AAAAI Work Group Report

American Academy of Allergy Asthma & Immunology

Developing a standardized approach for assessing mast cells and eosinophils on tissue biopsies: A Work Group Report of the AAAAI Allergic Skin Diseases Committee

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Mast cells and eosinophils are commonly found, expectedly or unexpectedly, in human tissue biopsies. Although the clinical significance of their presence, absence, quantity, and quality continues to be investigated in homeostasis and disease, there are currently gaps in knowledge related to what constitutes quantitatively relevant increases in mast cell and eosinophil number in tissue specimens for several clinical conditions. Diagnostically relevant thresholds of mast cell and eosinophil numbers have been proposed and generally accepted by the medical community for a few conditions, such as systemic

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mastocytosis and eosinophilic esophagitis. However, for other mast cell– and eosinophil-associated disorders, broad discrepancies remain regarding diagnostic thresholds and how samples are processed, routinely and/or specially stained, and interpreted and/or reported by pathologists. These discrepancies can obfuscate or delay a patient's correct diagnosis. Therefore, a work group was assembled to review the literature and develop a standardized consensus for assessing the presence of mast cells and eosinophils for a spectrum of clinical conditions, including systemic mastocytosis and cutaneous mastocytosis, mast cell activation syndrome, eosinophilic esophagitis, eosinophilic gastritis/enteritis, and hypereosinophilia/hypereosinophilic syndrome. The intent of this work group is to build a consensus among pathology, allergy, dermatology, hematology/oncology, and gastroenterology stakeholders for qualitatively and quantitatively assessing mast cells and eosinophils in skin, gastrointestinal, and bone marrow pathologic specimens for the benefit of clinical practice and patients. (J Allergy Clin Immunol 2021;■■■:■■■-■■■.)

Key words: *Systemic mastocytosis, cutaneous mastocytosis, biopsy, mast cells, eosinophils, bone marrow, skin, gastrointestinal tract, work group, consensus, allergy, dermatology, pathology, gastroenterology*

Abbreviations used

CEL, NOS:	Chronic eosinophilic leukemia, not otherwise specified
CM:	Cutaneous mastocytosis
CSU:	Chronic spontaneous urticaria
CTCL:	Cutaneous T-cell lymphoma
EoE:	Eosinophilic esophagitis
FISH:	Fluorescent *in situ* hybridization
GI:	Gastrointestinal
HαT:	Hereditary α-tryptasemia
H&E:	Hematoxylin and eosin
HE:	Hypereosinophilia
HES:	Hypereosinophilic syndrome
IBS:	Irritable bowel syndrome
IBS-D:	Diarrhea-predominant irritable bowel syndrome
IHC:	Immunohistochemistry
IHES:	Idiopathic HES
IQR:	Interquartile range
MCAS:	Mast cell activation syndrome
MLNeo:	Myeloid/lymphoid neoplasms associated with eosinophilia
NGS:	Next-generation sequencing
SM:	Systemic mastocytosis
SM-AHN:	SM-associated hematologic neoplasms
WDSM:	Well-differentiated SM
WHO:	World Health Organization

Mast cells and eosinophils are commonly present in human tissue biopsies, but the clinical meaning of their presence, absence, quantity, and quality continues to be researched in homeostasis and disease. Current gaps in knowledge include what constitutes quantitatively relevant increases in mast cell and eosinophil numbers in tissue specimens for several clinical conditions. Diagnostically relevant thresholds of mast cell and eosinophil numbers have been proposed and generally accepted by the medical community for a few conditions, such as mast cells in systemic mastocytosis (SM) and eosinophils in eosinophilic esophagitis (EoE).[1-5] However, for other mast cell– and eosinophil-associated disorders, broad discrepancies remain in diagnostic thresholds and how samples are processed, routinely and/or specially stained, and interpreted and/or reported by pathologists. These discrepancies can obfuscate or delay a patient's correct diagnosis. Moreover, the diagnostic relevance of mast cell and/or eosinophil numbers and features in human biopsy specimens of different sampling locations (skin, gastrointestinal [GI] tract, bone marrow) and disease conditions is often undefined. In addition to the density, the activation status and degranulation of these cells likely have diverse roles in pathophysiology, but how these features should be assessed and interpreted for diagnostic purposes is poorly understood. Although there is an expansive literature pertaining to mast cell and eosinophil involvement in a spectrum of pulmonary disorders and SM, the literature pertaining to mast cell activation syndrome (MCAS) and skin, GI, and respiratory symptoms is less robust. Therefore, a work group was assembled to review the literature and develop a standardized consensus for assessing the presence of mast cells and eosinophils for a spectrum of clinical conditions, including SM and cutaneous mastocytosis (CM), MCAS, EoE, eosinophilic gastritis/enteritis, and hypereosinophilia (HE)/hypereosinophilic syndrome (HES). The intent of this work group is to build a consensus among pathology, allergy, dermatology, hematology/oncology, and gastroenterology stakeholders for qualitatively and quantitatively assessing mast cells and eosinophils in skin, GI, and bone marrow pathologic specimens for the benefit of clinical practice and patients. We first discuss general principles in evaluating human biopsies for mast cells and eosinophils for clinical purposes and then tissue-specific recommendations by the location of tissue sampling (skin, GI tract, bone marrow).

GENERAL PRINCIPLES IN EVALUATING HUMAN BIOPSIES FOR MAST CELLS AND EOSINOPHILS FOR CLINICAL PURPOSES

Mast cells have differing morphology depending on whether they are nonneoplastic or neoplastic. Nonneoplastic mast cells are generally round cells with a central round nucleus and relatively abundant granular cytoplasm, but they may have somewhat different phenotypes in different tissues and different locations (eg, mucosa, submucosa). Mast cell granules are not very conspicuous on hematoxylin and eosin (H&E)–stained slides and thus can be missed if cells are individually dispersed; after they start forming aggregates, their recognition becomes easier. In contrast to nonneoplastic mast cells, which are usually individually scattered in tissues, neoplastic mast cells tend to form multifocal dense aggregates of >15 cells (see the World Health Organization [WHO] major criterion for SM in Table I), are more likely to be spindle-shaped, and may have decreased to absent granule content. Specific morphologic alterations of mast cells seen on bone marrow aspirate smears in mast cell disorders have been previously described.[6] For a detailed description of

TABLE I. 2016 WHO classification criteria for mastocytosis

- Cutaneous mastocytosis
- Systemic mastocytosis
- Mast cell sarcoma (localized mast cell tumors)

For SM, there are 2016 WHO criteria for diagnosis and subclassification.

Diagnostic criteria

Major: Multifocal dense infiltrates of mast cells in bone marrow biopsies and/or sections of other extracutaneous organs

Minor:

Twenty-five percent of all mast cells are atypical on bone marrow smears or are spindle-shaped in mast cell infiltrates detected on sections

KIT point mutation at codon 816 in the bone marrow or another extracutaneous organ

Mast cells in bone marrow or blood or another extracutaneous organ exhibit CD2 and/or CD25*

Baseline serum tryptase level ≥20 ng/mL (in case of an unrelated myeloid neoplasm, this is not valid as an SM criterion)

To diagnose SM, 1 major and 1 minor or 3 minor criteria should be met.

Subclassification of SM

Indolent SM (low mast cell burden, no C findings; see below)

Smoldering SM (high mast cell burden, ≥ 2 B findings; see below)

SM-AHN

Aggressive SM (≥1 C finding; see below)

Mast cell leukemia (diffuse infiltrate, >20% mast cell in bone marrow aspirate that are atypical and immature, ± circulating mast cells)

B findings: Indicate a high burden of mast cells and expansion of the neoplastic process into multiple hematopoietic lineages, without visible impairment of organ function

Mast cell infiltration grade in the bone marrow >30% by histology and the basal serum tryptase level is >200 ng/mL

Hypercellular bone marrow with loss of fat cells, discrete signs of dysmyelopoiesis without substantial cytopenias or WHO criteria for an MDS or MPN

Organomegaly: palpable hepatomegaly, palpable splenomegaly, or palpable lymphadenopathy (on CT or ultrasound: >2 cm) without impaired organ function

C findings: Are indicative of organ damage produced by mast cell infiltration (should be confirmed by biopsy if possible)

Cytopenia(s): ANC <1,000/μL or hemoglobin <10 g/dL or platelet count <100,000/μL

Hepatomegaly with ascites and impaired liver function

Palpable splenomegaly with associated hypersplenism

Malabsorption with hypoalbuminemia and weight loss

Skeletal lesions: large-sized osteolysis with pathologic fractures

Life-threatening organ damage in other organ systems that is caused by local mast cell infiltration in tissues

*Of note, CD25+ mast cells can appear in JAK2 myelodysplastic syndrome or FIP1L1-PDGFRA hypereosinophilic syndrome (HES) mutations and serum baseline tryptase levels >20 ng/mL can be seen in hereditary alpha tryptasemia (HαT) or advanced renal failure.

histologic features and criteria for neoplasms of mast cell lineage, see past and current WHO books and review articles.[7-12]

Mast cells can be stained histochemically or immunohistochemically; however, using immunohistochemical stains is recommended. Regarding histochemical staining, mast cell granules stain metachromatically with toluidine blue and Giemsa, orange-red with chloroacetate esterase (also known as Leder stain), and intense purple with pinacyanol-erythrosinate.[13-15] However, histochemical stains suffer from low sensitivity (eg, mast cell subtypes have been reported to be chloroacetate esterase–negative) and specificity (chloroacetate esterase stains other granulocytes); thus in clinical practice, these histochemical stains have been largely replaced by immunohistochemical stains, namely CD117/KIT and mast cell tryptase.[16] CD117/KIT, a surface receptor that is highly sensitive for detecting mast cells, is involved in mast cell development and survival. However, KIT is also involved in the development of germ cells, melanocytes, hematopoietic stem cells, and interstitial cells of Cajal in the GI tract; thus, KIT is not a specific marker for mast cells. Therefore, KIT expression needs to be interpreted in the appropriate clinical and histological context. In contrast, immunohistochemistry (IHC) for mast cell tryptase is a granular cytoplasmic stain that has great specificity for mast cells. However, its expression is more variable than that of CD117/KIT; for example, in SM, neoplastic mast cells have less cytoplasm and fewer granules and thus may completely lose expression of tryptase.[3] Thus, in many clinical contexts, it is recommended to use both immunohistochemical stains, CD117/KIT and mast cell tryptase, to obtain optimal sensitivity and specificity or, if cost limitations exist, first to screen with the more sensitive stain, CD117/KIT, and second to confirm with the more specific one, mast cell tryptase.

