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 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;148:964-83.)

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

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
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 \( \geq 20\) 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, \( \geq 2 \) B findings; see below)
- SM-AHN
- Aggressive SM (\( \geq 1 \) C finding; see below)
- Mast cell leukemia (diffuse infiltrate; \( >20\% \) mast cell in bone marrow aspirate that are atypical and immature, \( \pm \) 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 \( >200 \) ng/mL
- Hypercellular bone marrow with loss of fat cells, discrete signs of dysmyelopoesis 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 \( <1,000/\mu\text{L} \) or hemoglobin \( <10 \) g/dL or platelet count \( <100,000/\mu\text{L} \)
- 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-PDGFRα hypereosinophilic syndrome (HES) mutations and serum baseline tryptase levels \( >20 \) ng/mL can be seen in hereditary alpha tryptasemia (HαT) or advanced renal failure.**

## TABLE I. 2016 WHO classification criteria for mastocytosis

<table>
<thead>
<tr>
<th>Classification</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>Cutaneous mastocytosis</td>
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<tr>
<td>Systemic mastocytosis</td>
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<tr>
<td>Mast cell sarcoma (localized mast cell tumors)</td>
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</table>

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

### Minor criteria
- Multifocal dense infiltrates of mast cells in bone marrow biopsies and/or sections of other extracutaneous organs
- 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 \( \geq 20 \) ng/mL (in case of an unrelated myeloid neoplasm, this is not valid as an SM criterion)

### Major criteria
- Indolent SM (low mast cell burden, no C findings; see below)
- Smoldering SM (high mast cell burden, \( \geq 2 \) B findings; see below)
- SM-AHN
- Aggressive SM (\( \geq 1 \) C finding; see below)
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### Notes

- **Histology:** 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
- **Immunohistochemistry:** Mast cells can be stained immunohistochemically using CD117/KIT, which has great specificity for mast cells. However, its expression is relatively 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.

### Clonality
- 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*DBV16 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.¹⁰ 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 instances³ and is easier to interpret, CD25 is the recommended marker to assess mast cell clonality by IHC. CD25 may be more relevant when using flow cytometry than when using IHC, as...
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. 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 is often negative, using CD30 in combination with CD25 has been shown to increase the diagnostic accuracy of SM. Thus, CD30 has recently been proposed as an additional minor diagnostic criterion of SM. 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 mm²), 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. 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). 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 mm²” 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 mm²” 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 = πr²) of 0.24 mm²; 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 mm². 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 mm² 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 mm²” 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 mm²” or provide equivalent “per mm²” 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. 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, both eosinophils and mast cells may be considered.
TABLE III. Mast cell density by tryptase IHC in normal skin

<table>
<thead>
<tr>
<th>Region</th>
<th>n</th>
<th>Mean Mast cells/mm²</th>
<th>SD Mast cells/mm²</th>
<th>Mean Mast cells/hpf*</th>
<th>SD Mast cells/hpf*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trunk</td>
<td>19</td>
<td>10.2</td>
<td>3.15</td>
<td>6</td>
<td>1.2</td>
</tr>
<tr>
<td>Forearm</td>
<td>27</td>
<td>11.5</td>
<td>3.2</td>
<td>4.9</td>
<td></td>
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<tr>
<td>Upper arm</td>
<td>44</td>
<td>11.5</td>
<td>3.2</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Upper leg</td>
<td>29</td>
<td>11.2</td>
<td>3.8</td>
<td>5.8</td>
<td></td>
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<tr>
<td>Lower leg</td>
<td>24</td>
<td>17.0</td>
<td>7.0</td>
<td></td>
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</tbody>
</table>

Data were modified from published work as noted.

ND, Not determined (the area of hpf was not reported in these studies; thus, density per mm² cannot be accurately determined).

*Converted values from Janssens et al assuming an hpf area of 0.15 mm².

†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. 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. 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) of the WHO guideline for SM classification.

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.

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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. 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. 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

<table>
<thead>
<tr>
<th>Stain</th>
<th>Count method</th>
<th>Data reported as</th>
<th>Location(s), count</th>
<th>Adult vs pediatric</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptase</td>
<td>400× magnification hpf (field size, 0.25 mm²)</td>
<td>Mean ± SD (range)</td>
<td>Colon, 19 ± 6.1 (7-39)</td>
<td>Adult</td>
<td>IBS and MCAS vs controls</td>
<td>Doyle et al²⁸</td>
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<tr>
<td>CD117</td>
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<td>CD25</td>
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<td>CD30</td>
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<tr>
<td>Tryptase</td>
<td>400× magnification hpf (field size, 0.25 mm²)</td>
<td>Mean (range)</td>
<td>Stomach, 12 (5-21) Duodenum, 27 (4-51) Terminal ileum, 32 (21-40) Colon, 21 (10-31) (Tryptase or CD117)</td>
<td>Adult</td>
<td>SM vs controls and varied abnormal diagnoses (IBD, eosinophilic gastrointestinal disorder, celiac disease, etc)</td>
<td>Hahn et al²⁶</td>
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<tr>
<td>CD117</td>
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<td>CD25</td>
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<tr>
<td>Giemsa</td>
<td>400× magnification hpf (field size, 0.55 mm²)</td>
<td>Mean (range)</td>
<td>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)</td>
<td>Pediatric</td>
<td>Normal control</td>
<td>Chernetsova et al²⁷</td>
</tr>
<tr>
<td>Tryptase</td>
<td>400× magnification hpf (field size, 0.28 mm²)</td>
<td>Mean ± SD (maximum)</td>
<td>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)</td>
<td>Pediatric</td>
<td>Normal control</td>
<td>Saad²⁸</td>
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<tr>
<td>CD117</td>
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<tr>
<td>Tryptase</td>
<td>400× magnification hpf (field size, 0.28 mm²)</td>
<td>Mean ± SD (maximum)</td>
<td>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</td>
<td>Pediatric</td>
<td>Normal control</td>
<td>Tison et al²⁹ (abstract)</td>
</tr>
<tr>
<td>Tryptase</td>
<td>Percent volumetric</td>
<td>Percentage of area ± SD</td>
<td>Distal colon, 3.3 ± 0.8</td>
<td>Adult</td>
<td>IBS vs controls. Complex volumetric evaluation not suitable for day-to-day use.</td>
<td>Barbara et al³⁰</td>
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(Continued)
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 scalpels 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. In some conditions, 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. 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. 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; 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. 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.  

