphoblastic leukemia/lymphoma (B-ALL) cases, and one T-lymphoblastic leukemia/lymphoma (T-ALL). Clinicopathologic, cytogenetic, and molecular findings are summarized in **Table 8**.

Acute Myeloid Leukemia

The 12 AML cases span the spectrum of AML classification based on the current WHO classification[35] (Table 8). AML with inv(16)(p13.1q22) or (16;16)(p13.1;q22);*CBFB-MYH11* represents the single most commonly represented subtype of AML, and it accounts for 6 of the 12 AML cases (26, 43, 67, 130, 155, and 272). AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*, acute promyelocytic leukemia, and AML with biallelic mutation of *CEBPA* were represented by one case each, respectively (cases 123, 261, and 281). There were two cases of AML with myelodysplasia-related changes (AML-MRC)[36] (217 and 274) and a single case of myeloid sarcoma with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1* (273).

Aside from marrow eosinophilia, the presence of aberrant, immature-appearing eosinophilic granules is an important observation in AML with inv(16)(p13.1q22) or

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Table 8

Clinical, Pathologic, Cytogenetic, and Molecular Findings of Acute Leukemia Cases Submitted to the Workshop

Case No.	Age (y)/ Sex	Diagnosis	Cytogenetic and Molecular Findings	Interesting Features
26	54/M	AML with inv(16)	46,XY,inv(16)(p13.1q22)(p13.1q22), add(18)(p11.3)[5]/46,XY[15]	Absolute monocytosis with aberrant monocytes in PB; atypical eosinophils and dysplastic neutrophils in BM; absent blasts
43	19/F	AML with inv(16), tryptase positive	46,XX, inv(16)(p13.1q22)(p13.1q22)	Mastocytosis-like presentation of urticaria, elevated tryptase with pancytopenia, and circulating blasts; tryptase-positive granules in blasts and in myelomastocytic cells
67	59/M	AML with inv(16)	46,XY,inv(16)(p13.1q22)(p13.1q22)	Abnormal eosinophils without increase in blasts in bone marrow at a relapse
130	57/M	AML with inv(16), with multiple relapse	46,XY,inv(16)(p13.1q22)(p13.1q22)	Aggressive clinical course with five relapses; cytogenetic and molecular evolution, with abnormalities shared by blasts and eosinophils
155	57/F	AML with inv(16)	46,XX,inv(16)(p13.1q22)(p13.1q22){20]/separate clone with loss of 18p	History of breast cancer; AML inv(16) as a therapy-related myeloid neoplasm
272	38/F	AML with inv(16)	46,XX, inv(16)(p13.1q22)(p13.1q22)	Acute myelomonocytic leukemia with BM eosinophilia and dysplasia
123	66/F	AML with t(8;21)	46,XX,t(8;21)(q22;q22)	Marked absolute eosinophilia (AEC 72 × 10^9/L), with only 6% PB and 4% BM blasts
261	72/F	APL with eosinophilic differentiation	46,XX,t(15;17)(q24;q21) FISH: *PML/RARA* fusion in 89% of cells in peripheral blood	Absolute eosinophilia of 2.57 × 10^9/L at day 21 after treatment with ATRA and arsenic trioxide; eosinophils are positive for *PML/RARA* fusion
281	42/M	AML with biallelic mutation of *CEBPA*	46,XY[20] Molecular studies: biallelic *CEBPA* mutation Negative for *NPM1*, *FLT* ITD and TKD mutations	PB eosinophilia (1.1 × 10^9/L) with immature basophilic granules in eosinophils and dysplastic features in neutrophils
217	63/M	AML with myelodysplasia-related changes	Complex aberrant male karyotype with several related clones, including –5 and –7 NGS: *TP53* mutation	BM eosinophilia and dysplastic features of the eosinophils in BM aspirate
274	71/M	AML with myelodysplasia-related changes	45,XY,–7[20] NGS: *ASXl1*, *EZH2*, *FLT3*, and *PTPN11* mutations	PB and BM eosinophilia with dysplastic features and increased blasts
273	3/M	Myeloid sarcoma with t(8;21)	FISH performed on orbital mass 88% of cells positive for *RUNX1-RUNXT1*	Orbital mass (2.8 × 2.6 × 1.3 cm), composed of sheets of blasts and eosinophils; there was no PB eosinophilia
248	10/M	B-ALL with t(5;14)	Complex male karyotype with t(5;14)(q31;q32) FISH positive for IGH rearrangement	WBC of 83 × 10^9/L with 75% eosinophils; progression and relapse heralded by increasing eosinophils
16	8/M	B-ALL with t(5;14)	47,XY,t(5;14)(q31;q32),+22 FISH positive for IGH rearrangement	Fever, WBC of 23.2 × 10^9/L with 56% eosinophils
278	25/F	B-ALL with t(5;14)	46,XX,t(5;14)(q31;q32)	Leukocytosis and 67% eosinophils
2	51/F	B-ALL, *BCR/ABL1*-like	46,XX B-ALL FISH shows *CRLF1/IGH* fusion, *ETV6* rearrangement at 12p13, and a *CDKN2A* deletion at 9p21	WBC of 61 × 10^9/L, with 52.46 × 10^9/L eosinophils and 9% blasts; eosinophilic myocarditis with heart failure, brain embolism, and hemiparesis due to the eosinophilia
93	11/M	B-ALL with iAMP21	46,XY,add(21)(q22)[1]/46,sl,del(6)(q21), add(11)(p11.2),–20,+mar[2]/46,XY[17]	Heart failure, AEC of 18.7 × 10^9/L and low-level circulating B-lymphoblasts
75	69/M	B-ALL, NOS	46,XY SNP microarray: homozygous loss of short arm of 9 and gain of the long arm of 9 consistent with i(9q)	AEC of 8.4 × 10^9/L with atypical eosinophils but no blasts Initially a myeloid neoplasm suspected
102	15/M	B-ALL, NOS	Complex abnormal karyotype with an i(9q) and two subclones with additional abnormalities	WBC of 31.2 × 10^9/L with AEC of 15.19 × 10^9/L; BM shows sheets of blasts and eosinophils
82	14/M	B-ALL, NOS	46,XY, aCGH/SNP microarray of CD19+ sorted cells: suspicious for monosomy 17 and several small regions of LOH/UPD of unknown significance	WBC of 136.4 × 10^9/L, with AEC of 107.56 × 10^9/L and no blasts in PB A myeloproliferative neoplasm suspected; flow cytometry demonstrated 0.01% abnormal B-lymphoblasts
209	43/M/	B-ALL, NOS	46,XY FISH: IGH rearrangement NGS: negative	History of eosinophilic granulomatosis with polyangiitis, WBC of 10.56 × 10^9/L, AEC of 4.75 × 10^9/L, and 4% B-lymphoblasts