Mast cell clonality is usually conferred by activating mutations in the *KIT* gene, which lead to enhanced downstream signaling, including the PI3K/AKT, JAK/STAT, and RAS/MEK/ERK pathways, and confer resistance to apoptosis and increased proliferation. However, *KIT*.D816V is a relatively "weak driver" mutation and is unable to transform a stem cell clone into a full-blown malignancy by itself. Clonality can be assessed using expression of CD25 and/or CD2 as surrogate markers, but CD25 is recommended. CD25 expression, assessed by IHC or flow cytometry (most commonly performed as part of bone marrow evaluation), is a relatively specific and sensitive marker of clonality.[16] Conversely, aberrant CD2 expression on mast cells can be challenging to interpret by IHC, especially when these aberrant mast cells do not demonstrate atypical clustering; CD2 is normally expressed on both T-cell lymphocytes and natural killer cells, making it difficult to differentiate scattered mast cells with aberrant CD2 expression from these other cells normally expressing CD2. One approach, albeit more subjective, is to perform IHC staining with CD3 in parallel with CD2 and compare levels of CD2- and CD3-positive cells. However, because CD25 is positive in almost all instances[5] and is easier to interpret, CD25 is the recommended marker to assess mast cell clonality by IHC. CD2 may be more relevant when using flow cytometry than when using IHC, as

TABLE II. Immunohistochemical markers used in evaluating mastocytosis

Marker	Interpretation
*CD117/KIT	Sensitive, but not specific, marker of mast cells with membranous staining pattern.
*Tryptase	Specific, but not sensitive, marker of mast cells with granular cytoplasmic pattern. Staining can be patchy or even negative in neoplastic mast cells. However, it is highly sensitive for identifying over 95% of nonneoplastic human body mast cells.
**CD25	Aberrantly expressed in neoplastic mast cells and thus can be used as surrogate of clonality. Mast cell expression of CD25 fulfills a minor WHO criterion for SM. CD25 is normally expressed in a subset of T cells.
CD2	Aberrantly expressed in neoplastic mast cells (smaller subset than CD25) and thus can be used as surrogate of clonality. However, CD2 is expressed in all T cells, and thus interpretation (confirming that CD2 is expressed on mast cells) can be difficult by IHC. Mast cell expression of CD2 fulfills a minor WHO criterion for SM.

*Given the high sensitivity and low specificity of CD117 IHC and the low sensitivity and high specificity of Tryptase IHC, it is recommended to perform both tryptase and CD117 IHC to detect all mast cells and distinguish them from other cell types.
**CD25 IHC is recommended to identify clonal mast cells.

flow cytometry technology more accurately distinguishes the specific cell types being assessed. In addition, other markers may be useful for specific conditions; for instance, CD30 is aberrantly expressed on mast cells in subsets of SM importantly including well-differentiated SM (WDSM), a condition in which other minor criteria are often not met.[17-19] While initial studies have suggested CD30 is preferentially expressed in cases of advanced SM, other studies have shown its expression on more indolent forms and thus it is not considered useful for grading disease severity. However, particularly due to its expression on WDSM where CD25 is often negative, using CD30 in combination with CD25 has been shown to increase the diagnostic accuracy of SM.[20] Thus, CD30 has recently been proposed as an additional minor diagnostic criterion of SM.[21] The main immunohistochemical markers and their interpretation are summarized in Table II.

Although immunohistochemical stains are widely used to confirm mast cell lineage and to assess their clonality, assessing cell density in tissue to help diagnose mast cell–related diseases has been beset with limitations, including variable use of histochemical versus immunohistochemical stains; format of reported microscopic data (per hpf vs per mm^2), microscopic field size (lack of standardization among microscopes), and hpf magnifications (eg, 200×, 250×, 400×); and use of average versus peak mast cell density counts.[22] Generally in normal tissues, histochemical stains are less sensitive for mast cells and yield lower mast cell densities than do immunohistochemical stains (Tables III and IV).[23-35] Thus, it is generally recommended that immunohistochemical stains (CD117/KIT, mast cell tryptase) should be used to assess mast cell density in tissue sections. Other considerations for assessing mast cell density include the variable section thickness and specific tissue areas examined (eg, deep vs superficial dermis, bowel epithelium vs lamina propria).

Variability in reported microscopic parameters is a substantial challenge that limits the utility of data to advance the field and clinical practice. Collectively, published studies (as summarized in Tables III and IV) evidence broad variability in cell density values because of cell density being expressed schismatically as "per mm^2" or "per hpf" in individual studies and because of frequent incomparability of "per hpf" among studies due to individual microscopes having different field sizes and magnifications. For instance, the same sample viewed on 2 different microscopes with the same magnification would yield a lower mast cell density "per hpf" for the microscope with the smaller microscopic field; however, these 2 values would be incomparable when reported as "per hpf" unless the field size and magnification were also reported. Even then, the reader would need to convert the published data to "per mm^2" to compare results among studies. The most commonly used microscopic combination is a 400× magnification with a field diameter of 0.55 mm and thus an area ($A = \pi r^2$) of 0.24 mm^2; however, other studies use 200× or 250×. Even within the studies using 400× magnification, the field areas may vary among 0.12, 0.2, 0.24, 0.3, or 0.44 mm^2. Therefore, the mast cell density when expressed as "per hpf" cannot be assumed to be comparable among studies; only when the magnification and area of the microscopic field are provided can values be converted into a standardized per mm^2 measurement by the reader and thus be comparable. It is thus critical that researchers and practicing pathologists provide all the necessary microscopic reading information in their reports (microscopic field size and magnification) so that a standardized conversion factor can be determined to provide homogeneous data. Though it is currently the practice to express density as "per hpf," there will likely be a transition in the future to "per mm^2" for standardization purposes and due to the growth of digital pathology, in which round fields are no longer relevant. It is recommended that, when feasible, investigators use "per mm^2" or provide equivalent "per mm^2" data within supplemental materials to facilitate broadly comparable results.

Similar to the lack of standardized reporting in microscopic parameters limiting comparability and utility of cell density results, variability in reporting either the mean or peak cell density also limits comparability and utility of study results. In some instances, it is not even specified whether the provided count is mean or peak, and if mean, how many fields were counted and how they were chosen (random vs continuous vs fields where tissue fills the entire field, etc). This lack of standardization hampers our ability to compare studies and determine diagnostically relevant cutoffs. Studies are needed to determine the approach with the least interobserver variability and most clinical relevance. Until then, it is recommended that both mean and peak are reported.

Both eosinophils and mast cells may degranulate when activated, leading to functional outcomes; however, this degranulation also affects our technical ability to detect and count cells in tissue biopsies. For instance, IHC for eosinophil granule proteins identified extracellular deposited content of eosinophils even in situations in which intact eosinophils were not seen.[36-41] Though clinically meaningful cutoffs have been established for EoE despite the possible limitations posed by degranulation, this caveat still needs to be considered in other eosinophil-mediated and mast cell–mediated diseases. Thus, if the index of suspicion is high, but eosinophils are not conspicuous (eg, when glucocorticoid

TABLE III. Mast cell density by tryptase IHC in normal skin

Region	n	Mast cells/mm^2		Mast cells/hpf*	
		Mean	SD	Mean	SD
Trunk[23]	19	ND	ND	10.2 6†	1.9 1.2†
Trunk[24]	8	ND	ND	12.5	3
Trunk[25]	17	78.6	31.5	11.8	4.7
Upper arm[25]	44	76.5	32.7	11.5	4.9
Forearm[25]	27	101.2	32.6	15.2	4.9
Upper leg[25]	29	74.9	38.8	11.2	5.8
Lower leg[25]	24	113.2	46.7	17.0	7.0

Data were modified from published work as noted.
ND, Not determined (the area of hpf was not reported in these studies; thus, density per mm^2 cannot be accurately determined).
*Converted values from Janssens et al[25] assuming an hpf area of 0.15 mm^2.
†Toluidine blue.

treatment was started prior to biopsy), ancillary testing with IHC for eosinophil granule proteins can be considered (eg, major basic protein, eosinophilic cationic protein, eosinophil-derived neurotoxin, eosinophil peroxidase). Similarly, recent studies have shown striking differences in levels of mast cell degranulation in diseased esophagus (specifically achalasia), prompting consideration for assessment of mast cell degranulation on tryptase-stained slides.[42] Finally, an experienced pathologist should review slides to ensure that crush artifact and nonspecific staining are not misinterpreted as degranulation. Additional research is needed to clarify the role of eosinophil and mast cell degranulation in evaluating biopsies for diagnostic purposes.

Depending on the patient's health care network's electronic medical record system, the pathologist evaluating the specimen may not have full access to clinical information. Thus, the referring physician should clearly communicate the patient's clinical history and diagnostic considerations to the pathologist.[43] For instance, for suspected SM, any clinical information critical to the diagnosis and classification of SM should be provided because the final diagnosis depends on a clinicopathologic correlation. Specifically, clinical signs and symptoms, serum tryptase level, presence or absence of organomegaly (spleen, liver, other), and any signs of organ dysfunction should be communicated by the referring physician to the pathologist, as this information comprises the B and C criteria (Table I)[10,44] for WHO SM guideline classification. Having more complete clinical information can help the pathologist determine between differential causes and evaluate the specimens within the relevant clinical context; for example, elevated levels of serum tryptase may not be due to increased mast cell numbers alone, but rather due to excess secretion of protryptases due to increased copy number of a gene that encodes α-tryptase *TPSAB1*, as seen in hereditary α-tryptasemia (HαT), or to degranulation of mast cells releasing mature tryptases, as seen in anaphylaxis.[45,46] Furthermore, depending on the institution, the pathologist evaluating the biopsy may or may not have additional training and certification in subspecialty pathology (hematopathology, dermatopathology) or expertise with rare mast cell– and eosinophil-associated diseases. Sending the specimen for an expert pathologist consultation, when available, should be considered. In this case, it is even more essential to provide pertinent clinical information or personally speak with the specialized consultant, who usually will not have access to any medical records from the referring institution. Critical information that should be provided to the pathologist includes (1) demographic information, including age, sex, and ethnicity; (2) clinical history, including organomegaly (spleen, liver, other) and signs of organ dysfunction: they comprise B and C criteria (Table I) of the WHO guideline for SM classification; (3) laboratory values, including serum tryptase, 24-hour urine measurements for methylhistamine, leukotriene E, or prostaglandin F_2-α; (4) all diagnoses, including working diagnosis, short differential diagnosis, and/or any diagnoses to be specifically evaluated and/or eliminated or to provide appropriate context to the pathologist; (5) biopsy sampling location (anatomic location, lesional vs nonlesional tissue); (6) differential diagnosis– or targeted therapy–specific stains and analyses to be requested or discussed with the pathologist.

TISSUE-SPECIFIC RECOMMENDATIONS BY THE LOCATION OF TISSUE SAMPLING: SKIN, GI TRACT, BONE MARROW

We discuss tissue-specific recommendations by the location of tissue sampling (skin, GI tract, bone marrow) and provide summary recommendation statements.

Skin

Skin biopsy: Indications and technique. Few skin diagnoses seen in the allergy/immunology clinic have pathognomonic findings on skin biopsy. If a cutaneous malignancy is suspected, skin biopsy is advisable and should be repeated at an alternative site (or sites) if the concern for malignancy persists despite a negative biopsy. A skin biopsy can be considered to support the diagnosis of a variety of common clinically diagnosed conditions as summarized in Table V.[47-53] An example of skin biopsies with different staining methods illustrating histologic features that confirm a diagnosis of mastocytosis in the skin are shown in Fig 1, *A*.[47] However, in most cases, it is advisable to discuss with patients the likelihood that a skin biopsy will not lead to a specific diagnosis, as many allergic skin disorders have similar histopathology.