<table>
<thead>
<tr>
<th>Stain</th>
<th>Count method</th>
<th>Data reported as</th>
<th>Location(s), count</th>
<th>Adult vs pediatric</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD117</td>
<td>400× magnification (1 mm² = 6.249 hpf)</td>
<td>Mean ± SD</td>
<td>Jejunum, 15.3 ± 4.4</td>
<td>Adult</td>
<td>IBS-D vs controls</td>
<td>Guillarte et al 31</td>
</tr>
<tr>
<td>Tryptase</td>
<td>20× magnification (mm²)</td>
<td>Mean ± SD</td>
<td>Cacum, 0.55 ± 0.14</td>
<td>Adult</td>
<td>IBS vs normal control</td>
<td>O’Sullivan et al 32</td>
</tr>
<tr>
<td>Uranyl nitrate azure A</td>
<td>400× magnification mm²</td>
<td>Mean ± SEM</td>
<td>Lamina propria</td>
<td>Adult</td>
<td>IBD vs normal control</td>
<td>Lloyd et al 33</td>
</tr>
<tr>
<td>Azure A</td>
<td>400× magnification Mean</td>
<td>Mean ± SD</td>
<td>Rectal, 2.3 ± 1.1</td>
<td>Adult</td>
<td>UC vs normal control</td>
<td>Sarin et al 34</td>
</tr>
<tr>
<td>Tryptase</td>
<td>400× magnification</td>
<td>Mean</td>
<td>Colon Tryptase, 14.2 ± 3.4</td>
<td>Adolescent/adult</td>
<td>Chronic diarrhea vs normal control</td>
<td>Zare-Mirzaie et al 35</td>
</tr>
<tr>
<td>Toluidine blue</td>
<td></td>
<td></td>
<td>Tryptase, 14.2 ± 3.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IBD, Inflammatory bowel disease; LP, lamina propria; UC, ulcerative colitis.
TABLE V. For a given “working diagnosis,” suggested information to give to the dermatopathologist and findings that may facilitate a diagnosis

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

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, neutromucocutaneous 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...
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 erup-
tiva perstans was not included as a separate entity in the most recent
guideline for CM, it is characterized as reddish-brown, maculo-
papular, 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 measure-
ment 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 bi-
opssies 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. Tissue obtained for direct immunofluorescence should be placed in Michel’s medium or Zeus medium (not formalin) for preservation or directly frozen. 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.

**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. 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. 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). 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. 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. 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. 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. 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; 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 mononuclear cells with abundant pale cytoplasm.81 Immunohistochemical staining with tryptase IHC are 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 hpf, 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.

**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.4,20,90 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.25 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.25 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 hpf, 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.

**What constitutes a normal number of mast cells in various segments of the GI tract has not been well established.**
(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. 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. Another study of 7 patients with SM also showed the colon being most commonly involved, followed by the small bowel. 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. Interestingly, a recent study found that mast cell numbers remained elevated in adults with EoE despite clinical remission with normalization of intraepithelial eosinophilia.

Mastocytic enterocolitis represents a controversial entity, which has yet to be established as a specific clinical diagnosis and has been associated with persistent diarrhea. As described, these patients have no evidence of CM or SM but have increased numbers of duodenal mast cells and often respond to H1 and H2 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.

**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; 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 despite clinical remission with normalization of intraepithelial eosinophilia.

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**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.
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 were not fully established, but they include persistence of skin disease, female sex, and familial mastocytosis.

Patients with Hct 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. In contrast to SM, HoT does not have an increased number of mast cells, but mast cells show subtle morphologic alterations and distribution differences.

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 HoT.

**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 F₂α) biomarker; and pediatric and adult patients with clinical suspicion for WDSM or HoT.

**Eosinophilia.** Eosinophil-associated disorders were classified by the Working Conference on Eosinophil Disorders and Syndromes. Eosinophilia is defined as an elevation of the eosinophil count in peripheral blood, usually above 0.5 × 10⁹ eosinophils/L, whereas HE is defined as eosinophil count above 1.5 × 10⁹ 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.

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 (IHS). 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 B₁₂. Other reasons based on clinical suspicion may prompt the need for a bone marrow biopsy.

**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. 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 1). 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. 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). 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. 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,18-122 bone marrow morphologic features are strong discriminators between reactive HES and neoplastic CEL.23 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 recommendations—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 recommendations—HIC 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. 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). In contrast, KIT mutations outside the tyrosine kinase domain, as well as wild-type KIT, are sensitive to imatinib.27-130

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 recommendations—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 PDGFRα are indicated.119 However, with recent recognition of an increasing number of cryptic PDGFRα rearrangements,138-140 it may be prudent to also perform FISH for PDGFRα 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 PDGFRα, PDGFRβ, 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
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).

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 cells. 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 diagnostic cutoff thresholds for mastocytosis and eosinophilia 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, mm²) 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. Examples include CD30 (brentuximab), CD33 (gentuzumab-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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