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Table 8
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Case No.	Age (y)/ Sex	Diagnosis	Cytogenetic and Molecular Findings	Interesting Features
233	7/M	B-ALL, NOS	46,XY aCGH/SNP of sorted B cells showed 2p microdeletion, 2p16.1 (1.2 Mb) including *BCL11A*, and an 8q microdeletion, 8q22.1 (534 Kb); sorted eosinophils and basophils were normal by aCGH/SNP	BM eosinophilia; BM aspirate flow cytometry showed 6% abnormal B-lymphoblasts and 43% eosinophils
222	15 M	B-ALL, not further characterized	No karyotype of FISH performed; RT-PCR was negative for *BCR-ABL1*, *PDGFRA-FIP1L1*, and *MLL-AF4* abnormalities	Eosinophilia, with a WBC of 42.86 × 10^9/L and AEC of 38.57 × 10^9/L; BM aspirate showed B-ALL with 45% B-lymphoblasts and increased eosinophils
158	10 M	Early T-cell precursor ALL	47,XY,t(10;11)(p12-13;q14), +19[2]/47,sl,add(22)(q13)[7]/46,XY[11]	Fever, joint pain, and WBC of 220.40 × 10^9/L with AEC of 26.48 × 10^9/L; BM aspirate showed 90% blasts and eosinophils with abnormal basophilic granules

aCGH/SNP, array comparative genomic hybridization/single-nucleotide polymorphism; AEC, absolute eosinophil count; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; ATRA, all-*trans* retinoic acid; B-ALL, B-lymphoblastic leukemia/lymphoma; BM, bone marrow; FISH, fluorescence in situ hybridization; IGH, immunoglobulin heavy chain gene; LOH/UPD, loss of heterozygosity/uniparental disomy; NGS, next generation sequencing; PB, peripheral blood; RT-PCR, reverse transcription polymerase chain reaction; SNP, single-nucleotide polymorphism.

(16;16)(p13.1;q22);*CBFB-MYH11*. This finding was featured in three cases, visible either in the BM aspirate (cases 26 and 67) and, in one case, in the PB, which also showed an absolute eosinophilia of 3.2 × 10^9/L (case 130) **Image 12A** and **Image 12B**. These immature eosinophilic granules are most evident in the late promyelocyte and myelocyte stages and usually are not present at later stages of eosinophil maturation. The eosinophilic granules are often larger than those normally present in immature eosinophils, are purple-violet in color, and in some of the cells may be so dense that they obscure the cell morphology. These cells are often referred to as "abnormal eosinophils with basophilic granules." The mature eosinophils in these cases occasionally show nuclear hyposegmentation. Recognition of these eosinophils with aberrant, immature granules may be valuable in suspecting the specific cytogenetic abnormality of inv(16) or t(16;16). On the other hand, the presence of aberrant basophilic eosinophilic granules is not unique for AML with inv(16), and in our workshop series, it was observed in a case of AML with biallelic mutation of *CEBPA* (case 281) and in a case of early T-cell precursor acute lymphoblastic leukemia (case 158).

While eosinophils with aberrant granules are important as a diagnostic clue, cells with coarse granules do not necessary represent eosinophils. The workshop received a single unusual case of tryptase-positive AML with inv(16)(p13.1q22) (case 43; **Image 12C** and **Image 12D**). This patient, a 19-year-old woman, had a mastocytosis-like clinical picture, including an urticarial rash and an elevated serum tryptase level of 102 µg/L. Later she developed pancytopenia. A BM aspirate showed many blasts, meeting criteria of an AML. Coarse tryptase-positive granules were seen in blasts and also in more mature myeloid cells. The cytogenetic evaluation revealed inv(16)(p13.1q22). This case is an example of a tryptase-positive AML, a finding that correlates well with the mastocytosis-like clinical presentation. In the past, the term myelomastocytic leukemia was used in the literature for patients with advanced myeloid neoplasms with increased immature atypical mast cells but not meeting criteria of systemic mastocytosis.[37] The term myelomastocytic leukemia is not used by the latest WHO classification; therefore, the Review Panel recommends such cases to be classified based on the WHO classification (in this case, as an AML with inv(16)(p13.1q22)), and then a disclaimer of "tryptase positive" or "with mastocytic differentiation" should be added.

Some AML cases may present with a blast percentage lower than the diagnostic threshold of 20%. This observation was demonstrated by three cases of the workshop, including two AMLs with inv(16)(p13.1q22) or (16;16)(p13.1;q22) (cases 26 and 67) and one AML with t(8;21)(q22;q22.1) (case 123). During a diagnostic evaluation, it might be difficult to interpret these cases as an acute leukemia in the absence of increased blasts. In this setting, the presence of increased eosinophils and/or aberrant immature-appearing eosinophil granules may represent an important morphologic warning sign that the BM might harbor an AML.

