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Mast Cell Numbers in Primary Eosinophilic Colitis Are Significantly Higher Than in Secondary Tissue Eosinophilia and Normal Controls: A Possible Link to Pathogenesis

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ABSTRACT

Background: Primary Eosinophilic Colitis (PEC) is one of the rare eosinophilic gastrointestinal diseases with a poorly understood pathogenesis. Eosinophilic esophagitis (EE) is the most common and best-understood disease in this category. Activated mast cells (MCs) have a role to play in the tissue damage in EE. It is not known if PEC shares this mechanism.

Objective: This cross-sectional study aimed to investigate the number of MCs in PEC and to compare them with cases of secondary colonic tissue eosinophilia (TE) and normal colon.

Methods: The study included 19 PEC cases, 47 cases of secondary tissue eosinophilia and 50 normal colon tissues. Histopathological slides of all cases were reviewed to confirm the diagnosis and count the number of eosinophils. Glass slides for all cases were stained for C-kit (CD117) to highlight and count the MCs.

Results: The mean number of the MCs in normal controls was 9.7 MCs per HPF (SD=4.6). The mean number of MCs in the PEC cases was 26.5 (SD=7.1) which was significantly higher than the normal counts (p-value <0.000). The mean number of MCs in the secondary TE group was 18.0 (SD=7.1), whichwas significantly higher than normal controls; P<0.000. Comparing MC counts in PEC and secondary TE also revealed a significant difference with a P value of <0.000.

Conclusion: MCs in PEC are significantly higher than those in secondary TE and normal controls. This suggests the role of the MCs in the pathogenesis of Primary Eosinophilic Colitis.

Keywords: Eosinophilia, Eosinophilic Colitis, Gastrointestinal Diseases, Mast Cells

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INTRODUCTION

Primary Eosinophilic Colitis (PEC) is a rare, poorly understood disease of the colon characterized by an inflammatory infiltrate rich in eosinophils. It can occur in isolation or as part of generalized eosinophilic gastrointestinal diseases (1) that include eosinophilic esophagitis (EE), eosinophilic gastritis, eosinophilic enteritis, and primary eosinophilic colitis (PEC) (2).

PEC is the rarest and least understood entity within the spectrum of eosinophilic gastrointestinal diseases. There is no consensus on the criteria for diagnosing PEC, however, most researchers define PEC as suggested by Tally et al (3) in1990 as the presence of tissue eosinophilia (TE) defined as increased eosinophils in colonic biopsies, which is present in symptomatic patients, and cannot be explained by secondary causes of eosinophilia, including infections, drug reactions, inflammatory bowel disease, autoimmune diseases and cancer (4), (5). The most common presentation in patients with PEC is diarrhea and/or abdominal pain.

Apart from eosinophilic esophagitis, the pathogenesis of eosinophilic gastrointestinal diseases is not well understood but food allergy is thought to have a role to play. The pathogenesis of eosinophilic esophagitis is believed to be related to non- IgE mediated food allergy caused by loss of tolerance to certain foods resulting in downregulation of regulatory T cells (Treg) and a shift to T helper 2 lymphocytes. This subgroup of T helpers produces cytokines including IL 5 which recruits eosinophils, (6, 7) IL9 which acts as a growth factor for mast cells (8), and IL4 and IL13 that stimulate mast cells (9). As such mast cells are increased in EE (10) and their chemical mediators play a vital role in cell damage via immunologic and inflammatory mechanisms.

The pathogenesis of PEC in adults is not properly known and the effect of MCs has not been investigated in the literature except in a case report describing the accumulation of MCs in a patient with PEC (11). If MCs were found to be significantly increased in PEC, this might shed light on the pathogenesis of PEC and help in investigating the effect of new therapies like mast cell stabilizers.

Due to the role of MCs in the pathogenesis of EE and their effect on stimulating eosinophils, we hypothesize that MCs increase in PEC and probably contributes in its pathogenesis. This study aims at investigating the number of MCs in PEC and to compare them to normal controls and to cases of secondary TE to find out if there is any association between MCs and PEC. If such an association exists this will strengthen the suggestion that PEC shares the same pathogenesis of EE, namely a non-IgE mediated food allergy modulated by T helper 2 lymphocytes and MCs.

