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HISTOLOGY AND HISTOPATHOLOGY

ISSN: 0213-3911
e-ISSN: 1699-5848

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DOI: 10.14670/HH-18-708
Article type: ORIGINAL ARTICLE
Accepted: 2024-01-11
Epub ahead of print: 2024-01-11

Title

Quantification of eosinophils in the lower gastrointestinal tract of adults: a review of surgical specimens with normal histology from the Free State province, South Africa

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Short title: Eosinophils in the lower GIT of adults

Abstract

Aim. Eosinophils are normal residents of the gastrointestinal tract (GIT). They are noted in small numbers with significant variation between anatomic locations. An idiopathic increase of eosinophils is known as eosinophilic gastrointestinal disease (EGID). EGIDs are a heterologous group of disorders that produce a range of enteric and colonic syndromes. Their incidence has been increasing worldwide. Our study aimed to quantify eosinophils in each segment of the GIT in surgical specimens with normal histology to facilitate the histological diagnosis of EGID. Similarly, we aimed to describe the effect of race and gender on gastrointestinal eosinophil numbers.

Methods. A retrospective, quantitative comparative study was performed. We assessed 360 surgical specimens with normal histology from the lower gastrointestinal tract of African and Caucasian adults from the Free State Province, South Africa. The number of eosinophils per mm^2 was counted.

Results. Overall, comparable eosinophil values were noted for both males and females, and African and Caucasian South Africans. However, Caucasians recorded a higher concentration of eosinophils in the appendix and the left colon. Eosinophils were most numerous in the lamina propria, with only small numbers present in the epithelium. Our results show that the South African population has similar eosinophil distribution trends to international studies. However, South Africans had far fewer eosinophils than Japanese and North American adults in each segment.

Conclusions. Specific eosinophil reference ranges were formulated to quantify reference ranges of eosinophils in the lower GIT, allowing for the accurate diagnosis of EGIDs in our population in future.

Keywords: eosinophils; gastroenteritis; eosinophilic gastrointestinal disease; density

List of abbreviations

AIDS	acquired immunodeficiency syndrome
EGID	eosinophilic gastrointestinal disease
GIT	gastrointestinal tract
HIV	human immunodeficiency virus
HPF	high-power field
HSREC	Health Sciences Research Ethics Committee
IQR	interquartile range
mm^2	millimeter squared
NHLS	National Health Laboratory Service
SNOMED	Systematized Nomenclature of Medicine
UFS	University of the Free State

Introduction

Eosinophilic gastrointestinal diseases (EGIDs), first described by Kaijser (1937), are complex disorders that include eosinophilic esophagitis, gastritis, enteritis and colitis. These conditions are characterised by the pathological increase of eosinophils in the gastrointestinal tract (GIT). Initially thought to be rare diseases limited to Western countries (Matshusita et al., 2015), the prevalence of EGIDs has been increasing worldwide. EGIDs are closely associated with allergic disease and are believed to be caused by an abnormal type 2 immune response to food antigens (De Brosse et al., 2006). As the rate of allergies increases, the incidence of EGIDs is expected to escalate (Strachan, 1989).

Eosinophilic esophagitis remains the only EGID with standardised histological criteria (Furuta et al., 2008). The diagnosis in other segments of the GIT is often subjective, relying on pathologist experience and the exclusion of secondary causes, such as parasitic infestation, inflammatory bowel disease, certain drugs and malignancies.

Accurate data regarding the normal distribution of eosinophils in the lower GIT are required to create reproducible histological criteria for EGIDs in our patient population. Several studies have investigated the possible association between eosinophil density, age, gender, race and geographic region. Pediatric studies described a similar distribution of eosinophils compared to their adult counterparts but revealed that children have lower concentrations (Turner et al., 2017; Silva et al., 2018). Additionally, studies have failed to show a statistically significant difference between genders for both adults and children (Okpara et al., 2009; Lwin et al., 2011; Saad, 2011). Studies investigating race and geographic region have noted that densities among Asian Japanese, Hawaiian and Japanese American populations are comparable for all regions of the GIT. However, a small significant difference has been described for esophageal biopsies. This may be due to variable allergen exposure, genetic polymorphisms and interfering medical conditions (Matsushita et al., 2015). A study from the United States of America (USA) described the distribution of eosinophils in children's gastric mucosa from the northern and southern states. They revealed similar values between the groups and found, at least for the stomach, that geographic region was not a significant variable (Polydorides et al., 2008). Most studies recommend that each laboratory produces a population-specific reference range for eosinophils in each GIT segment (Pascal et al., 1997; Polydorides et al., 2008; Matsushita et al., 2015; Turner et al., 2017). However, to our knowledge, no such study has been conducted in South Africa or the

remainder of the continent.