AML with inv(16)(p13.1q22) or (16;16)(p13.1;q22); *CBFB-MYH11* provides an important model of the relationship between the myeloid leukemia clone and the eosinophils. Are eosinophils parts of the myeloid clone? If they are, cytogenetic and molecular abnormalities would be shared between the myeloid blasts and the eosinophils. Workshop case 130, a (previously published)

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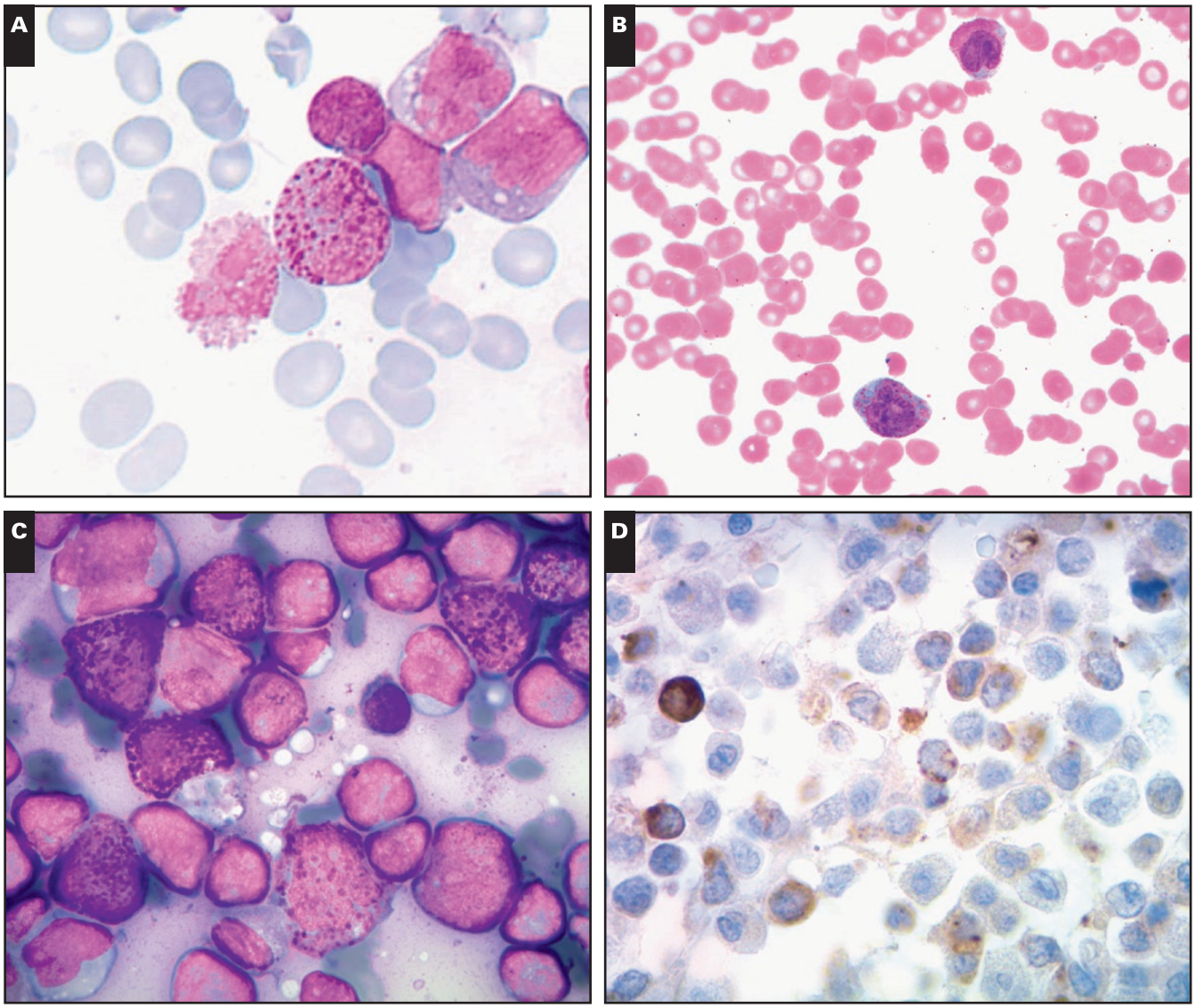

Image 12 **A-D**, Immature-appearing abnormal granules in eosinophils, observed in two cases of acute myeloid leukemia (AML) with inv(16)(p13.1q22);*CBFB-MYH11*. **A**, Case 67, bone marrow aspirate smear (Wright-Giemsa, ×500). **B**, Case 130, peripheral blood smear (Wright-Giemsa, ×1,000). **C**, **D**, Tryptase-positive granules in a case of AML with inv(16)(p13.1;q22), with mastocytic differentiation. Tryptase positivity is seen in blasts and more mature myelomastocytic cells (**C**, Wright-Giemsa, ×500; **D**, tryptase immunostain, ×200).

case of AML with inv(16) that was characterized over multiple relapses, offered direct insights into this relationship. This case demonstrated that blasts and eosinophils sorted by flow cytometry shared the inv(16) cytogenetic abnormality. Furthermore, as the myeloid blasts acquired additional cytogenetic abnormalities of trisomy 8 and trisomy 20 during the fourth relapse and then an additional *KRAS* G12D mutation during the fifth relapse, the same cytogenetic and molecular abnormalities were also demonstrated in the eosinophils.[38]

The workshop's single case of acute promyelocytic leukemia (APL), case 261, demonstrates that eosinophils may represent a main line of differentiation in AML. The case features a 71-year-old woman with a diagnosis of APL established based on classical morphologic and cytogenetic features. She was treated with all-*trans* retinoic acid and arsenic trioxide and then developed a marked absolute eosinophilia that peaked at day 22 after treatment (WBC count of 5.47×10^9/L, AEC of 2.57×10^9/L). FISH analysis of the PB demonstrated 89% of the leukocytes to contain a *PML*/*RARA* fusion. Therefore, the eosinophils represent differentiated forms of the leukemia, as opposed to an allergic reaction or drug-induced eosinophilia after therapy.

The two cases of AML with MRC share many similarities (cases 217 and 274). Both were male patients, 63

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and 71 years of age, respectively, and both had pancytopenia, absolute eosinophilia, and circulating blasts. Cytogenetic findings included a complex karyotype and a monosomy 7, respectively. The BM biopsy specimens showed increased blasts, multilineage dysplasia, and sheets of eosinophils. Case 217 is notable for the unusual florid dysplastic features of the eosinophils, including bizarre nuclear hypersegmentation and hyposegmentation.