There are various methods for obtaining a skin biopsy and multiple factors that should be considered in selecting the most appropriate biopsy technique. Among the most important factors are location of the lesion and how deep and wide of an excision should be taken. Biopsies are categorized as incisional, in which only a portion of a lesion is sampled, or excisional, in which the

TABLE IV. Normal mast cell counts in GI tract from varied articles and abstracts

Stain	Count method	Data reported as	Location(s), count	Adult vs pediatric	Comment	Reference
Tryptase CD117 CD25 CD30	400× magnification hpf (field size, 0.25 mm^2)	Mean ± SD (range)	Colon, 19 ± 6.1 (7-39)	Adult	IBS and MCAS vs controls	Doyle et al[4]
Tryptase CD117 CD25	400× magnification hpf (field size, 0.25 mm^2)	Mean (range)	Stomach, 12 (5-21) Duodenum, 27 (4-51) Terminal ileum, 32 (21-40) Colon, 21 (10-31) (Tryptase or CD117)	Adult	SM vs controls and varied abnormal diagnoses (IBD, eosinophilic gastrointestinal disorder, celiac disease, etc)	Hahn et al[26]
Giemsa	400× magnification hpf (field size, 0.55 mm^2)	Mean (range)	Antrum, 0.3 (0-2) Stomach body, 0.3 (0-7) Duodenum, 0.3 (0-7) Duodenal cap, 0.04 (0-1) Ileum, 0.9 (0-11) Cecum, 0.5 (0-7) Ascending colon, 0.2 (0-3) Transverse colon, 0.3 (0-3) Descending colon, 0.4 (0-7) Sigmoid, 0.3 (0-3) Rectum, 0.6 (0-5)	Pediatric	Normal control	Chernetsova et al[27]
Tryptase	400× magnification hpf (field size, 0.28 mm^2)	Mean ± SD (maximum)	Lamina propria Cecum, 17.4 ± 7.4 (32) Ascending colon, 15.3 ± 4.9 (22) Transverse colon, 15.9 ± 8.2 (31) Descending colon, 17.6 ± 7.3 (32) Rectosigmoid, 14.5 ± 6.4 (31) Crypt epithelium Cecum, 0.3 ± 0.5 (2) Ascending colon, 0.2 ± 0.4(1) Transverse colon, 0.04 ± 0.2 (1) Descending colon, 0.02 ± 0.2 (1) Rectosigmoid, 0.2 ± 0.4 (1)	Pediatric	Normal control	Saad[28]
Tryptase CD117	400× magnification hpf (field size, 0.28 mm^2)	Mean ± SD (maximum)	Distal esophagus, 0.18 ± 0.31 (3) Stomach body and antrum (surface or glandular epithelium) (2) Stomach body and antrum (lamina propria), 11.5 ± 4.3 (29) Duodenal villous (lamina propria), 3.5 ± 2.4 (18) Duodenal intercryptal (lamina propria), 14.5 ± 4.5 (36) Terminal ileum villous (lamina propria), 3.4 ± 1.5 (19) Terminal ileum intercryptal (lamina propria), 16.1 ± 6.7 (42) Cecum/ascending colon LP (19.4) Transverse colon, 17.9 ± 4.4 Descending colon, 17.7 ± 6 Sigmoid/rectum, 12.7 ± 4.3 CD117 stain demonstrated similar counts throughout the GI tract	Pediatric	Normal control	Tison et al[29] (abstract)
Tryptase	Percent volumetric	Percentage of area ± SD	Distal colon, 3.3 ± 0.8	Adult	IBS vs controls. Complex volumetric evaluation not suitable for day-to-day use.	Barbara et al[30]

(Continued)

TABLE IV. *(Continued)*

Stain	Count method	Data reported as	Location(s), count	Adult vs pediatric	Comment	Reference
CD117	400× magnification (1 mm^2 = 6.249 hpf)	Mean ± SD	Jejunum, 15.3 ± 4.4	Adult	IBS-D vs controls Atypical collection method (Watson's capsule)	Guilarte et al[31]
Tryptase	20× magnification (mm^2)	Mean ± SD	Caecum, 0.55 ± 0.14	Adult	IBS vs normal control	O'Sullivan et al[32]
Uranyl nitrate azure A	400× magnification mm^2	Mean ± SEM	Lamina propria Ileum, 211.05 ± 8.4 Colon, 80.35 ± 2.95 Rectum, 40.5 ± 2.6 Submucosa Ileum, 84.37 ± 2.2 Colon, 86.78 ± 3.26 Rectum, 98.2 ± 3.4	Adult	IBD vs normal control	Lloyd et al[33]
Azure A	400× magnification	Mean ± SD	Rectal, 2.3 ± 1.1	Adult	UC vs normal control	Sarin et al[34]
Tryptase Toluidine blue	400× magnification	Mean	Colon Tryptase, 14.2 ± 3.4 Toluidine blue, 7.1 ± 2.4	Adolescent/adult	Chronic diarrhea vs normal control	Zare-Mirzaie et al[35]

IBD, Inflammatory bowel disease; *LP*, lamina propria; *UC*, ulcerative colitis.

entire lesion is removed. An incisional biopsy can be performed as a shave, scissor, curettage, or punch specimen. An excisional biopsy is usually performed as a full-thickness scalpel excision or with a deeper oblique (shave or scoop) excision. An incisional punch biopsy is appropriate in most skin disorders seen in an allergy/immunology clinic, as it allows visualization of the epidermis, dermis, and subcutaneous tissue. In some circumstances, such as differentiating between toxic epidermal necrolysis and staphylococcal scalded skin syndrome, a shallower incisional biopsy will suffice. For most conditions, biopsy of the active lesion is appropriate; however, with bullous lesion it is recommended to biopsy the edge of the lesion for light microscopy and the nonlesional skin for immunofluorescence microscopy.[54-56] Maximizing diagnostic information from skin biopsy involves discussion with the reading dermatopathologist to prevent diagnostic error.[57]

Overall skin biopsy indications and technique recommendation. The majority of dermatoses seen in allergy clinic have no specific histologic findings on skin biopsy; therefore, patients should be counseled on the likelihood that a skin biopsy will not lead to a specific diagnosis.

Skin biopsy: Interpretation. As mentioned, most of the common dermatoses (eg, atopic dermatitis, allergic contact dermatitis, irritant contact dermatitis, most drug eruptions, chronic spontaneous urticaria) seen in an allergy/immunology clinic have no specific histologic findings on skin biopsy.[58-60] For instance, the presence or absence of mast cells, neutrophils, and/or eosinophils in biopsied wheals is neither sensitive nor specific to the diagnosis of chronic spontaneous urticaria;[61] however, urticarial vasculitis can be distinguished from chronic spontaneous urticaria by H&E staining because a urticarial vasculitis biopsy demonstrates leukocytoclastic vasculitis of the small vessels characterized as vascular damage caused by nuclear debris from infiltrating neutrophils.[62] Furthermore, rashes that can be caused by a drug have overlapping histopathology with infectious, autoimmune, inflammatory, or malignant etiologies. Dermatopathology reports should be written in a way to reflect these uncertainties. A solitary diagnosis should only be included if the biopsy is diagnostic for that disorder. Alternately, descriptive histopathology, including the predominant pattern of injury with or without a differential diagnosis can be presented.

Healthy human skin contains mast cells most prominently found around blood vessels and adnexa.[23] However, few studies have attempted to quantify the upper limit of mast cell numbers in normal human skin. Among the studies performed, older studies used toluidine blue and other histochemical stains that are less sensitive and specific than immunohistochemical staining.[23] In more recent studies summarized in this review, mast cell numbers have been reported as mast cells/hpf or as mast cells/mm^2. As discussed in the general principles and considerations, the size of hpf varies among microscopes; thus, the use of area in mm^2 (calculated for each microscope from the field diameter) is recommended to facilitate comparability of results between and among studies.[63] We summarize the findings of studies using skin biopsy samples in healthy and diseased human skin as follows. O'Neill et al[23] reported an average of 10.2 ± 1.9 (mean ± SD) mast cells/hpf (400×) with a range of 4 to 16 mast cells in truncal skin from 19 adults undergoing excision for the removal of a previously biopsied neoplasm. Ribatti et al[24] reported 20 ± 5 mast cells/hpf (250×), which is approximately 12.5 ± 3 mast cells/hpf (400×). Durham et al[64] found no statistical difference between the numbers of mast cells in normal skin from 11 and 12 adult subjects with and without atopy, respectively, when comparing medians and interquartile ranges (IQR) of mast cells/hpf in extensor forearm biopsies; normal skin contained a median of 11.2 (IQR, 8.8, 14.9) mast cells/hpf (400×) in patients with atopy and 11.4 (IQR, 9.1, 14.6) mast cells/hpf (400×) in patients without atopy. Minimal variation of mast cell numbers in skin have been seen between sexes and among different age groups.[23,63] Janssens et al[25] reported that normal adult skin contains approximately 75 to 113 mast cells/mm^2 (mean values) depending on the biopsy site, which converts to 12 to 17 mast cells/hpf (assuming an area of 0.15 mm^2). The variability of mast cell density by skin sampling location was previously reported using toluidine blue staining;[63] however, these studies were small and suffered from population

TABLE V. For a given "working diagnosis," suggested information to give to the dermatopathologist and findings that may facilitate a diagnosis