This study aimed to quantify eosinophils in the lower GIT of adults from the Free State Province in central South Africa and describe the relationship between eosinophilic density, gender and race. With this data, proposed eosinophilic reference ranges could be formulated for our population.

Materials and methods

Study design

A retrospective, quantitative comparative study was performed. We reviewed histologically normal surgical specimens and biopsies of the lower GIT submitted to the Department of Anatomical Pathology, National Health Laboratory Service (NHLS) and Faculty of Health Sciences, University of the Free State (UFS), Bloemfontein, South Africa, for possible inclusion in the study.

According to Statistics South Africa, the two largest racial groups in the Free State Province are Africans (88.7%) and Caucasians (8.5%) (Statistics South Africa, 2016). Therefore, these groups were included in the study, while other race groups were excluded due to insufficient patient numbers for statistical analysis.

For the purpose of this study, the right colon extended from the cecum to the beginning of the splenic flexure, and the left colon extended from the splenic flexure to the rectum.

Cases were identified by searching the NHLS laboratory information system for appropriate Systematized Nomenclature of Medicine (SNOMED) codes. Cases were selected from most to least recent until 30 patients were collected for each race group (30 x 2 = 60), gender (60 x 2 = 120) and gastrointestinal segment (120 x 3 = 360). Consequently, a total of 360 cases were selected from October 2014 to March 2020. In the event of multiple biopsies from a single patient (whether from numerous locations or performed on different days), only one biopsy was selected at random.

Surgical specimens obtained from patients with underlying medical conditions were included in our study. To minimise variables, our study design closely followed several international studies that aimed to determine eosinophil numbers from surgical specimens (De Brosse et al., 2006; Polydorides et al., 2008; Matsushita et al., 2015; Turner et al., 2017; Silva et al., 2018). Firstly, in the case of large excision specimens, we only evaluated sections from surgical margins that

represented macroscopically normal regions. Secondly, we only included cases where the final pathological diagnosis for the segment did not indicate gastrointestinal disease.

Laboratory procedure

Potential cases without an available haematoxylin and eosin-stained section or without available tissue for obtaining a section for histological assessment were excluded. Furthermore, patients with conditions known to influence eosinophil concentrations, such as organic gastrointestinal diseases, parasitic infections, atopy or allergy, haematopoietic malignancies, or patients using steroids or immune modulators, were excluded.

Demographic data, including age, sex, race, biopsy location, chronic conditions and the indication for surgery or colonoscopy, were collected from the pathology reports. Patients with incomplete data were excluded from the study.

Slides were evaluated for adequacy by two investigators (JD and JG). Slides were recut and stained as necessary. Once optimal slides were obtained, JD counted eosinophils from four randomly selected high-power fields (HPFs) (at 400X power representing 0.245 square millimeter [mm^2]) with both lamina propria and epithelium. Only eosinophils with both an identifiable nucleus and eosinophilic granules were counted, and those within lymphoid follicles or blood vessels were excluded. The number of eosinophils in the epithelium and lamina propria were quantified and entered into a data sheet. The total number of eosinophils per mm^2 was calculated by adding the number in four random HPFs and multiplying it by 1.02.

Statistical analysis

Statistical analysis was performed by the Department of Biostatistics, UFS. Results were summarised by frequencies and percentages (categorical variables) and medians and interquartile ranges (IQRs) (numerical variables due to skew distributions). Reference ranges (2.5^{th} to 97.5^{th} percentiles) were calculated non-parametrically. All analyses were performed using statistical analysis software SAS, version 9.4 (SAS Institute Inc.; Cary, NC).

Ethical considerations

Approval to conduct the study was obtained from the Health Science Research Ethics Committee (HSREC) of the University of the Free State (reference number UFS-HSD2019/0670/0110).

Permission to access patient slides and data was obtained from the business manager of the NHLS, Universitas Academic Laboratories.