Acute Lymphoblastic Leukemia

Lymphoblastic leukemias represented 12 of the 24 total acute leukemia cases, with 11 being B-ALL, with only one case of T-ALL received (case 158) (Table 8). The single most common entity in this category was B-ALL, not otherwise specified (B-ALL, NOS, five cases, 75, 102, 82, 209, and 233), followed by B-ALL with t(5;14)(q31.1;q32.1); *IGH*/*IL3* (three cases, 16, 248, and 278). B-ALL, *BCR*/*ABL*-like (case 2), B-ALL with iAMP21 (case 93), and B-ALL not further characterized (case 222) were represented by a single case each, respectively.

From the clinical point of view, B-ALL cases associated with eosinophilia had a remarkably similar clinical presentation: they were characterized by a very high AEC combined with paucity or even absence of circulating B-lymphoblasts in the PB (cases 16, 22, 82, 278, and 209). Therefore, these cases were commonly suspected to represent a myeloid malignancy with eosinophilic proliferation. Circulating blasts were often detectable only with a highly sensitive flow cytometric analysis. A typical case of this type of presentation is case 82, a case of a 14-year-old boy who had fevers, dizziness, weakness, and difficulty in following commands. The CBC and PB differential showed a hemoglobin of 12.1 g/dL, a platelet count of 137×10^9/L, and a WBC count of 136.4×10^9/L, with 79% eosinophils (AEC of 107.7×10^9/L), 14% neutrophils, 3% lymphocytes, 3% monocytes, and 1% basophils. Initially, an MPN was suspected. Flow cytometric analysis of the PB identified a very small (0.01% of events) population of circulating abnormal immature B cells. Cytogenetic studies in this case showed a normal male karyotype. FISH analysis was negative for the following probes: *CEP4*, *FIP1L1*/*CHIC2*/*PDGFRA*, *PDGFRB*, *FGFR1*, *ABL1*, *CEP10*, *KMT2A*, *ETV6*, *CBFB*, *RUNX1*, and *BCR*. Molecular genetic studies were negative for *BCR-ABL1* fusion by RT-PCR and for clonal T-cell gene rearrangement. The diagnosis of B-ALL, NOS was rendered. This case emphasizes the importance of a highly sensitive flow cytometric analysis in suspected cases with marked eosinophilia but no conspicuous blasts in PB.

Eosinophils in B-ALL and T-ALL, unlike in AML, are thought to be reactive and not part of the neoplastic clone. The classical model of this relationship is B-ALL with t(5;14)(q31.1;q32.1);*IGH*/*IL3*. In this type of B-ALL, a chromosomal rearrangement results in overexpression of the cytokine interleukin (IL) 3 under the promoter of *IGH*, and the excess of the cytokine results in eosinophilia.[39] This association is so strong that a disease relapse is commonly heralded by increasing eosinophil counts. The workshop received three cases of B-ALL with t(5;14)(q31.1;q32.1);*IGH*/*IL3* (cases 16, 248, and 278), and they appeared similar in their clinical and pathologic features. All three patients were young (8-year-old boy, 10-year-old boy, and a 25-year-old woman) and had high absolute eosinophil counts (10.9×10^9/L, 11.62×10^9/L, and 46.36×10^9/L). The BM showed sheets of eosinophils and blasts in all three cases.

Case 233, a case of B-ALL, NOS, had a detailed evaluation of the relationship between eosinophils and B-lymphoblasts. In this study, B-lymphoblasts, eosinophils, and basophils were sorted by flow cytometry, and the separate populations were compared by an array genomic hybridization/single-nucleotide polymorphism method. The authors demonstrate that the B cells had a 2p microdeletion at 2p16.1 (1.2 Mb), including *BCL11A*, and an 8q microdeletion, at 8q22.1 (534 kb). Eosinophils and basophils had no evidence of chromosomal aberrations or significant loss of heterozygosity/uniparental disomy. This is an elegant demonstration that the B-ALL and the eosinophils or basophils are unrelated, and the increase in eosinophils is likely reactive.

While the eosinophilia in B-ALL with t(5;14)(q31.1;q32.1) has a very clear pathogenesis, overall, this specific type of B-ALL is very rare, and the most common cases of B-ALL and eosinophilia encountered in clinical practice represent B-ALL, NOS. Therefore, eosinophilia can be driven by several other mechanisms in B-ALL, some of which may not be known yet. Of note, in our workshop series, three cases of B-ALL demonstrated homozygous loss of the 9p21.3 region, including two cases of B-ALL, NOS, both with isochromosome 9q (cases 75 and 102) and one case of B-ALL, *BCR-ABL1*–like, with *CDKN2A* deletion demonstrated by FISH (case 2). These cases suggest that a specific gene linked to eosinophilia may be localized in the 9p21.3 chromosomal region, in proximity of the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene. Similar cases of B-ALL with eosinophilia have been described in the literature.[40,41]

Germline Disorders Associated With Eosinophilia

As with many inherited diseases, germline disorders with associated eosinophilia commonly present in infants and children. The differential diagnosis of eosinophilia in a pediatric patient is similar to that in adults, and etiologies

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other than inherited syndromes include infection, atopic conditions, medications, toxins, autoimmune diseases, gastrointestinal disorders, and eosinophilic neoplasms, although the latter are much less frequent in younger age patients.[42,43] Thus, determining the cause of eosinophilia requires a detailed patient history, family history of eosinophilia, and physical examination with emphasis on specific signs and symptoms such as rashes, gastrointestinal or pulmonary symptoms, and petechiae. Laboratory evaluation includes basic metabolic studies and a full CBC with a differential and peripheral smear evaluation. Further studies depend on clinical suspicion and, in the case of germline causes, may include assessment of the presence of expected PB lymphocyte subsets and cytokine production, BM evaluation, and gene sequencing.