Working diagnosis	Signs/symptoms	Specific requests	Pathologic findings*
Atopic dermatitis, atypical	Lesions typical of atopic dermatitis but without pruritus	R/O T-cell lymphoma T-cell receptor clonality Expression of T-cell–specific antigens (consider flow cytometry if available)	Spongiotic dermatitis pattern of injury with or without eosinophils and mast cells
Autoinflammatory disorders with skin involvement	Difficult to treat urticaria with systemic symptoms (fever, malaise) and elevated CRP (USA) or PCR (not USA)	Assess for evidence of autoinflammatory disease	Variable depending on autoinflammatory condition and not necessarily definitive
Contact dermatitis, atypical	Unusual distribution Poor response to standard therapy Suspect drug reaction	Consider contact dermatitis vs drug reaction	Spongiotic dermatitis pattern of injury Eosinophil and neutrophilic infiltrates are more suggestive of a drug reaction Mononuclear infiltration is more suggestive of atopic dermatitis
Cutaneous mastocytosis/ urticaria pigmentosa	Positive Darier sign	Tryptase, CD117 ± CD25 staining; optional: D816V mutation	Sheets/clusters of mast cells Aberrant expression of CD25 by mast cells if neoplastic Positive finding of the D816V mutation
Drug hypersensitivity reactions/DRESS	Skin and multiorgan involvement	R/O DRESS	Highly variable Perivascular infiltration with lymphocytes, eosinophils, neutrophils and atypical lymphocytes
Eczema herpeticum/Kaposi varicelliform eruption	Skin lesions with vesicles High concern but with recent negative swab for virus	PCR and viral culture for Zoster	Positive PCR and positive viral culture
Eosinophilic fasciitis	Symmetrical swelling and induration	Standard H&E stain	Thickening of the fascia Eosinophilic infiltrate
Eosinophilic cellulitis (Wells syndrome)	Markedly edematous plaques and nodules	Standard H&E stain	Eosinophilic infiltrate
Grover disease (transient acantholytic dermatosis)	Severe pruritic papules or nodules and/or bullous lesions	Standard H&E stain	Epidermal acantholysis with or without dyskeratosis
Herpetiform dermatitis	Skin lesions with vesicles	Immunofluorescence for IgA	Subepidermal blister with neutrophils Evidence of deposition of IgA
Neutrophilic urticaria with or without IgM monoclonal gammopathy (Schnitzler syndrome)	Nonpruritic burning wheals	Semiquantitation of the identity of inflammatory cells R/O neutrophil predominance	Neutrophil predominance
Prurigo nodularis	Patients with chronic itching/ neurodermatitis	Lichen planus-like lesions with epithelial reaction	Hyperkeratosis and acanthosis with a nonspecific inflammatory infiltrate
Seborrheic dermatitis, atypical	Scaling scalp dermatitis nonresponsive to usual treatment	Distinguish severe seborrheic dermatitis from scaling psoriasis	Inflammation of scalp psoriasis vs seborrheic dermatitis
T-cell lymphoma/mycosis fungoides	A more mature adult patient with difficult to treat atopic dermatitis	T-cell receptor clonality by PCR Loss of expression of T-cell specific antigens: CD2, CD3, CD5, and CD7 (by IHC or flow cytometry)	Epidermotropic infiltration of atypical T cells T-cell receptor clonality Loss of expression of T-cell–specific antigens: CD2, CD3, CD5, and CD7
Vasculitis, urticarial, or other	Urticarial lesions that last >24 h, leave a bruising lesion, and are more painful than pruritic	Immunofluorescence for IgG and complement	Leukocytoclastic vasculitis

CRP, C reactive protein; *DRESS*, drug reaction with eosinophilia and systemic symptoms; *R/O*, rule out. For consideration of the following diagnoses, recommend referral to a dermatologist: bullous pemphigoid, pemphigus foliaceus, ichthyosis vulgaris, dyskeratosis follicularis, pityriasis rubra pilaris, neuromucocutaneous disease/Melkersson-Rosenthal syndrome, and other diagnoses not mentioned.

*Here are definitions for some pathologic findings: *acantholysis*, keratinocyte separation due to disruption of their junctions; *acanthosis*, thickening of spinous layer of the epidermis; *dyskeratosis*, necrotic or apoptotic keratinocytes; *hyperkeratosis*, thickening of surface keratin layer of the epidermis; *spongiosis*, widened spaces between keratinocytes.

homogeneity. On the basis of the modified data from Janssens et al[25] (Table III), a working cutoff value of <31 mast cells/hpf (400×) was proposed as a conservative estimate of the maximum density of mast cells in normal adult patient skin (>97th percentile). However, further studies are required to confirm this cut point.

It is encouraged that increases in mast cell density be interpreted in the context of the patient's clinical history and that this estimate

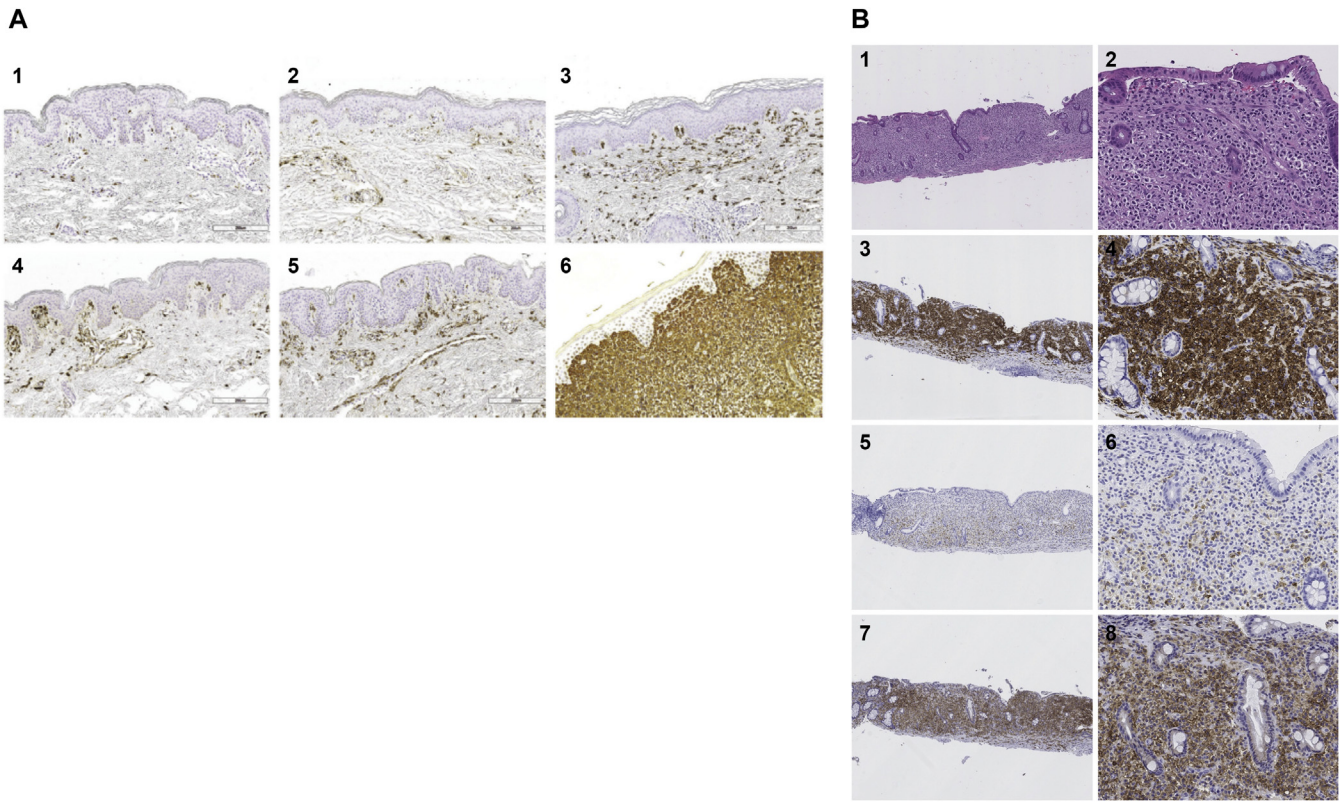

FIG 1. (A, 1-6) Histologic features of CM. Compared with healthy skin **(1)**, dermal mast cell numbers are increased in skin from patients with CM **(2-6)** and are shown here stained with tryptase antibody. Mast cell numbers vary greatly from patient to patient. Monomorphic, adulthood-onset, maculopapular CM **(2, 3)** is typically associated with a less pronounced increase in mast cell numbers than that of patients with polymorphic, childhood-onset, maculopapular CM **(4, 5)**. Mast cell numbers are particularly increased in patients with diffuse CM **(6)**. Reproduced with permission from Hartmann et al.[47] **(B, 1-8)** Patient with systemic mastocytosis involving the gastrointestinal tract. Biopsy of colon shows diffuse infiltration by mast cells **(1, 2)**, which are positive for CD117 **(3, 4)** and CD25 **(7, 8)**, and weakly positive for tryptase **(5, 6)**. Magnifications are 10× **(1, 3, 5, 7)** and 40× **(2, 4, 6, 8)**.

be continually reassessed as new research evolves. To reach a diagnosis, the distribution (scattered vs clusters/sheets), shape (round vs spindle), aberrant antigen expression (eg, CD25) of mast cells, and associated findings (eg, basal hyperpigmentation in urticaria pigmentosa) are more important than specific mast cell counts. If clinical suspicion is high, repeat biopsies may be indicated because the infiltrate is sparse in some instances. This is particularly true for telangiectatic lesions of CM (previously termed telangiectasia macularis eruptiva perstans), which can present with subtle increases in mast cells around slightly dilated superficial blood vessels. Although telangiectasia macularis eruptiva perstans was not included as a separate entity in the most recent guideline for CM, it is characterized as reddish-brown, maculopapular, telangiectatic lesions of 2- to 6-mm in size and located on the trunk and extremities. It occurs almost exclusively in adults and is uncommonly seen in patients with SM.[65]

Skin biopsy interpretation recommendation. Few studies have attempted to quantify the upper limit of mast cell numbers in normal human skin, but based on limited data, a working cutoff value of <31 mast cells/hpf (400×) is proposed as a conservative estimate of the maximum density of mast cells in normal skin (>95th percentile) in adult patients. This measurement should be performed in the dermis; at the current time, there is insufficient evidence to more precisely define the recommended depth and area for the measurement.

Skin biopsy: Stains and analyses. Specific stains and analyses can be performed on skin biopsies, depending on the differential diagnosis, which is information that we recommend that the physician should provide to the pathologist (see further discussion in the general principles and guidelines). Below we discuss indications and caveats of specific stains and analyses for mast cell and eosinophil assessment in skin biopsies.

Tryptase and/or CD117/KIT. Tryptase and/or CD117/KIT can confirm and highlight mast cells.

CD25. As in other locations, expression of CD25 on mast cells is a marker of their neoplastic rather than reactive nature and is predictive of SM in adults.[66,67] However, CD25 is not a consistent marker for mutated mast cells in skin, especially in children's biopsies for mastocytosis in the skin, in which less than 25% of mast cells were CD25 positive.[68]

Fungal stains. Fungal stains, such as periodic acid–Schiff or Grocott's methenamine silver, may be indicated if a cutaneous fungal infection is suspected and a direct KOH stain is not revealing.[68]

Immunofluorescence. Immunofluorescence is indicated if cutaneous vasculitis, bullous skin disease (eg, pemphigoid,

linear IgA bullous dermatosis), dermatomyositis, or another pathologic process mediated by antibodies or complement is suspected.[69,70] Tissue obtained for direct immunofluorescence should be placed in Michel's medium or Zeus medium (not formalin) for preservation or directly frozen.[70,71] Discussion with the dermatopathologist will ensure that an appropriate method is used.

KIT *D816V mutation.* Assessing mutational status on the skin specimen is not part of the routine evaluation for CM or SM. If there is concern about mastocytosis in the skin with clonality, analysis for the *KIT* D816V mutation can be ordered on a formalin-fixed, paraffin-embedded block of the skin biopsy through commercial laboratories licensed under the Clinical Laboratory Improvement Amendments of the 1988 statute. In a study of 29 patients with SM and evidence of mastocytosis in the skin, 100% had the *KIT* D816V mutation in skin.[72]

T-cell studies. If there is concern about cutaneous T-cell lymphoma (CTCL), it may be helpful to assess T-cell lymphocyte cell receptor clonality by PCR and to examine the tissue for loss of expression of T-cell lymphocyte–specific antigens, such as CD2, CD3, CD5, and CD7.[73] As specific recommendations may change over time, it is prudent to communicate with the reading dermatopathologist in highly suspicious cases.