The second aim of this study is to establish the number of MCs in normal colonic biopsies among the Jordanian population, an area that was not studied before.

MATERIALS AND METHODS

Study Design and Ethical Considerations

This is a retrospective cross-sectional study that was conducted at the Jordan University Hospital (JUH) and covered the period from the first of January 2016 to the first of Mach 2019.

The study has been approved by the School of Medicine, the University of Jordan, and the Committee of the Institutional Hospital Council of Jordan University (IRB). The authors paid for the project themselves; no outside funding was obtained.

Samples

The study included a total of 116 cases; 19 PEC, 47 secondary tissue eosinophilia, and 50 normal controls.

The computerized system in the Histopathology Department at JUH was searched for colonic biopsies diagnosed with tissue eosinophilia (130 cases). Patients' electronic clinical records were then checked

out to find out the final diagnosis of these patients. Tissue eosinophilia in the majority of them was because of secondary causes, leaving 22 patients qualified for the diagnosis of PEC, defined as tissue eosinophilia of at least 20 eosinophils per high power field (HPF) in the colonic biopsies of symptomatic individuals who underwent a full clinico-pathological assessment to rule out secondary causes of TE. This assessment included discussion at the regular clinico-pathological meetings held in the gastrointestinal unit at JUH. Because parasitic infections are relatively common in Jordan, wet mount stool analysis is a part of the routine investigation of patients with diarrhea and abdominal pain; all 22 cases had negative stool analysis for ova and parasites. Of the 22, two paraffin blocks were missing (cases referred from the private sector and blocks returned after histological diagnosis) and in one case, there was not enough tissue in the block for immunohistochemical assessment. As such 19 cases of PEC were included in this study.

In the same period, 108 cases of secondary TE were diagnosed in our lab, 12 of which were drug-induced, and these were included in this study. Among the others, 60 were inflammatory bowel disease cases, 33 of these were Ulcerative Colitis (UC) type and 27 were Crohn's disease (CD) type. To reduce the costs, we randomly selected around 60% of each type, as such 19 UC and 16 CD cases were included.

The normal controls were selected from normal margins of colorectal cancer resection specimens. The histopathological reports and patients' electronic clinical data were reviewed to exclude cases where there was underlying inflammatory bowel disease or any history of inflammatory or functional gastrointestinal disorders.

For all categories, the PEC, secondary TE, and normal controls, essential demographic data, age, gender, clinical history, and biopsy site were recorded. For the PEC cases, clinical records were reviewed, and a history of allergy and blood eosinophilic count (peripheral eosinophilia) were recorded.

In the majority of cases, the exact site of the biopsies was not available. The routine practice in our hospital is to send colonic biopsies in a single container labeled as random colonic biopsies which would include biopsies from the right and left sides of the colon.

Histopathology Assessment and Eosinophil Counts.

The Hematoxylin and Eosin (H& E) slides for all cases were retrieved from the histopathology lab archives and reviewed to confirm the diagnosis. The number of eosinophils was counted in five high power fields and the mean number of the 5 fields was recorded The eosinophilic count was performed applying a 10X ocular lens and a 40X objective lens resulting in 400-fold magnification with a field area of 0.24 mm^2 via an Olympus BX51 microscope.

Immunohistochemistry

As MCs cannot be reliably identified on slides stained with the routine Hematoxylin and Eosin stains, immunohistochemical methods are needed to detect them. In this study, a C-Kit stain has been made use of to highlight mast cells. A C-kit (CD117) is a proto-oncogene that encodes a transmembrane tyrosine kinase receptor which is important for mast cell differentiation and maturation (10, 12). A C-kit is expressed in all MCs regardless of their state of maturation and activation (13, 14). Four-micrometer sections from each paraffin block were cut and mounted on coated glass slides and stained with C-kit (CD117) using a rabbit's 100-200 microliter primary antibody CD117 cloneYR145 (Biogenex company) and visualized by Biogenix detection kit. The normal control slides were prepared by tissue microarray using array-mold kit IW-110 to obtain 2 mm fragments from each paraffin block. Positive controls from gastrointestinal stromal tumors were done with each immunohistochemistry run. Membranous staining was counted as

positive staining. The number of MCs was counted in five high power fields and their mean was recorded in the same manner and the same microscope was used for counting eosinophils