Despite the higher frequency of eosinophilic germline disorders within the pediatric population, they still remain quite uncommon and likely account for less than 10% of eosinophilia cases in children.[42] Many of the inborn causes of eosinophilia are associated with primary immunodeficiencies that can involve T cells, B cells, natural killer cells, or phagocytic cells on their own or in combination. A recent retrospective review of the medical literature identified approximately 40 loci that were implicated in inherited immunodeficiencies with associated eosinophilia.[44] Not surprisingly, the patterns of inheritance are autosomal dominant, autosomal recessive, or X-linked depending on the locus involved. Given the relatively large number of mutations that give rise to immunodeficiencies and eosinophilia, grouping them by related presentations and pathophysiology is useful in directing clinical studies.

In the 2019 workshop, 14 cases of germline disorders with associated eosinophilia, including primary immunodeficiency diseases, were received **Table 9**. The panel subdivided these cases into three main groups: primary immunodeficiencies with eosinophilia, BM failure syndromes with associated eosinophilia, and myeloid neoplasms with eosinophilia arising from germline predisposition. The grouping of these diseases is somewhat arbitrary, since mutations may function in multiple cell types and have various phenotypes. For instance, patients with mutations in the Wiskott-Aldrich syndrome protein (WASP) have eosinophilia associated with a combined immunodeficiency, failure of platelet production, and an increased risk of developing solid and hematopoietic cancer.[45]

Primary Immunodeficiencies With Associated Eosinophilia

Within the subcategory of primary immunodeficiencies with associated eosinophilia, three of the cases are representative of autosomal-dominant (case 284) and autosomal-recessive (cases 90 and 256) hyper-IgE syndrome and provide a framework to consider a number of the other submitted primary immunodeficiency cases.

The hyper-IgE syndromes include a group of primary immunodeficiency disorders displaying the classic triad of eczema, recurrent skin and pulmonary infections, and elevated IgE levels.[46] The pathogenesis of these syndromes is linked, in part, to skewing of the T-helper inflammatory responses to T-helper 2 (T_H2) differentiation.[47] T_H2 cells are responsible for immune responses to parasitic infections and elaborate proinflammatory cytokines, including IL-4, IL-5, and IL-13.[48] IL-4 promotes antibody class switching to IgE, while IL-5 and IL-13 promote eosinophil proliferation and chemotaxis, respectively.[49,50] The effects of these cytokines and the inhibited function of T-helper 1 (T_H1) and T-helper 17 (T_H17), which regulate immune response to viruses and extracellular bacteria, respectively, contribute to the triad of clinical findings in patients with hyper-IgE syndrome.

The most common form of autosomal-dominant hyper-IgE syndrome results from dominant negative mutations in *STAT3*.[51] STAT3 is involved in signal transduction cascades initiated by IL-6, IL-10, IL-11, and IL-21, which normally promote T_H17 differentiation and regulate interferon-γ production by T_H1 cells. The net result of STAT3 inhibition by dominant negative mutations is skewing toward T_H2 differentiation and the resultant clinical triad.[46] Case 284 described a 7-year-old girl with a *STAT3* mutation who had the classic features of eczema, recurrent skin infections, a markedly elevated IgE level, and eosinophilia. This patient also displayed bone abnormalities, including retained primary teeth craniosynostosis, two common findings in patients with autosomal-dominant hyper-IgE syndrome since STAT3 is also involved in bone formation.[52]

Autosomal-recessive hyper-IgE syndrome can result from mutation in a number of genes either directly or indirectly involved in cytokine signaling. These include the nonreceptor tyrosine kinase *TYK2* and the transcription factor *ZNF384*, which is involved in *STAT3* transcription.[53,54] However, the most common genetic lesion in autosomal-recessive hyper-IgE syndrome is the biallelic mutation in the dedicator of cytokinesis 8 (*DOCK8*) gene.[55] *DOCK8* is a GTPase that forms a complex with WASP and the WASP interacting protein (WIP). This complex regulates the actin related protein 2/3 (ARP2/3) complex to control actin nucleation and cytoskeletal remodeling.[56] Disruption of this DOCK8/WIP/WASP complex through *DOCK8* mutations results in multiple cellular defects, including defective immune synapse formation between T cells and antigen presenting cells. Suboptimal immune synapse formation, in turn, leads to

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Table 9

Clinical, Laboratory, and Genetic Findings of Germline Disorders With Eosinophilia Cases