Results

The median age of the patients was 50 years (IQR 34-63) and ranged from 18 to 95 years. Males had a median age of 50 years (IQR 36-64) and females 49 years (IQR 31.5-61). The median age of African and Caucasian patients was 44 (IQR 30-56) and 58 (IQR 39-69) years, respectively. The median age of patients with suspected appendicitis was 29 years (IQR 22-40), which differed markedly from those who underwent surgery for other indications ($p<0.0001$). The median age for other indications ranged from 53 years for general surgery to 61 years for colonic malignancy. Overall, Caucasian patients were older than the African group. This difference was most striking in the ascending colon. The most notable gender difference was observed in the cecum, where males were older than female patients. The difference between male and female patients for the ascending colon and sigmoid was negligible.

The indication for surgery or biopsy is summarised in Table 1. Most specimens were retrieved from patients with suspected appendicitis (n=93; 25.8%). General surgery included emergency surgery for bowel obstruction, trauma surgery and urological and gynaecological procedures (excluding those performed for malignancies). This group comprised the second largest group (n=91; 25.3%). Examples of non-specific gastrointestinal symptoms included dysphagia, diarrhea and weight loss, evaluation for infection, surveillance of carcinoma and therapeutic polypectomies. Carcinomas of the bladder, ovary and kidney comprised the extra-colonic malignancy group.

The most common indications for surgery in African female patients were suspected appendicitis and general surgery (n=29; 32.2%). Caucasian females most frequently underwent surgery or biopsy for non-specific gastrointestinal symptoms (28.9%). In males, African patients underwent general surgery (n=30; 30.0%) most frequently, whereas more Caucasian males underwent surgery for colonic malignancy (n=25; 27.8%). The least common indication for surgery in all groups was extra-colonic malignancies (n=18; 5.0% overall).

Specimens from the right and left colon were further subdivided into anatomic locations. Biopsies from the right colon included 42 (35.0%) specimens from the cecum and 78 (65.0%) from the ascending and proximal transverse colon. Biopsies from the left colon included 32

(26.7%) from the distal transverse and descending colon, 60 (50.0%) from the sigmoid and 28 (23.3%) from the rectum. Biopsies from the appendix were not specified further. The number of biopsies collected from each location was similar with regard to race and gender. Table 2 demonstrates the number of eosinophils in the lamina propria, epithelium and the total number per mm^2 for all patients.

Eosinophils were present in the lamina propria in varying concentrations. The highest concentration was observed in the appendix (median 59.2 eosinophils/mm^2), followed by the cecum. Moving distally, fewer eosinophils were present. In general, a small peak was noted in the sigmoid colon, followed by a sharp decrease in the rectum. Overall, the total density of eosinophils in the right colon was significantly higher than those in the left colon ($p=0.0016$).

As shown in Figure 1, numerous eosinophils were identified within the lamina propria (green arrows), but were scanty in the epithelium (see inset). Overall, very few eosinophils were present within the epithelial compartment of all GIT segments. The highest epithelial concentration of eosinophils was noted in the appendix of a single patient (5/mm^2).

The number of intraepithelial eosinophils was similar between races, genders and GIT locations, and no statistically significant differences were identified ($p>0.05$). Table 3 compares the number of eosinophils for both race groups. Each race had comparable median values for the majority of locations. However, differences between races for the left colon and appendix were statistically significant. In both these sites, more eosinophils/mm^2 were recorded among Caucasian patients.

Median values and quartiles were comparable between genders, and no statistically significant differences were identified (Table 4). In addition, no significant gender differences were found per race group.

Per gender, significant differences between racial groups were found. In females, Caucasian patients had higher eosinophil concentrations in the appendix than African patients ($p=0.0032$). In males, Caucasian patients had higher values in the appendix ($p=0.0413$), ascending colon ($p=0.0294$) and rectum ($p=0.0492$).

Figure 2 demonstrates the eosinophil density based on the indication for surgery or biopsy. The highest number of eosinophils was noted in a patient with suspected appendicitis (256 cells/mm^2). Indication groups with larger eosinophil densities included extra-colonic malignancies and suspected appendicitis. Non-specific colonic symptoms and colonic malignancies had the lowest concentration. These differences were statistically significant

($p<0.001$).

Proposed reference ranges

We proposed the 2.5th and 97.5th reference range for each GIT segment as shown in Table 5. As a statistically significant difference has not been noted, these reference ranges applied to both genders. The appendix and left colon values showed a statistically significant difference between Africans and Caucasians; therefore, their total and individual values are presented for each race.