Statistical Analysis

The data was presented on a Microsoft Excel sheet, version 16.12. Categorical data were presented as numbers and percentages. The mean, median, and standard deviation were calculated for continuous data. Two-tailed t-test has been utilized to compare the means of variables between the cases and the controls. A significant p-value was considered to be <0.05. The Receiver Operating characteristic curve (ROC curve) for mast cell numbers was plotted using the pivot table in an excel sheet. Sensitivity and false-positive values were calculated to determine the cutoff point of normal mast cells. To determine the significance of the ROC curve values, the area under the curve (AUC) was divided into five categories, 0.90–1 which is considered as excellent, 0.80–0.90 as good, 0.70–0.80 as fair, 0.60–0.70 as poor, and 0.50–0.60 as fail (15).

RESULTS

Cases Included

A total of 66 TE cases (19 primary, 47 secondary) as well as 50 normal controls, were included in this study. PEC was diagnosed after a full clinico-pathological review and discussion in the clinicopathological meetings to rule out secondary causes of TE. Eight (42%) of the PEC cases had an allergy, 3 (38%) of which were as a result of milk. Four cases (21%) had peripheral eosinophilia. Table 1 describes the demographic features, history of allergy, and peripheral eosinophilia in the PEC cases.

Table 2 describes the demographic data of all cases and controls.

Eosinophil Counts

Eosinophils were counted in all cases in five high power fields (Figure 1). The mean number of eosinophils in the PEC cases was 34.8, which was significantly higher than that in secondary TE cases (26.5), P=0.032

Table 3 details eosinophil counts in PEC, secondary TE, and each category of TE as well as the P values.

Table 1. Peripheral eosinophilia and allergic history for the PEC cases.

Case Number	Age	Gender	Allergy	PE %
1	44	F	None	1.5
2	47	F	Milk	1.3
3	31	M	None	0.5
4	46	F	None	0.9
5	49	F	Eczema	17.2
6	67	F	None	3.8
7	35	M	Milk asthma	7.4
8	15	M	None	2.9
9	65	F	None	0.9
10	67	M	Drug	2.1
11	50	M	Milk	0.4
12	15	M	None	2.6
13	55	M	Egg	5.4
14	58	M	None	3.8
15	25	M	Fish	13.9
16	62	M	None	2.1
17	16	F	Atopy	1.3
18	87	F	None	5
19	21	F	None	3.6

PE: peripheral eosinophilia; Normal eosinophils in blood 5%

Table 2. Demographic features of cases and controls included in the study.

Category	Number of cases	M: F	Age range	Mean age	Median age	SD
PEC	19	1:0.9	15-87	45	47	20.6
Drug induced	12	1:1.4	19-71	43.3	43	18.2
Ulcerative colitis	19	1:1.1	18-60	41.5	44	13.4
Crohn disease	16	1:1.6	15-74	39.5	43	15.5
Normal controls	50	1:0.9	28-72	54.7	54.5	10.8

PEC; Primary eosinophilic colitis, M: Male, F: Female, SD: Standard deviation

Table 3. number of eosinophils per HPF in cases of primary and secondary TE.

	Maximum	Minimum	Mean	Median	SD	P value PEC vs category
PEC	66.6	18	34.8	26	14.5	
Drug induced	49.4	20.6	30.8	30	8.0	0.393
UC	47	8	29.5	27.2	12.3	0.232
CD	66.2	4.6	20.0	15.2	16.2	0.007*
All	66.2	4.6	26.5	27.2	13.6	0.032*

PEC: Primary eosinophilic colitis, UC: Ulcerative Colitis, TE: Tissue eosinophilia, SD: Standard deviation, *significant