Case No.	Age/Sex	Clinical History	Laboratory Findings	Pathologic Findings	Genetic Findings	Diagnosis
284	7 y/F	Eczema, recurrent skin infections, thrush, H3N2 influenza, craniosynostosis, retained primary teeth	WBC 4.9 × 10^9/L AEC 0.56 × 10^9/L IgE 930 UI/mL (normal <200)	BM with trilineage hematopoiesis Increased eosinophils	Karyotype: 46,XX[20] *STAT3* c.1909 G>A; p. V637M	Hyper-IgE syndrome with *STAT3* deficiency
90	5 y/F	Food allergy, atopic dermatitis, chronic otitis media, chronic cryptosporidium, HSV1 infection, eosinophilic pneumonia, eosinophilic esophagitis	WBC 40.9 × 10^9/L AEC 36 × 10^9/L IgE 3,320 UI/mL	BM with trilineage hematopoiesis 61% eosinophils	Array CGH: Heterozygous partial deletion of *DOCK8* *DOCK8*: c.5815_5816insT; p.Y1939LfsX12	Hyper-IgE syndrome with *DOCK8* deficiency
256	5 y/F	Food allergy, atopic dermatitis, GI candidiasis, pyelonephritis, HSV gingivostomatitis, HSV pneumonitis, lymphadenopathy at age 9	CBC Not provided AEC 6 × 10^9/L IgE 2700 UI/mL T- and B-cell lymphopenia	Lymph node (age 9): Large Reed-Sternberg–like cells, CD2+ CD3+ CD5+ CD30+ ALK1–	*DOCK8*: c.5490-5512del/5499-5520del	Hyper-IgE syndrome with *DOCK8* deficiency Anaplastic large cell lymphoma ALK1 negative
19	6 mo/M	Failure to thrive, eczema, neutropenia, respiratory infection, petechiae, hematochezia	WBC 5.79 × 10^9/L ANC 0 × 10^9/L AEC 1.6 × 10^9/L Hb 9.8 g/dL PLT 18 × 10^9/L	BM with trilineage hematopoiesis Increased eosinophils	*WAS*: c.424C>T; p.Gln142*	Wiskott-Aldrich syndrome
138A	Newborn/M	Eczema on arms, diffuse petechial rash Age 4: asthma, splenectomy for thrombocytopenia	WBC 15.4 × 10^9/L AEC 0.46 × 10^9/L Hb 13 g/dL PLT 11 × 10^9/L Positive urine CMV	Spleen: B-cell follicles with attenuated marginal zones	*WAS*: c.134C>T; p.T45M	Wiskott-Aldrich syndrome
138B	4 y/F	Developmental delay, dysphagia aspiration pneumonia, skin infections, hematochezia	WBC 14.4 × 10^9/L AEC 2.5 × 10^9/L Hb 11 g/dL PLT: 329 × 10^9/L	Stomach: EBV+ smooth muscle tumor Colon and esophagus: Increased eosinophils	*CARMIL2*: c.1942delC	Primary immunodeficiency with *CARMIL2* mutation
280	14 y/F	Atopic dermatitis, recurrent infections, asthma, GERD, eosinophilic esophagitis, molluscum contagiosum, spontaneously perforated nasal septum, destructive sinopulmonary, granulomatous inflammation	WBC 15.5 × 10^9/L AEC 7.48 × 10^9/L Hb 10.9 g/dL PLT 519 × 10^9/L IgE 52,511 UI/mL	BM with relative myeloid hyperplasia 36% eosinophils	*PIK3CD*: c.1546G>A; p.E522K	Eosinophilia and granulomatous polyangiitis with *PIK3CD* mutation
56	1 y/M	Eczema, fever, EBV-associated hemophagocytic syndrome, thrombotic thrombocytopenic purpura, cerebral infarcts, heart failure	WBC 113 × 10^9/L AEC 96.5 × 10^9/L Hb 11.0 g/dL PLT 50 × 10^9/L T-cell lymphopenia	BM with trilineage hematopoiesis 44% eosinophils Mild reticulin fibrosis	No pathogenic mutations identified	Eosinophilia likely associated with a primary immunodeficiency
65	7 wk/F	Failure to thrive, rash, fever, seizures, metabolic acidosis; patient died	CBC Not provided Eosinophils 43%	Autopsy findings: GI: Absent plasma cells Thymus: Eosinophil infiltration, decreased thymocytes, EMH Lymph nodes: Architectural effacement, decreased CD20 and CD3 staining Liver/spleen: EMH	No pathogenic mutations identified; variant of unknown significance in *PSMB8*	Eosinophilia associated with a primary immunodeficiency

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Table 9
(cont)

Case No.	Age/Sex	Clinical History	Laboratory Findings	Pathologic Findings	Genetic Findings	Diagnosis
17	5 mo/M	*Staphylococcus aureus* pustules	WBC 13.9 × 10^9/L ANC: 0 × 10^9/L AEC: 0.13 × 10^9/L Hb 11.6 g/dL PLT: 537 × 10^9/L	BM: Myeloid series with mostly promyelocytes and myelocytes Markedly decreased neutrophils Increased eosinophils	No pathogenic mutations identified	Severe congenital neutropenia with eosinophilia
264	Neonate/M	Papules that become hemorrhagic vesicles over entire body	WBC: 1.8 × 10^9/L AEC: 400 × 10^9/L Hb: 8.8 g/dL PLT: 242 × 10^9/L	BM with trilineage hematopoiesis Increased eosinophils Hyplobated megakaryocytes Almost no glycophorin A+ cells Skin with spongiosis, parakeratosis, bleeding under epidermis Perivascular eosinophils	*MYSM1*: c.1943G>T; p.Gly648Va	Eosinophilia and bone marrow failure in setting of *MYSM1* mutation
74	37 y/F	Skin abscesses, *Mycobacterium kansasii* lymphadenitis infection, history of VZV pneumonia Warts on hands and feet, Bell's palsy	WBC: 13.8 × 10^9/L AEC: 1.5 × 10^9/L ABS MONO: 0 × 10^9/L Hb: 7.3 g/dL PLT: 217 × 10^9/L	BM with trilineage dysplasia and eosinophilia	*GATA2*: c.1192C>T; p.R398W (constitutional) Karyotype: Loss of X, trisomy 1, trisomy 8	MDS/MPN with eosinophilia and germline *GATA2* mutation
12	68 y/F	History of breast carcinoma Angioedema, eosinophilic myocarditis	WBC: 46.0 × 10^9/L AEC: 20.2 × 10^9/L Blasts: 4%, Hb: 9.6 g/dL PLT: 69 × 10^9/L	Hypercellular BM with trilineage dysplasia and eosinophilia Areas with 10%-15% blasts	Complex karyotype *TP53*: c. 733G>A (VAF ~0.5); c. 818G>A (VAF: ~0.4)	Chronic eosinophilic leukemia in the setting of Li-Fraumeni syndrome
140	11 mo/F	Diffuse violaceous patches Periorbital swelling Hepatosplenomegaly MRI: orbital mass and separate cerebellar mass	WBC: 94.8 × 10^9/L AEC: 77.7 × 10^9/L Hb: 9.3 g/dL PLT: 67 × 10^9/L IgE: 106 UI/mL IL5: 53,920 pg/mL (normal <10 pg/mL)	BM with granulocytic hyperplasia, 78% eosinophils Orbital mass: Cells CD33+ lysozyme+ CD117+ Cerebellar mass: CD33– vimentin+ SMARCB1–	PB and tumors *SMARCB1*: c.110delG; p.R37Lfs*18	Myeloid sarcoma with eosinophilia in setting of *SMARCB1* germline mutation Atypical teratoid/rhabdoid tumor