Skin biopsy stains and analyses recommendation. Specific stains and analyses should be performed, depending on the differential diagnosis, which the physician should communicate to the dermatopathologist along with other recommended information (discussed further in the "General principles in evaluating human biopsies for mast cells and eosinophils for clinical purposes" section).

Skin biopsy: Site-specific and time-specific results. A skin biopsy is limited to 1 location obtained at 1 point in time on a patient's body. A newly evolving understanding of resident, noncirculating immune cells in human skin emphasizes that any inference about the presence or absence of similar cells in other lesional or nonlesional biopsy sites cannot be made on the basis of results of 1 biopsy site.[74] Local factors can also allow for recirculating immune cells to preferentially exit vessels into certain skin areas and not others. Therefore, results from a skin biopsy should be interpreted specifically with respect to the biopsy site and not be applied broadly to other areas of the body, particularly if only 1 lesion was biopsied.

Notably, skin biopsy results may differ over the course of a disease, and this time specificity of findings should be considered by physicians and pathologists. For instance, one of the most common reasons eczematous lesions are biopsied is to eliminate the differential diagnosis of CTCL because nonspecific eczematous inflammation can be seen in early CTCL. If clinical suspicion for CTCL remains, repeating the biopsy to assess for T-cell lymphocyte clonality and loss of T-cell lymphocyte–specific markers should be considered, as the condition may no longer be within the early stage of CTCL.

Skin biopsy site-specific and time-specific results recommendation. Results from a single skin biopsy should be interpreted specifically on the basis of the biopsy site location and not be applied broadly to lesions in other parts of the body, and physicians and pathologists should consider the differential findings and methods over the course of a suspected disease.

Skin biopsy: Eosinophils in human skin. Eosinophils do not reside in the extravascular space of healthy human skin; however, skin eosinophilia is a nonspecific finding in a wide variety of syndromes, meaning that their mere presence in the human skin is not diagnostic for any particular skin disorder nor can it differentiate whether a skin process is allergic or parasitic in nature. Examples of skin pathologies featuring eosinophils include eosinophilic cellulitis, eosinophilic fasciitis, and severe drug reactions, including drug reaction with eosinophilia and systemic symptoms, which is an uncommon but potentially life-threatening drug reaction involving the skin and other organ symptoms (Table V).[75-80] Eosinophils also can be seen in various dermatologic diseases, many of which are also associated with peripheral blood eosinophilia and HE, including pemphigus and pemphigoid (and variants), eruptions associated with radiotherapy, erythema toxicum neonatorum, Kimura disease, Langerhans cell histiocytosis, and pregnancy-related dermatoses. Although some of these entities have typical histologic findings (eg, Langerhans cell histiocytosis), others have nonspecific histologic findings and thus require clinicopathologic correlation for diagnosis. Little information is available regarding what constitutes abnormal numbers of eosinophils in skin, and proposed eosinophil density cutoffs have not shown diagnostic utility at this point. As a detailed discussion of the differential diagnoses of dermatologic conditions with eosinophils is beyond the scope of this review, we refer interested readers to a previous review on the topic.[78] In summary, the presence of eosinophils in skin is nonspecific and should be interpreted in the context of other histopathologic and clinical findings.

Skin biopsy eosinophils in human skin recommendation. The presence of eosinophils in the human skin is not diagnostic for any particular skin disorder nor can it differentiate whether a skin process is allergic or parasitic in nature.

GI tract

GI biopsy: Indications and techniques. Upper and/or lower endoscopy with biopsies for evaluating mast cells and eosinophils may be considered if there is concern for MCAS, SM, urticaria pigmentosa, mastocytic enterocolitis, diarrhea predominant irritable bowel syndrome (IBS-D), EoE, HES, or eosinophilic gastritis/enteritis. For example, the diagnosis of SM in a patient who presents primarily with GI symptoms may be challenging to make, but abnormal mast cells can be seen in 70% to 80% of patients with SM, with symptoms ranging from abdominal pain, diarrhea, gastroesophageal reflux, peptic ulcer disease, steatorrhea, and malabsorption.[81] During routine upper endoscopy, also known as esophagogastroduodenoscopy, biopsies are typically obtained in the esophagus, stomach, and duodenum, and less frequently from the jejunum. Notably, there currently are no consensus guidelines regarding the number of biopsies that should be obtained or the optimal time and location for a biopsy of the upper GI tract. However, as per prior guidelines for EoE, multiple biopsy specimens from 2 or more locations, targeting areas of apparent inflammation, are suggested to increase the diagnostic yield.[82] Some conditions may require biopsies from lower GI locations; for example, when SM involves the GI tract, the most common sites include the colon and ileum.[4] For colonoscopy, there appears to be less consensus regarding an appropriate biopsy site from the colon and ileum; furthermore, there is no consensus on how many biopsies of the lower GI tract should be obtained at each location to increase the likelihood that mast cell or eosinophil density is representative. Importantly, similar

to eosinophil infiltrate in the esophagus of EoE, the mast cell infiltrate in mastocytosis may be patchy;[3] thus, multiple, systematic biopsies are recommended, with special instructions to pathology to perform appropriate staining. If referring to another specialist to perform the biopsy, it is important to provide clear direction on what information is being requested.

GI biopsy indications and techniques recommendation. Endoscopy with biopsies for evaluating mast cells and eosinophils should be considered in the context of the patient's clinical history and the clinician's differential diagnosis. Multiple systematic biopsies and clear communication with the specialist performing the biopsy and the pathologist are recommended to accommodate potential patchiness of mastocytosis and eosinophilia.

GI biopsy: Interpretations for diagnostic biopsies for GI mast cells. Mast cells are normally present in the GI tract, albeit scattered as single round/ovoid cells with pale, granular cytoplasm, and they are not prominent on H&E staining of normal biopsies. Increased numbers of mast cells with absent features of mast cell clustering and dysmorphology is not consistent with clonality but can be seen as a reactive process in eosinophilic GI disorders or a finding suggestive of a mast cell–predominant process.[83-86] There are no consensus guidelines regarding the number of GI biopsies that should be obtained or optimal time and location for a biopsy to enumerate mast cells, but similar to EoE guidelines, multiple biopsy specimens from 2 or more sites, targeting areas of apparent inflammation, are recommended to increase the diagnostic yield. Mast cell phenotypes have been characterized previously by anatomic location (intramucosal vs connective tissue) along with protease content, dividing them into 2 subsets: (1) Mast cell-tryptase, mast cells containing tryptase but little or no chymase, and (2) Mast cell tryptase-chymase, mast cells containing tryptase, chymase, and carboxypeptidase.[87,88]

Notably, mucosal biopsies in patients with SM have infiltrates in the lamina propria of mast cells in aggregates or sheets. These aggregates are often present directly under the surface epithelium, but they can also be scattered throughout the lamina propria. Aggregates of >15 mast cells seen on GI biopsy fulfill the major criterion of SM.[44,89-92] There is a wide variety of morphology seen in patients with SM, including round to oval to spindle-shaped cells with pale, granular cytoplasm; small cells with irregular nuclei; and medium-sized monotonous cells with abundant pale cytoplasm.[81] Immunohistochemical staining with tryptase and CD117 is recommended when there is clinical suspicion for SM or with an observed abnormal eosinophil-rich infiltrate, as mast cell infiltrates are often admixed with prominent eosinophilia. Furthermore, immunohistochemical staining is helpful in fulfilling the minor criteria for SM, namely aberrant expression of CD25 and/or CD2.[89,90] A representative case of SM involving the GI tract is shown in Fig 1, *B*.

Recently, unique histologic features of mast cells in the GI tract of patients with HαT were defined.[93] Compared with control patients and patients with MCAS without elevated baseline tryptase, mast cells in patients with HαT were increased in density, extended into the villous lamina propria, and formed small (2-15 cells) clusters. Genetic testing for HαT is suggested if these features are observed.

GI biopsy interpretations for diagnostic biopsies for GI mast cells recommendation. There are no consensus guidelines regarding the number of GI biopsies that should be obtained or optimal time and location for a biopsy to enumerate mast cells, but similar to EoE guidelines, multiple biopsy specimens from 2 or more sites, targeting areas of apparent inflammation, are recommended to increase the diagnostic yield.

GI tract: Stains and analyses for GI mast cells. Because there is no consistent method of mast cell enumeration noted across a variety of studies with respect to staining (eg, tryptase, CD117, Giemsa, azure A, toluidine blue, chloroacetate esterase)—counts per hpf or per mm^2, peak counts versus average counts of multiple fields—it is difficult to make comparisons across studies. In general, as elaborated in the "General principles in evaluating human biopsies for mast cells and eosinophils for clinical purposes" section, immunohistochemical stains (tryptase and CD117/KIT) are superior to histochemical stains (toluidine blue, chloroacetate esterase, Giemsa) and H&E alone and, therefore, should be used for enumeration of mast cells.

GI tract stains and analyses for GI mast cells recommendation. Immunohistochemical stains should be used to quantify mast cell levels.

GI tract: Mastocytosis. What constitutes a normal number of mast cells in various segments of the GI tract has not been well established. Similar to identifying mast cells in different organ systems, mast cell counting in the GI tract should be done on slides stained by IHC for tryptase or CD117; however, these stains are expressed in normal and neoplastic mast cells.[4,26,90] One study found that in control subjects, the average peak colonic mast cell count in a single hpf was 26 (range, 11-55).[4] In other studies in which counts were separated by location, mean findings were as follows: stomach, 12 (range, 5-21); duodenum, 27 (range, 4-51); terminal ileum, 32 (range, 21-40); and colon, 21 (range, 10-31) mast cells per hpf (Table IV).[26] The number of mast cells have also been examined in children. One Canadian study investigated both the upper and lower GI tract of healthy children.[27] The number of mast cells determined by insensitive Giemsa staining at different locations found that mean mast cell numbers per hpf were as follows: antrum, 0.3 (range, 0-2); stomach body, 0.3 (range, 0-7); duodenum, 0.3 (range, 0-7); duodenal cap, 0.04 (range, 0-1); ileum, 0.9 (range, 0-11); cecum, 0.5 (range, 0-7); ascending colon, 0.2 (range, 0-3); transverse colon, 0.3 (range, 0-3); descending colon, 0.4 (range, 0-7); sigmoid, 0.3 (range, 0-3); and rectum, 0.6 (range, 0-5). Another pediatric study examined 41 healthy children in Arkansas.[28] The mean ± SD of mast cells per hpf determined by tryptase IHC were as follows: cecum, 17.4 ± 7.4; ascending colon, 15.3 ± 4.9; transverse colon, 15.9 ± 8.2; descending colon, 17.6 ± 7.3; and rectum, 14.5 ± 6.4. As seen in Table IV, there is large study-to-study variability that likely stems from use of different stains to highlight mast cells, the differing size of hpfs, and whether average of representative fields or peak count were reported. Similar to eosinophil levels varying within the GI tract, it is possible that different sections within the GI system have differing normal levels of tissue-resident mast cells.[1]

Although there are several studies comparing mast cells in the GI tract in different GI conditions, the results have been mixed. A systematic review and meta-analysis showed that compared to controls, patients with IBS had higher number of mast cells in the rectosigmoid and descending colon in both constipation and diarrhea-predominant IBS.[94] Another study compared GI biopsies from 100 patients who were asymptomatic, 100 patients with IBS-D, and 10 patients with MCAS.[4] They found that the mean highest colonic mast cell count in a single hpf for patients who were asymptomatic was 26 (range, 11-55), IBS-D was 30

(range, 13-59), and MCAS was 28 (range, 14-48). The difference between the IBS group and the asymptomatic group was statistically significant ($P < .001$), albeit without a clear cutoff threshold that could be established between the overlapping groups, whereas the difference between the MCAS group and the asymptomatic group was not statistically significant.