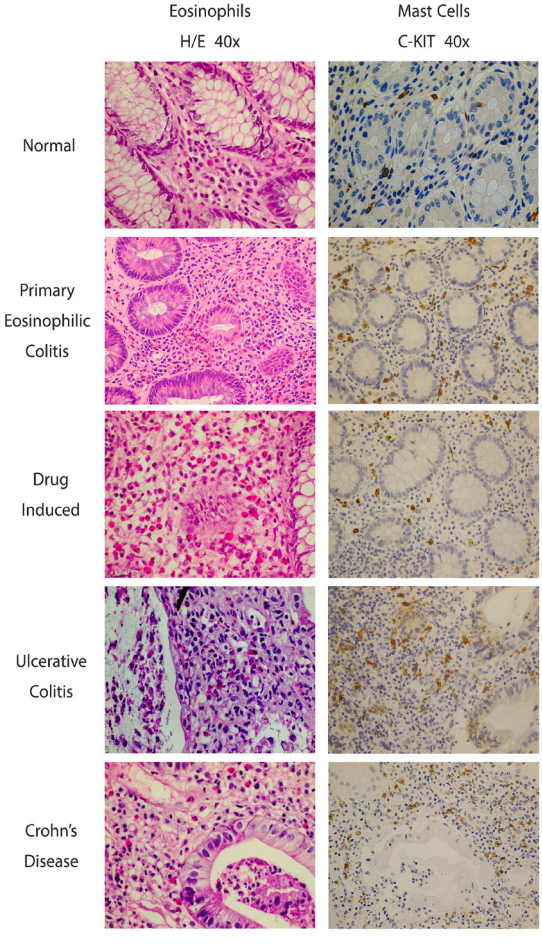

Figure 1. Eosinophils and mast cells in all categories included in the study.

Mast Cell Counts

Mast cells have been counted in all cases in five high-power fields using a C-kit stain (Figure 1). In the 50 normal control cases, the mean number of mast cells in colonic mucosal biopsies was 9.7 MCs per HPF. Table 4 details mast cell counts in normal biopsies and compares their numbers among genders. In the normal biopsies, mast cells tended to accumulate in the deeper mucosal layers, close to the muscularis mucosa. They were scarce within the superficial mucosal layers, close to the lumen.

The mean of normal MC +2SD was 18.9 eosinophils /HPF. ROC curve graph (Figure 2) was plotted to determine sensitivity (true positive values distinguishing abnormal from normal) and specificity (1-fasle-positive). The area under the curve (AUC) was 0.82 regarded as excellent. (15). ROC curve suggests that the mean+2SD (19 eosinophils per HPF) achieves a low sensitivity (56%) but only a 4% false-positive rate (96% specificity). The mean of normal MCs+1SD is 14.3. This point gives an 80% sensitivity and 22% false-positive rate (78% specificity). This latter point seems to be a better cut-off point to define

Table 4. Mast cell numbers in normal colonic mucosa

	All Cases	Males	Females
Maximum	21.2	21.2	18.2
Minimum	3.6	4.1	3.6
Mean	9.7	10.5	8.9
Median	8.6	9.5	7.7
Standard deviation	4.6	5.0	4.2

P value between male and female counts: 0.21

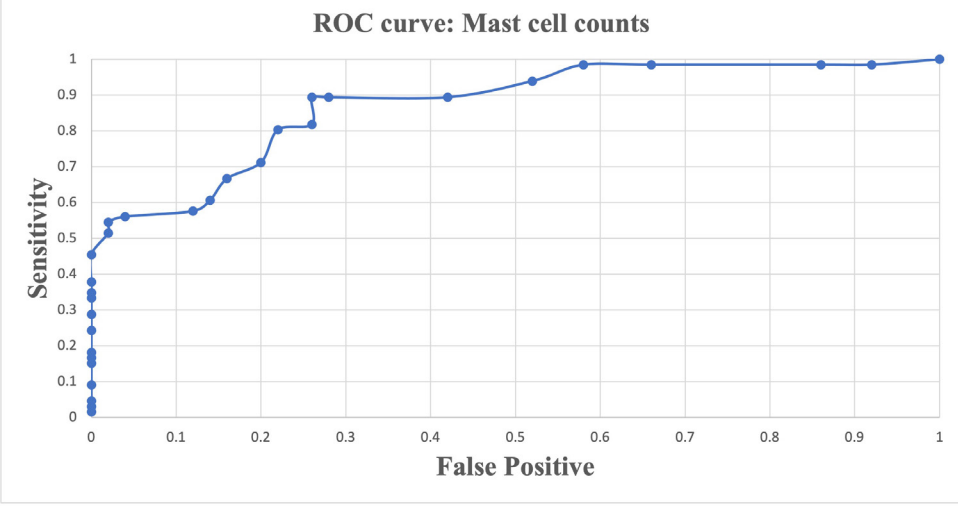

Figure 2. ROC CRVE: Mast cell numbers in normal biopsies versus in primary and secondary TE cases.