ABS MONO, absolute monocyte count; AEC, absolute eosinophil count; ANC, absolute neutrophil count; BM, bone marrow; CGH, comparative genomic hybridization; EBV, Epstein-Barr virus; CMV, cytomegalovirus; EMH, extramedullary hematopoiesis; GERD, gastroesophageal reflux disease; GI, gastrointestinal; Hb, hemoglobin; HSV, herpes simplex virus; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; MRI, magnetic resonance imaging; PB, peripheral blood; PLT, platelet count; VAF, variant allele frequency; VZV, varicella zoster virus.

weakened T-cell receptor signaling, which favors T_H2 cell differentiation.[57]

Two cases submitted for the workshop (cases 90 and 256) showed eosinophilia in the setting of *DOCK8* mutations. Both cases described 5-year-old girls with signs of atopy, food allergies, multisystem chronic infections, and very high IgE levels. Patients with DOCK8 deficiency are at high risk for developing malignancies, including B- and T-cell lymphomas and squamous cell carcinoma.[58] Case 256 highlighted this cancer predisposition, as the patient developed an ALK1-negative anaplastic large cell lymphoma 4 years after her initial diagnosis.

Although not technically classified as a hyper-IgE syndrome, Wiskott-Aldrich Syndrome (WAS), resulting from a mutation in *WASP*, has many overlapping clinical features.[59] This phenotypic relationship is not surprising given involvement of the DOCK8/WIP/WASP complex in actin regulation and immune synapse formation. Similar to patients with hyper-IgE syndrome, patients with WAS typically have eczema, are highly susceptible to recurrent infections, and have an increased risk of autoimmunity and malignancies.[60] Patients with WAS typically have petechiae due to microthrombocytopenia since platelet production, function, and clearance are altered secondary to biallelic WASP mutations.[61,62] Since WASP is on the X chromosome, evaluation for WAS should be undertaken in male infants with eczema or recurrent infections in association with thrombocytopenia.[63]

Two cases of WAS were submitted to the conference (cases 19 and 138A). Both cases describe male infants

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with eczema and petechiae. The patient in case 19 also had neutropenia and recurrent infections, while the patient in case 138 was shown to have increased IgE levels. Notably, a presumptive diagnosis of WAS was made on case 19 by PB flow cytometry for the WASP antigen protein, a test that has been shown to have ~90% sensitivity and 100% specificity.[64]

Two additional cases of germline mutations resulting in eosinophilia with an associated immunodeficiency were submitted to the conference (cases 138B and 280). Case 138 involves a 4-year-old girl with recurrent skin infections, aspiration pneumonia, anemia, and a marked eosinophilia. Sequencing studies revealed a homozygous mutation of capping protein, ARP2/3 regulator, and myosin 1 linker 2 (*CARMIL2*). Homozygous *CARMIL2* mutations are a rare autosomal-recessive cause of inherited immunodeficiencies and are associated with eczema, recurrent cutaneous *Staphylococcus aureus* infections, respiratory infections, and esophageal candidiasis.[65] As implied by its name, CARMIL2 is involved in actin filament capping and cytoskeletal homeostasis, and it regulates the same ARP2/3 complex that is regulated by the DOCK8/WIP/WASP complex.[66] Additional immune dysfunction may be attributable to decreased CD28 costimulation of T cells with loss of CARMIL2.[67] At least one case series of patients with *CARMIL2* mutations shows a high frequency of Epstein-Barr virus–positive smooth muscle tumors,[65] and this type of neoplasm was found in the submitted case. Both case 138B and the WAS case 138A have been published in a small case series.[68]

The patient described in case 280 was a 14-year-old girl who displayed the classic features of hyper-IgE syndrome, including atopic dermatitis, recurrent skin infections, and markedly increased levels of IgE. She was also diagnosed with granulomatous polyangiitis that likely led to a perforated nasal septum. Sequencing studies revealed an activating mutation in the δ isoform of the p110 subunit of phosphoinositide-3-kinase (*PIK3CD*). Immunodeficiencies resulting from PI3-kinase activating mutations are inherited in an autosomal-dominant manner and have their own syndromic classification (activated PI3 kinase δ syndrome or APDS).[69] Patients with APDS frequently have primary immunodeficiency characterized by recurrent respiratory tract infections, herpes virus infections, developmental delay, and increased risk of autoimmune diseases and lymphoma. A recent report shows increased T_H1 and T_H2 cytokines (at the expense of T_H17 cytokines) with activating *PIK3CD* mutations, the latter of which may contribute to the eosinophilia found in the submitted case.[70]

An additional two cases that were submitted to the conference had signs of a primary immunodeficiency, but an underlying genetic abnormality was not identified. The clinical and laboratory features of these cases (56 and 65) are summarized in Table 9.

Primary BM Failure Syndromes With Associated Eosinophilia

Primary BM failure can be associated with eosinophilia, as illustrated by two of the cases submitted to the workshop. One of these (case 17) involved a 5-month-old boy with features highly reminiscent of severe congenital neutropenia, including *S aureus*–induced pustules and agranulocytosis (absolute neutrophil count of zero). The AEC was 0.13×10^9/L. Although increased eosinophil counts have been reported in patients with mutations in genes associated with severe congenital neutropenia,[71,72] no such mutation was detected in this patient. Additional causes of eosinophilia, including infections and myeloid growth factor administration, should also be considered potential etiologies for the relative eosinophilia.

The other case submitted with BM failure and eosinophilia (case 264) involved a male neonate of consanguineous parents with papules over his entire body that became hemorrhagic vesicles upon contact. PB showed anemia and eosinophilia, while marrow studies showed eosinophilia, hypolobated megakaryocytes, and almost no glycophorin A–positive erythroid precursors. Sequencing studies revealed a mutation in the gene encoding the Myb-like, SWIRM, and MPN domains containing protein 1 (*MYSM1*). MYSM1 is an H2A deubiquitinase involved in chromatin remodeling and transcriptional regulation.[73] The essential role of MYSM1 is shown in mouse models, in which deletion of *Mysm1* leads to a significant reduction in BM stem and progenitor cells.[74] Reports of patients with biallelic MYSM1 are limited, but all patients have hematopoietic defects ranging from transient anemia, mild thrombocytopenia, and lymphocyte anomalies to severe BM failure with myelodysplastic features.[75-77] To our knowledge, this is the first association of MYSM1 mutation with eosinophilia.