Another study looked at adult patients with various GI conditions to compare their mast cell density.[26] For patients with eosinophilic gastritis (n = 4), enteritis (n = 3), and colitis (n = 1), they found that the mean number of mast cell numbers per hpf were as follows: stomach, 14 (range, 9-17); small intestine, 15 (range, 6-22); and colon, 12 (n = 1). In patients with urticaria pigmentosa, mean mast cells per hpf were as follows: stomach, 14 (range, 10-17); small intestine, 22 (range, 12-32); and colon, 13 (range, 8-19). In patients with SM, mean mast cells per hpf were as follows: stomach, 57 (range, 24-90); small intestine, 175 (range, 74-339); and colon, 209 (range, 110-301). The only condition with a significantly higher number of mast cells was SM, with a mean of 116 (range, 20-278) mast cells/hpf.[4] One study showed that in patients with SM, the colon was the most commonly involved site, followed by the ileum, duodenum, and stomach.[4] Another study of 7 patients with SM also showed the colon being most commonly involved, followed by the small bowel.[81] There are several case studies of adult patients with SM who reportedly have an "increased" number of mast cells, but the specific numbers were not reported.[81-92,95] Interestingly, a recent study found that mast cell numbers remained elevated in adults with EoE despite clinical remission with normalization of intraepithelial eosinophilia.[96]

Mastocytic enterocolitis represents a controversial entity, which has yet to be established as a specific clinical diagnosis and has been associated with persistent diarrhea.[97] As described, these patients have no evidence of CM or SM but have increased numbers of duodenal mast cells and often respond to H_1 and H_2 receptor antagonism or mast cell stabilizing agents. More research is needed before mastocytic enterocolitis can be established as a specific clinical entity that requires differentiation from other conditions with overlapping symptoms, such as microscopic colitis (collagenous and lymphocytic) and IBS. Histologic differences between these conditions can be enigmatic without special staining for mast cells. In the meantime, clinical judgment and clinicopathologic correlation are recommended.

In summary, in the absence of clustering, atypical morphology (eg, spindle-shaped mast cells) and/or aberrant expression of markers, such as CD25, the number of mast cells largely overlap between physiologic states, reactive processes, and SM. At this point a clear cutoff threshold with strong positive and negative predictive values has not been established for increased mast cell counts. The latest mast cell disorder work group report does not address the occurrence of local mast cell activation, including what constitutes an increase in mast cell numbers in the GI tract, which is a gap in knowledge that requires more research.[98]

GI tract mastocytosis recommendation 1. Multifocal, dense aggregates of >15 mast cells seen on GI biopsy fulfill the major criterion of SM. However, what constitutes a normal number of mast cells and whether there is a clinically useful cutoff threshold of mast cell density to support diagnoses of MCAS or mastocytic enterocolitis requires additional research, standardization, and development of validated reference ranges. In the meantime, clinicopathologic correlation is recommended.

GI tract mastocytosis recommendation 2. Special requests must be made by the endoscopist or ordering physician for the pathologist to perform special mast cell staining, as it is not standard of care and should only be performed when there is clinical suspicion.

GI tract mastocytosis recommendation 3. Mastocytic enterocolitis associated with persistent diarrhea represents a controversial entity that has yet to be established as a specific clinical diagnosis. Thus, more research is required to justify routine staining of mast cells on GI biopsies in suspected cases. In the meantime, clinical judgment and clinicopathologic correlation are recommended.

GI biopsy: Interpretations for diagnostic biopsies for GI eosinophils. GI eosinophilia can occur in single or multiple locations of the GI tract. H&E staining can identify eosinophils to assess for evidence of GI eosinophilia. We discuss GI eosinophilia for eosinophilic GI disorders.

EoE is the most common of the eosinophilic GI disorders and evidences eosinophilia in the esophagus. In EoE, an esophageal eosinophil count of >15 eosinophils/hpf is the histologic criterion for EoE. However, EoE is a clinicopathologic diagnosis; thus, signs/symptoms of esophageal dysfunction and exclusion of other diseases with eosinophilia are required for diagnosis. Besides eosinophils, other histologic changes, including increased numbers of mast cells, are seen in EoE;[2] the utility of a recently developed EoE histologic scoring system is under evaluation. Excess mast cells are also present, and reactive increases in mast cells have been identified in EoE.[82-85] Furthermore, a recent publication demonstrated that esophageal mast cells can remain elevated in patients with EoE who remain symptomatic in spite of apparent control of esophageal eosinophilia.[99] Another study reported that tryptase staining of mast cells has been found to differentiate EoE from reflux esophagitis.[100] In addition, the clinical symptom of dysphagia correlates best with esophageal gene transcripts predominantly involving mast cell–specific genes.[101]

For other eosinophilic GI disorders, such as eosinophilic gastritis, eosinophilic enteritis, and eosinophilic colitis, the diagnostic criteria are less well defined. For eosinophils, baseline counts have been established in pediatric patients across the GI tract.[102] One proposed criterion for eosinophilic gastritis is an average of ≥30 gastric eosinophils/hpf in 5 separate hpfs, and 1 study showed a distinct gastric transcriptome compared to EoE and evidence of increased gastric mast cells.[103] Studies to determine the clinical utility of this and other histologic criteria for eosinophilic gastritis and eosinophilic colitis are in progress. Pathology reports should be written in a way to emphasize these uncertainties.

GI biopsy interpretations for diagnostic biopsies for GI eosinophils recommendation–EoE. For EoE, peak esophageal eosinophil count of >15 eosinophils/hpf is a well-established criterion, but a diagnosis should be made considering esophageal dysfunction and other GI disorders with eosinophilia.

GI biopsy interpretations for diagnostic biopsies for GI eosinophils recommendation–eosinophilic gastrointestinal disorders (non-EoE). For eosinophilic GI disorders other than EoE, further research is required to establish the diagnostic criteria for increased numbers of mast cells or eosinophils.

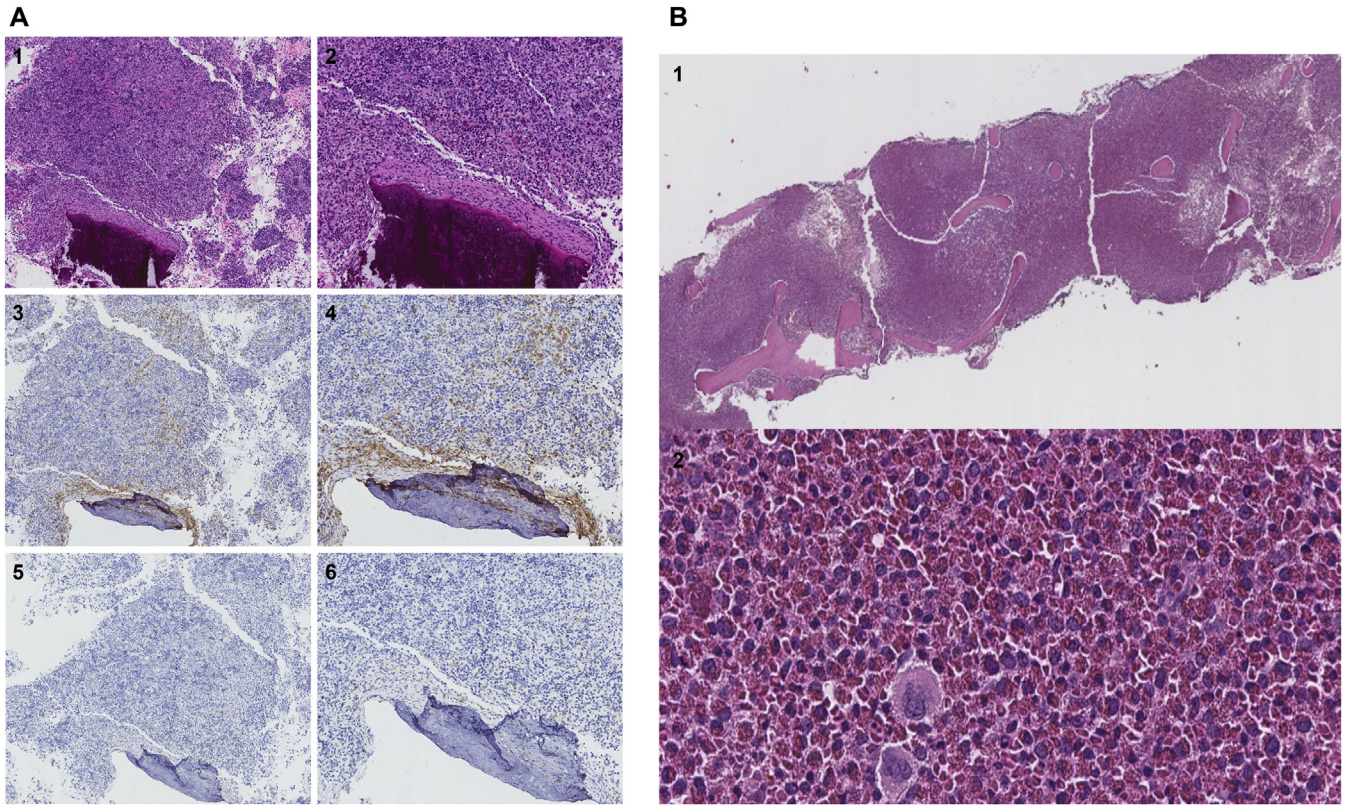

FIG 2. (A, 1-6) Patient with SM-AHN. Shown is IHC of bone marrow biopsies of a patient with SM-AHN, specifically myelodysplastic syndrome with excess blasts. Hematoxylin and eosin staining of the bone marrow (**1, 2**) shows hypercellular marrow with maturing, left-shifted myelopoiesis with excess blasts, dysplasia in the erythroid and megakaryocyte lineage, consistent with myelodysplastic syndrome with excess blasts. Additionally, clusters of mast cells are seen, highlighted by CD117 **(3, 4)**, but only weakly positive for tryptase **(5, 6)**. NGS identified mutations *IDH1*.R132 and *KIT*.D816V. Magnifications are 10× **(1, 3, 5)** and 40× **(2, 4, 6)**. **(B, 1, 2)** Myeloid/lymphoid neoplasm with eosinophilia and *PDGFRA* rearrangement. Bone marrow core biopsy from a patient with *FIP1L1-PDGFRA* fusion. The core biopsy is hypercellular with little residual hematopoiesis (note the 2 megakaryocytes) and proliferation of eosinophils and their precursors. H&E staining. Magnifications are 2× **(1)** and 40× **(2)**.