Table 5. Comparison between mast cell counts among PEC and secondary tissue eosinophilia cases

	Maximum	Minimum	Mean	Median	SD	P value PEC vs category
PEC	44.6	14	26.5	27	7.1	
Drug induced	33.8	8.4	18.4	15.1	9.1	0.010*
UC	30.3	8.1	17.8	16.2	5.6	0.000*
Crohn	32.3	7.6	20.1	21.1	7.5	0.013*
All	33.8	7.6	18.0	17.2	7.1	0.000*

PEC: Primary eosinophilic colitis, UC: Ulcerative colitis, SD: Standard deviation, *significant

the upper limit of normal mast cells. Using counts less than 14 as a cut-off point achieves higher sensitivity (for example 82% for 13/HPF and 90 for 10 /HPF, however, these will result in higher false-positive rates (26% and 42% respectively) whereas employing higher points decrease false-positive (20% for 15 and 0% for 22 but the sensitivity will drop to 71% and 45% respectively).

In the TE group, mast cells appeared to be more evenly distributed with no predilection for the deeper mucosal layers. This applied to both primary and secondary TE cases. The mean number of MCs in the PEC cases was 26.5. This is significantly higher than the normal counts with a P<0.000. The mean number of MCs in the secondary TE group was 18.0, again this is significantly higher than normal controls; P<0.000.

As Table 5 shows, comparing MC counts among PEC and secondary TE also revealed a significant difference with a P<0.000. The table also demonstrates the statistically significant difference between the number of MCs between PEC and each subgroup of secondary TE.

In all TE cases, MCs were present within the lamina propria and only occasionally seen attacking colonic crypts (mast cell cryptitis). They were not present within the crypts (crypt abscesses) in any of the cases.

DISCUSSION

This study which included 19 PEC cases, 47 secondary TEs, and 50 normal controls, indication that mast cell counts are significantly higher in PEC than in secondary TEs and normal controls which suggests a role of mast cells in the pathogenesis of PEC. The mean number of mast cells in PEC is 26.5 compared to 9.7 in normal controls ($P<0.000$) and 18.0 in secondary TEs ($P<0.000$).

Mast cells (MCs) are bone marrow-derived inflammatory cells that are present in all vascularized tissues, except the retina and the central nervous system. MCs are concentrated in mucosal barriers, including the gastrointestinal tract, skin, and respiratory mucosa (12, 16). They are involved in the adaptive immune response where they are activated by immunoglobulin E (17) and in the innate immunity where their activation is initiated by microbial antigens and products of cell damage (16). Once activated, the preformed and newly synthesized mediators act to perform diverse mast cell functions, one of which is the recruitment of eosinophils (16). MCs also have a phagocytic activity mainly against bacteria (18). Within the gastrointestinal tract, in addition to their role in immunity and inflammation, MCs are important for intestinal homeostasis; their chemical mediators maintain epithelial integrity and regulate muscle peristalsis, blood flow, and coagulation as well as healing and fibrosis (12, 16, 19).

The role of mast cells in the pathogenesis of PEC has not been adequately studied in the literature. Several studies documented an increase in Mast cell numbers in several gastrointestinal diseases including inflammatory and functional gastrointestinal disorders such as irritable bowel syndrome (20, 21).Ulcerative Colitis (22) and Crohn's disease (23). Besides their increased numbers, the activation and degranulation of MCs were demonstrated by electron microscopy in cases of irritable bowel syndrome (24). Increased mast cell numbers were reported in eosinophilic esophagitis but have not been studied in the other entities of eosinophilic gastrointestinal diseases including PEC.

The pathogenesis of EE is believed to be the outcome of a non-IgE mediated food allergy, where there is a loss of tolerance to food allergens that downregulate regulatory T cells (Treg) and stimulate T helper 2 lymphocytes. These stimulated T helpers produce cytokines including the IL9 which acts as a growth factor for mast cells (8, 9), the IL4 and 13 that stimulate mast cells (9), and the IL 5 which recruits eosinophils (6, 7).