Eosinophilia-Associated Myeloid Neoplasms Arising From Germline Predisposition

Finally, three of the cases submitted for the conference (cases 74, 12, and 140) were examples of eosinophilia associated with myeloid neoplasms that arose from a germline cancer predisposition, including two affecting adults. The eosinophilia in these cases is somewhat unique, as a significant association between the involved genetic loci and eosinophilia has not been established for any germline alterations within this subclassification of cases. The patient in case 74 was a 37-year-old woman with a germline *GATA2* mutation and a history consistent

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with this alteration, including a *Mycobacterium kansasii* infection, warts, and recurrent skin abscesses.[78] She subsequently developed a MDS characterized by trilineage dysplasia, eosinophilia, and multiple chromosomal aberrations. Germline *GATA2* mutations can either arise spontaneously or are transmitted in an autosomal-dominant fashion. Clinical manifestations are highly variable yet frequently include severe infections (including viral, disseminated nontuberculous mycobacterial and invasive fungal infections), pulmonary dysfunction, sensorineural hearing loss, multiple cutaneous and genital warts, panniculitis, venous thrombosis, lymphedema, and hypothyroidism.[79] Patients have a strong propensity to develop MDS or AML, which may in some cases be the initial manifestation of the disease. Although phenotypic associations of myeloid neoplasms in patients with *GATA2* germline mutations have been described, eosinophilia is not noted.[80]

Case 12 involves a 68-year-old woman with Li-Fraumeni syndrome who developed a CEL upon mutation of the second *TP53* allele. Case 140 describes an 11-month-old girl who developed both a myeloid sarcoma with associated eosinophilia and a concurrent atypical teratoid, rhabdoid tumor in the setting of a germline *SMARCB1* mutation. Both of these cases have been described elsewhere.[81,82]

Germline mutations must be considered when evaluating patients with eosinophilia. These disorders most frequently present in the pediatric population, yet—as evidenced by some of the cases presented in this session—should not be completely discounted in the evaluation of adults with eosinophilia. A complete family history and appropriate genetic testing should be emphasized. Although the number of germline mutations that can lead to eosinophilia is highly diverse, they lead to similar alterations in lymphocyte distribution and overproduction of eosinophil-stimulating cytokines. This pathophysiology is not restricted to germline alterations, as many of the somatic causes of eosinophilia result in a similar skew in lymphocyte differentiation and cytokine dysregulation. Thus, despite their relative rarity, germline disorders with associated eosinophilia provide an excellent framework by which to understand more common eosinophilia etiologies.

Conclusions

- Eosinophilia may result from a broad range of neoplastic and nonneoplastic entities.
- Reactive causes of eosinophilia are by far the most common; in some cases, the diagnosis can be challenging and often leads to bone marrow examination.
- HE in the PB requires demonstration of persistent eosinophilia (>1.5 × 10^9/L) on two separate examinations at least 1 month apart.
- HES requires that a diagnosis of HE be established, and there must also be evidence of organ damage/dysfunction that is directly attributable to the infiltrating eosinophils.
- Idiopathic HE/HES are diagnoses of exclusion.
- Distinction of single-organ disease accompanied by HE (eg, as seen in eosinophilic lung disease) from IHES with extramedullary single-organ involvement can be challenging and in almost all cases requires a multimodality approach, including clinical impression, pertinent laboratory findings (eg, features of rheumatologic disease), and radiology.
- Idiopathic HE/HES, by definition, lack features of a clonal abnormality.
- CEL, NOS is classified as MPN but has clinical, laboratory, and morphologic features partially reminiscent of MDS/MPN. It is a clinically aggressive multisystem disorder, frequently associated with organ damage and constitutional symptoms. Persistent eosinophilia is a dominant feature. BM morphology helps to distinguish it from HES but is currently not a defining WHO diagnostic criterion. Molecular genetic findings can be used to define clonality but with caution; more weight should be given to mutations associated with myeloid neoplasms and at higher allele frequency.
- CEL-like disease presentations in association with AML/MDS, ALL, or lymphoblastic lymphoma strongly suggest one of the myeloid/lymphoid neoplasms with eosinophilia and specific gene rearrangements. Molecular testing is often necessary to identify the genetic fusions seen in these cases. RNA assessment studies might be indicated in the presence of negative karyotype and FISH results.
- Many chronic myeloid neoplasms are still not molecularly defined. Eosinophilia is rare in typical MDS, being more commonly seen as a feature of disease progression, and in classical MPN, except for CML, but is not uncommon in MDS/MPN, for example, CMML, both de novo, therapy related, and JMML. There are still difficulties in separating CEL, NOS from MDS/MPN-U with eosinophilia. In the presence of persistent HE, a diagnosis of CEL, NOS may be preferable in most instances.
- An acute leukemic onset may suggest CBF-AML, blast phase of CML, t(5;14) B-ALL, or one of the myeloid/lymphoid neoplasms with eosinophilia and specific gene

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rearrangements. In AML, the eosinophils are part of the neoplastic clone as opposed to lymphoblastic leukemia, in which the eosinophilia is likely reactive. The presence of eosinophilia and aberrant eosinophilic granules is an important morphologic clue of CBF-AML, especially when a CBF-AML presents with a blast percentage less than 20%.

- More extensive usage of molecular testing will cause a "decrease" in the number of myeloid neoplasms with eosinophilia included in this session, as well as a decrease in cases of CEL, NOS. This will be beneficial in identifying patients who are candidates for targeted treatments (eg, tyrosine kinase inhibitor therapy).
- Germline mutations must be considered in the differential diagnosis of eosinophilia, especially in infants and children, but also with increasingly recognized germline mutations associated with myeloid neoplasms in adults (eg, *GATA2* mutation).
- Family history and additional clinical findings can aid in directing diagnostic genetic studies.
- The spectrum of germline mutations leading to eosinophilia is diverse, yet alterations in lymphocyte development give useful insights into pathobiology, such as the association with increased T_H2 function.

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