Bone marrow

Bone marrow biopsy: Indications and techniques. *Mastocytosis.* Bone marrow biopsy is performed as part of workup in patients with suspected mast cell–mediated disease. We further discuss disease-specific findings.

Bone marrow is the most common site affected by SM and is thus the most common site to biopsy to assess for SM.[44] In patients with known mastocytosis in skin, indications for bone marrow biopsy differ in children and adults. Children may have CM alone, and bone marrow examination should be performed to exclude SM in patients with organomegaly or if mutation in *KIT* is detected by a sensitive method in the peripheral blood.[104] Pediatric patients with a tryptase level >20 ng/mL but without organomegaly (a common scenario in patients with diffuse CM) are unlikely to have systemic disease;[104] thus, bone marrow biopsy may not be warranted. Adults with mastocytosis in skin are more likely to have SM, and thus bone marrow biopsy is warranted.[105] Although detection of *KIT* mutation alone in peripheral blood with a sensitive method can be sufficient to diagnose SM,[106-108] a bone marrow biopsy is critical for proper disease classification, from indolent SM to mast cell leukemia. Prognosis in patients with SM is variable, ranging from indolent having normal life expectancy to rapidly deteriorating courses; thus, properly classifying cases guides treatment and prognosis.[109-112] Furthermore, ~40% of adults with SM in a referral center have an associated hematopoietic neoplasm (SM-AHN),[109] which requires specific treatment of the neoplastic component in addition to the SM and additionally highlights the need for bone marrow biopsy. A representative case of SM-AHN involving the bone marrow is shown in Fig 2, *A*.

WDSM is a rare form of SM typically presenting with mastocytosis in the skin of children that persists; its systemic nature is eventually demonstrated with bone marrow examination.[17] However, mast cells in WDSM are less likely to have *KIT* mutations, are often morphologically normal (round), and do not show aberrant expression of CD2 and CD25; thus, they often do not fulfill the WHO criteria for SM. However, establishing the diagnosis of WDSM is important because this form of SM is often responsive to imatinib,[113] and thus alternative diagnostic criteria were proposed.[17] Clinical criteria for suspecting WDSM are not fully established, but they include persistence of skin disease, female sex, and familial mastocytosis.

Patients with HαT who have inherited extra copies of the alpha tryptase gene (*TPSAB1*) and evidence a spectrum of symptoms involving multiple organ systems and resembling dysautonomia also present with increased serum tryptase levels, which require

a bone marrow biopsy to differentiate from SM.[46] In contrast to SM, HαT does not have an increased number of mast cells, but mast cells show subtle morphologic alterations and distribution differences.[114]

In summary, bone marrow examination is indicated in pediatric patients with mastocytosis in skin accompanied by organomegaly or peripheral blood *KIT* mutation, adult patients with suspected SM, and in situations in which there is clinical suspicion for WDSM or HαT.

Bone marrow biopsy indications and techniques recommendation–mastocytosis. Bone marrow is the most appropriate screening site for systemic involvement in SM, and a biopsy should be performed in adult patients with mastocytosis in the skin; pediatric patients with mastocytosis in the skin with organomegaly or with a c-KIT mutation detected by a sensitive method in peripheral blood; pediatric and adult patients with unexplained severe symptoms of mast cell activation, especially if there is an elevated blood (tryptase) or urine (methylhistamine, prostaglandin F_2-α) biomarker; and pediatric and adult patients with clinical suspicion for WDSM or HαT.

Eosinophilia. Eosinophil-associated disorders were classified by the Working Conference on Eosinophil Disorders and Syndromes.[115] Eosinophilia is defined as an elevation of the eosinophil count in peripheral blood, usually above 0.5×10^9 eosinophils/L, whereas HE is defined as eosinophil count above 1.5×10^9 eosinophils/L. Tissue eosinophilia is defined as >20% eosinophils in the bone marrow; a local, marked increase in tissue eosinophils; and/or marked deposition of eosinophil-derived proteins even in the absence of intact eosinophils. Once there is organ damage or dysfunction caused by eosinophils, the condition is considered HES.[115]

Eosinophilia and HE can be observed in a variety of conditions, including neoplastic and nonneoplastic disorders. The majority of cases are reactive nonneoplastic, including parasitic and fungal infection, hypersensitivity reactions, and collagen vascular disease. Bone marrow evaluation is used primarily to assess for presence of neoplastic conditions, which fall into several WHO categories:(1) Myeloid/lymphoid neoplasms associated with eosinophilia (MLNeo) and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1* or with *PCM1-JAK2*. A representative case of MLNeo and rearrangement of *PDGFRA* is shown in Fig 2, *B*. (2) Myelodysplastic syndrome, myeloproliferative neoplasm, and myelodysplastic syndrome/myeloproliferative neoplasm with eosinophilia, which includes chronic eosinophilic leukemia, not otherwise specified (CEL, NOS). (3) Acute myeloid leukemia with inversion at chromosome 16. (4) Reactive eosinophilia seen in other neoplastic hematopoietic conditions (eg, Hodgkin lymphoma, T-cell lymphomas).

If neither reactive nor neoplastic causes are identified, the process is characterized as idiopathic HES (IHES). Thus, bone marrow biopsy is indicated in evaluation of eosinophilia when there is (are) (1) persistent eosinophilia without obvious reactive etiology; (2) changes in the complete blood count other than eosinophilia (eg, leukocytosis due to other cell type, granulocytic left shift, circulating blasts, anemia, thrombocytopenia); (3) peripheral blood smear that shows atypical eosinophils or dysplasia in other lineages; (4) organomegaly; (5) an elevated tryptase; and (6) an elevated serum vitamin B_{12}. Other reasons based on clinical suspicion may prompt the need for a bone marrow biopsy.[116]

Bone marrow biopsy indications and techniques recommendation—eosinophilia. Bone marrow biopsy should be performed in patients with HE in which reactive etiologies have been excluded or if complete blood count/lab data raise suspicion for the likelihood for neoplastic process (eg, circulating blasts, dysplasia on peripheral smear, leukocytosis with increase in other lineages, anemia, thrombocytopenia).

Bone marrow biopsy: Interpretation

A bone marrow biopsy is usually performed at the iliac crest and involves 2 stages: an aspirate and a core biopsy. In pediatric populations, often only an aspirate is performed. After local anesthesia, a syringe is used to aspirate cellular content of the marrow. For adequacy, at least a 1.5-cm length of the bone marrow core is required.[117] It is essential to collect peripheral blood for a complete blood count with differential and peripheral blood smear evaluation before or shortly after the bone marrow biopsy for correlation of findings.

The bone marrow aspirate is smeared and stained with a Romanowsky-type stain, which is composed of a mixture of oxidized methylene blue (azure) dyes and eosin Y. The azures are basic dyes that bind acid nuclei and result in a blue to purple color, whereas the acid dye, eosin, is attracted to the alkaline cytoplasm, producing red coloration and thereby permitting detailed assessment of hematopoietic cell morphology. Due to the excellent cellular detail for morphologic assessment and single-cell suspension of the aspirate smear, it is the preferred specimen for performing a differential cell count and determining the percentage of individual cell types in the bone marrow, including mast cells. The remaining aspirate can be used to prepare a bone marrow clot section, which is fixed and processed similarly to the biopsy. In addition, the aspirate can be used for other cellular and molecular diagnostic testing, such as flow cytometry, fluorescent *in situ* hybridization (FISH), PCR, and next-generation sequencing (NGS), when indicated.

Preparing the core biopsy and clot section involves tissue fixation, decalcification, processing, sectioning, and staining by H&E. The core biopsy allows for assessment of cellularity, architecture, bone trabeculae, and other bone marrow elements that are not able to be assessed in the aspirate. This is especially important for situations such as bone marrow fibrosis or the presence of paratrabecular aggregates, such as the mast cell aggregates in SM, which is an important component of the WHO diagnostic criteria (Table I). The clot section and/or core biopsy can also be used for special stains (eg, Giemsa, toluidine blue) and IHC (eg, tryptase, CD117/KIT, CD25, CD2) to assess for mast cell clonality, which are additional diagnostic criteria for SM.

Bone marrow biopsy interpretation recommendation. Both bone marrow core biopsy and aspirate should be performed when feasible.

Mastocytosis. Mast cells are normally found in the bone marrow in small numbers and scattered throughout the interstitium as round cells with central round nuclei and granular cytoplasm. However, clonal or neoplastic mast cells tend to aggregate, are more likely to be spindle-shaped, and may have altered granule content.[6] In the core biopsy, neoplastic mast cells form dense aggregates (defined as >15 cells) and are spindle-shaped. Thus, special stain with Giemsa and/or IHC with tryptase

and CD117/KIT may highlight additional atypical mast cells not seen by H&E. This is especially important in morphologically occult SM, in which mast cells do not form dense aggregates and SM is diagnosed on the basis of the presence of ≥3 minor criteria (Table I).[5] Therefore, it is important for the physician to provide the pathologist the clinical information that would prompt performing these studies. The rationale for performing IHC to assess for mast cell clonality using specific markers separately or together has been discussed in the Bone marrow biopsy: Interpretation section. Flow cytometry correlates well with IHC staining in that no false negatives have been seen despite low mast cell burden in aspirates.[5] Some experts advocate that flow cytometry is not necessary and suggest its use be restricted to scenarios in which IHC is indeterminate; however, in most laboratories, flow cytometry is performed before IHC as a screening tool. For instance, if aberrant CD2 or CD25 are seen on CD117-positive cells by flow cytometry, this can prompt further investigation for evidence of SM in the core biopsy by IHC. Although cytologic abnormalities of eosinophils lack specificity to differentiate a neoplastic process from reactive eosinophilia,[118-122] bone marrow morphologic features are strong discriminators between reactive HES and neoplastic CEL.[123] Specific features, such as cellularity, abnormal morphology of megakaryocytes, erythroid precursors or myeloid cells, elevated myeloid/erythroid ratio, marrow fibrosis, abnormal eosinophil morphology in >20% of cells (eg, sparse granulation, hypo- or hypersegmentation, increased cell size), when assessed in combination and by an experienced observer can serve as indicators of clonal hematopoiesis. Ancillary studies, discussed next, should then be performed for further classification. It is important to emphasize that having an unremarkable bone marrow by histology does not exclude a clonal process and necessitates further evaluation.[124]

Bone marrow biopsy interpretation recommendation—mastocytosis. Although H&E can easily detect clusters of mast cells, they are not always present; thus, one should have a low threshold for using additional special stains (Giemsa) or IHC to detect mast cell–mediated disease.