Our study documents an increase in mast cells in PEC, a feature known to be seen in EE. Previous literature has shown that the plasma levels of two T helper 2 cytokines (IL5 and IL15) are elevated in cases of eosinophilic gastroenteritis (25). These findings support the assumption that these diseases share the same pathogenesis, and the clinical resemblances bolster this argument even further between EE and PEC which include association with atopic conditions (26). Notably, the PEC cases in our study show a high association with allergic conditions, mainly food allergies.

As we hypothesize that PEC is aided by mast cells, management options might be included utilizing mast cell stabilizers. Sodium cromoglycate (cromolyn) which blocks the release of mast cell mediators has been reported to be effective in treating eosinophilic gastroenteritis in some studies (27, 28). Ketotifen which is another mast cell stabilizer was applied to treat eosinophilic gastroenteritis and was reported to improve symptoms and decrease IgE antibodies and tissue eosinophilia (29). To assess the role of such therapeutic approaches, more prospective trials are needed in cases of PEC.

A secondary result of our study was to

establish the number of mast cells in normal colonic biopsies among our population. In our sample of 50 normal control cases, the mean number of mast cells was 9.7 MCs per HPF. (SD=4.6 and mast cells tended to accumulate in the deeper mucosal layers, close to the muscularis mucosa. They were scarce within the superficial mucosal layers, close to the lumen. ROC curve analysis shows that the best cut-off point to define normal MCs is 14 MCs per HPF, this is almost equal to the mean of normal MCs+1SD (14.3). This point gives an 80% sensitivity and 78% specificity (22% false-positive rate). The mean of normal MC+2SD is 18.9, this point achieves a low sensitivity (56%) but only a 4% false-positive rate (96% specificity). This is a point that we do not advocate using as it has a low specificity and will result in a high false-negative rate.

Previous studies have tried to establish the number of normal MCs within colonic biopsies using different immunohistochemical methods to demonstrate them, as they cannot be reliably spotted on hematoxylin and eosin-stained slides. Using mast cell tryptase as an immunohistochemical marker for mast cells, Jakate et al reported a mean number of 13.3 MCs per high power field (HPF) in 50 normal biopsies, the standard deviations (SD) was 3.5. Patients with more than 20 MCs /HPF (mean plus two SD) were considered to have increased mast cells (30). Other researchers also consider 20 mast cells per HPF as the upper normal limit (13). Sethi et al reported the mean number of MCs in 76 cases of chronic diarrhea of unknown etiology as 31 per HPF, compared to 24/ HPF in 89 normal controls ($P<0.001$) (20). Saad et al found a mean of 17.56±7.28 mast cells per HPF in the descending colon and 14.5±6.35 in the rectosigmoid among the pediatric age group (31).

The variation IN in normal numbers between studies can be explained by geographical variation or by the method of detecting them by various unohistochemical stains.

Our research is significant since it is the first of its kind in the literature to document a statistical difference in mast cell numbers in PEC cases compared to secondary TEs and normal controls. It is also the first study to investigate it in our region concerning the number of normal mast cells in colonic mucosal biopsies.

LIMITATIONS

This is a single institution study with a relatively small number of cases; however, our sample is comparable to those in the previously published literature. Acquiring larger samples in a rare disease like PEC is difficult. Another drawback is that we were unable to pinpoint the specific location of the biopsies. Colonic biopsies are routinely sent in a single container at our institution labelled as random colonic mucosa which includes biopsies from the right and left sides of the colon. This technique is widely used in normal histopathological practice. Although eosinophil counts are known to be higher in the right than in the left colon (32) MC counts were reported to vary among segments of the colon but with no specific pattern (31). Our findings are consistent with what many histology laboratories do on a daily basis.

CONCLUSION

MCs and eosinophils contribute to the pathogenesis of PEC. The increase in mast cells documented in our study, paired with the previously reported increase in T helper 2 cytokines in eosinophilic gastroenteritis, suggest that PEC shares the same pathogenesis of EE which is a non-IgE mediated food allergy. Our results explain the previously reported effect of mast cell stabilizers in treating eosinophilic gastroenteritis and open up the possibility of further trials to study the role and effectiveness of this therapy line in PEC.

The mean of normal MCs+1SD is 14.3.

This point gives an 80% sensitivity and 22% false-positive rate (78% specificity) and seems to be a sensible cut-off point to define the upper limit of normal mast cells. This needs to be evaluated carefully, however, as the actual biopsy location is not taken into account.

Conflicts of Interest: None declared.

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