Bone marrow biopsy interpretation recommendation—IHC for mast cells. When performing IHC for mast cells, use both CD117/KIT and tryptase, either simultaneously or sequentially. For sequential staining, use CD117/KIT to screen and then subsequently use tryptase to confirm any positive results. It is particularly important to confirm mast cells by tryptase in the bone marrow, where myeloid and erythroid precursors express CD117 and in the context of left-shifted maturation of either lineage. To establish mast cell clonality, use primarily the CD25 marker; if CD2 is used, interpret mast cell presence with caution, as CD2 is also expressed on T cells.

Ancillary testing for mastocytosis. Depending on the institution, ancillary testing (cytogenetics, molecular analysis, flow cytometry) are ordered by either the clinician or pathologist. Thus, both need to be aware of the indications for obtaining these tests.

When considering mastocytosis, mutation status of c-KIT is part of the diagnostic criteria and should always be performed. However, the specific mutation is also important for guiding treatment, as different KIT mutations have different sensitivity to tyrosine kinase inhibitors.[44] For example, *KIT*.D816V mutation, which is found in >80% of patients with SM, and other A loop mutations (D816V/H/Y/N) confer resistance to imatinib but responsiveness to some of the newer tyrosine kinase inhibitors, such as midostaurin and others currently in clinical trials for this indication (eg, avapritinib).[125,126] In contrast, KIT mutations outside the tyrosine kinase domain, as well as wild-type KIT, are sensitive to imatinib.[127-130]

The prognostic significance of cytogenetics is lost in multivariate analysis as long as molecular analysis (mutations in genes commonly involved in myeloid malignancies) is performed. Several studies have investigated the role of cytogenetic alterations in SM,[131,132]which are rare in SM other than in SM-AHN; thus, cytogenetic analysis should be performed routinely only in SM-AHN. In contrast, mutations in myeloid-associated genes have been shown to have prognostic importance.[133-136] This is especially true for mutations in *ASXL1*, *RUNX1*, and *SRSF2* genes. Specifically, using the mutation-augmented prognostic scoring system, it has been possible to stratify patients with these mutations into groups with very different survival curves. This rapidly expanding area of research has made it increasingly clear that mutational analysis beyond *KIT* is important for risk stratification of these patients.

Bone marrow biopsy interpretation recommendation—ancillary testing for mastocytosis. When SM is suspected, mutational analysis, including but not limited to c-KIT, should be performed routinely. Cytogenetics should only be performed for SM-AHN.

Ancillary testing for eosinophilia. Specifically related to workup of eosinophilia/HE, important ancillary testing includes cytogenetic and molecular testing. On the basis of current recommendations, karyotype and FISH for *PDGFRA* are indicated.[137] However, with recent recognition of an increasing number of cryptic *PDGFRB* rearrangements,[138-140] it may be prudent to also perform FISH for *PDGFRB* and/or molecular analysis capable of detecting fusion genes, such as DNA and/or RNA NGS. *FGFR1* rearrangement is seen by karyotype analysis and thus FISH for *FGFR1* is currently not recommended initially; however, if karyotyping fails or there are other morphologic or clinical suspicions for this rearrangement, it can be performed. Furthermore, there is an increased spectrum of tyrosine kinase–activated neoplasms with eosinophilia, including *FLT3*- and *ABL1*-rearranged cases.[141-146] Thus, molecular analysis can identify a much broader range of abnormal gene mutation–associated neoplasms than can cytogenetics alone.

Another situation in which NGS analysis is changing the way that we diagnose myeloid neoplasms with eosinophilia is the demonstration of clonality to meet the criteria for CEL, NOS. Typically, cases are classified as CEL, NOS if there is (1) eosinophilia, (2) increase in blood and/or bone marrow blasts or evidence of clonality, and (3) no specific recurrent molecular abnormalities (eg, rearrangement in *PDGFRA*, *PDGFRB*, or *FGFR1*) identified. Clonality has traditionally been determined by the presence of cytogenetic abnormalities (karyotype and/or FISH) or skewed expression of X chromosome genes in female patients. With increased use of NGS, somatic mutations suggesting clonality are being detected in an increasing number of IHES cases.[147-149] The most commonly mutated genes include *ASXL1*, *TET2*, *EXH2*, *SETBP1*, *CBL*, *NOTCH1*, *SCRIB*, *STAG2*, *SH2B3*, *PUF60*, *CDH17*, *LMLN*, *AQP12A*, and *PCSK1*. The challenge is to determine the biologic significance of the molecular findings, as many of the gene mutations are also present in healthy, aging

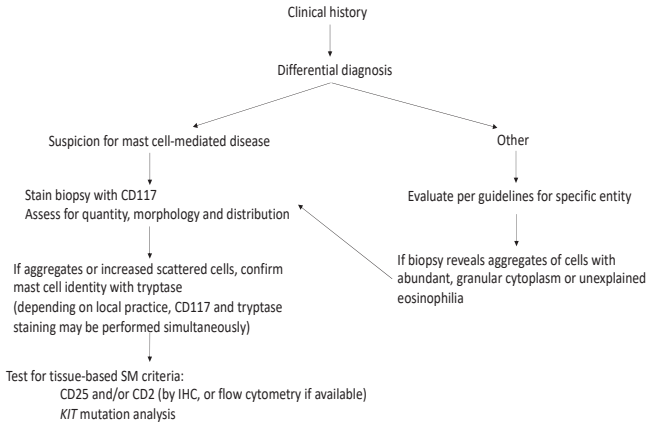

FIG 3. Algorithmic approach for evaluation and histologic staining of skin, GI tract, and bone marrow biopsies for mast cells.

individuals and have been termed clonal hematopoiesis of indeterminate potential.[150] Interpretation of their biologic significance needs to take into consideration the context of specific mutations, allele frequency, number of mutations present in an individual patient, and association of the mutation(s) with outcomes. However, ≥1 study demonstrated that mutation-positive disease in IHES exhibited biologic behavior closer to cytogenetically determined CEL, NOS than mutation-negative IHES cases, which have superior survival rates.[147] Thus, expanded use of novel molecular approaches, including NGS, may lead to many IHES cases being reclassified as clonal CEL, NOS and thereby impact prognosis and possibly therapeutic options.

Bone marrow biopsy interpretation—ancillary testing for eosinophilia recommendation 1. When working up HE, cytogenetic studies (karyotype and FISH for *PDGFRA* and *PDGFRB* gene rearrangement at minimum) should be performed even if bone marrow eosinophilia is the only morphologic abnormality.

Bone marrow biopsy interpretation—ancillary testing for eosinophilia recommendation 2. When working up HE, there should be a low threshold for molecular analysis (eg, NGS for myeloid gene panel), as it can aid in distinguishing CEL, NOS from IHES and in identifying MLNeo with cryptic rearrangements not seen by FISH analysis.

Bone marrow biopsy interpretation—ancillary testing for eosinophilia recommendation 3. When working up HE, there should be a low threshold for flow cytometry for T-cell lymphocyte analysis to assess for possible lymphocytic HES and/or T-cell lymphocyte clonality.

Differentiating MLNEO and SM. There is overlap between SM and MLNeo.[151] Thus, in patients with HE for which bone marrow assessment does not yield a specific diagnosis, it is indicated to perform IHC for tryptase, CD117, and CD25 to assess for SM.[124] Because abnormal mast cells can be seen in both MLNeo and SM, positive IHC should be followed by further testing, including *KIT* mutation assessment as discussed in the "Ancillary testing for mastocytosis" section.

Bone marrow biopsy interpretation recommendation–differentiating MLNEO and SM. When working up HE in cases of isolated bone marrow eosinophilia, there should be a low threshold for assessing SM using IHC for tryptase, CD117, and CD25.

Comprehensive reporting. The College of American Pathologists provides protocols (templates) for the examining and reporting of hematologic malignancies in bone marrow, including SM and neoplastic processes associated with HE. The use of this template has been recommended but not required for laboratory accreditation purposes. The use of templates provides completeness and clarity and is based on latest diagnostic guidelines. Any pathology report should include all the critical information outlined in the template, including specimen type and adequacy, bone marrow cellularity, morphologic findings, and ancillary studies (IHC, flow cytometry, cytogenetics, molecular genetics), and the final integrated diagnosis after all the ancillary studies have been completed. In the case of an SM diagnosis, the type of SM should be clearly stated in the final report on these findings. The College of American Pathologists template for reporting of bone marrow biopsy findings can be found online (https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates).[117]

Bone marrow biopsy interpretation recommendation—comprehensive reporting. Findings should be reported using the College of American Pathologists template or at a minimum should include all information from the template in the final integrated report, which includes results from morphologic, immunophenotypic, and cytogenetic/molecular studies.

GAPS IN KNOWLEDGE

Fig 3 summarizes an algorithmic approach for evaluating and staining of skin, GI tract, and BM biopsies for mast cell– and eosinophil-associated conditions. There are still many gaps in knowledge that require further research to optimize this algorithm. We highlight 4 gaps of high importance—standardizing measurements and units for assessing cell density, establishing norms for mast cell and eosinophil levels in the GI tract in homeostasis and disease, testing for targeted therapies, and researching mastocytosis enterocolitis—as follows.

Standardizing measurements and units for assessing cell density

Progress toward consensus techniques, interpretation, and diagnostic cutoff thresholds for mastocytosis and eosinophilia in human skin, GI tract, and bone marrow biopsies has been hindered by the limited comparability of reported data due to differing measurements and units. Currently, there are no clear standards of mast cell enumeration in the skin or GI tract except for the diagnosis of SM, which utilizes the measures similarly as for bone marrow analyses. Therefore, standardizing IHC techniques with antibodies for tryptase and CD117 staining in both pediatric and adult populations and utilizing a consistent measurement of area (eg, mm^2) is needed to improve assessments of mast cell–related disorders. Similarly, standardizing reported measurement of area for eosinophilia is needed to provide comparable results between and among studies to facilitate advancing the field and clinical practice.

Establishing norms for mast cell levels in the GI tract in homeostasis and disease

Reference ranges for what constitutes normal mast cell numbers in different portions of the GI tract, using the above

standardized approaches, need to be established. Additionally, whether there are clinically actionable cutoff values, which reliably differentiate disease states and predict response to therapy, needs to be determined.

Testing for targeted therapies

Neoplastic mast cells aberrantly express surface molecules, some of which are targets of antibody-based therapies.[18,19,152-154] Examples include CD30 (brentuximab), CD33 (gemtuzumab-ozogamicin), and CD52 (alemtuzumab). Studies are needed to determine whether these antibodies are useful for diagnosis of SM, and what clinical, histologic, or biologic markers are optimal for determining which patients will respond to a specific treatment. In addition, more research is needed to investigate monoclonal MCAS versus indolent SM to better understand the differences in natural history, prognosis, and diagnostic biomarkers for differentiating these conditions. (Eg, Is CD25 positivity enough to diagnose monoclonal MCAS if *KIT* mutation is negative?)

Researching mastocytic enterocolitis

As mentioned earlier, mastocytic enterocolitis is a controversial entity, and additional research is required. Standardizing mast cell density assessment, as previously mentioned, is an important first step. However, additional research is required to correlate histology features with clinical symptoms and outcomes related to mast cell–directed therapies and to further understand the pathophysiology.

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