In contrast to the lamina propria, the reference ranges for intraepithelial eosinophils for all segments, races and genders were comparable. The ascending colon had the narrowest range (0-1 eosinophil/mm^2), and the appendix the widest range (0-4 eosinophils/mm^2). For each of the remaining regions, a range of 0-3 eosinophils/mm^2 was reported. These values were small and had little effect on the total number of eosinophils per mm^2, meaning that the reference ranges for the lamina propria and total number were comparable. Subsequently, only the reference ranges for the total number of eosinophils per mm^2 have been discussed in Table 5. The proposed reference range for intraepithelial eosinophils was < 5 /mm^2 for all locations, races and genders.

Discussion

To our knowledge, this study is the first of its kind to assess eosinophil reference ranges in the GIT of South African adults. In keeping with international studies (Furuta et al., 2008; Saad, 2011; Turner et al., 2017), eosinophil density is greatest in the cecum, appendix and ascending colon, with a gradual decline towards the sigmoid colon. International studies identified a small peak in the rectum; however, we noted a peak in the sigmoid. The trend of increased counts in the right colon results from maximal food antigen exposure in the proximal colon (Matsushita et al., 2015). However, several murine studies have identified that eosinophil homing occurs early in prenatal development and that antigen and bacterial exposure are unlikely to influence eosinophil concentration (Polydorides et al., 2008), which may explain the eosinophil peak in the rectosigmoid.

A comparison of our median eosinophil value revealed a ratio of 20:8:5 for the appendix, right colon and left colon, respectively. Polydorides et al. (2008) found that eosinophils in the ascending colon's lamina propria were three times more numerous than in the descending colon. Turner et al. (2017) found this ratio to approximate 2:1, while Matsushita et al. (2015) noted that

eosinophils in the right colon were seven times more numerous than the left. Collins et al. (2018) found that values were more numerous in the right colon than in the rectosigmoid with a ratio of 56:1. It could therefore be agreed that values in the right colon are at least one and a half times as numerous as the left. International studies describing the eosinophil content within the appendix are lacking. However, our findings suggest that the appendix has the highest eosinophil concentration overall, which can be explained by the proposed immunological function of the appendix (Vitetta et al., 2019).

Intraepithelial eosinophils were maximal in the appendix, with the second peak in the sigmoid colon. However, in both locations, counts were far fewer than those of the lamina propria. The number of intraepithelial eosinophils did not significantly differ with regard to location, race or gender, which is in keeping with data from international studies (Matsushita et al., 2015; Turner et al., 2017).

When comparing the role of race on eosinophil density, it is clear that there is a substantial difference between South African and Japanese populations (Matsushita et al., 2015), as shown in Table 6. This information demonstrates that Japanese patients have greater eosinophil concentrations in the right colon than the South African groups. The opposite has been found in the left colon. Wider ranges have also been noted in South African patients.

Table 7 illustrates that eosinophil density has been reported to be far greater in the lamina propria of North American adults (Tuner et al., 2017) than individuals in South Africa. However, the range was wider in South African populations, with the highest concentration in the right colon ($211.1/mm^2$). The intraepithelial concentration was similar between the two nationalities. However, North American adults had more intraepithelial eosinophils in the right colon than the left, with the opposite identified in South African patients (Turner et al., 2017).

Although most GIT segments failed to show a statistically significant difference between African and Caucasian South Africans, the appendix and left colon of Caucasians showed higher median eosinophil counts and upper quartile values. Many studies attempted to explain these differences by investigating the role of genetic polymorphisms (Lamousé-Smith and Furuta, 2006), food antigen and pollen exposure (Lowichik and Weinberg, 1996; Polydorides et al., 2008), infections (Silva et al., 2018) and atopic conditions (Gonsalves, 2007; Ridolo et al., 2016), but concluded that a complex interplay between these factors was most likely.

In the South African setting where certain conditions are more prevalent in Caucasian

populations, such as diverticulosis (Segal and Walker, 1982; Imaeda and Hibi, 2018) and chronic constipation (Sun et al., 2011), it is plausible that these diseases may influence eosinophil concentrations in the lower GIT either through shared risk factors or the disease itself. However, as these conditions become more prevalent in non-Caucasian groups, it is expected that these comorbidities will cease to contribute to racial differences in future. In keeping with previously published data (Okpara et al., 2009; Lwin et al., 2011; Saad, 2011), our study showed that eosinophil count was comparable among genders.

We found a statistically significant difference among patients with different indications for biopsy or surgery. The highest median values were present in patients with suspected appendicitis (59.2/mm^2) and extracolonic malignancy (60.2/mm^2), and the lowest in patients with non-specific gastrointestinal symptoms (19.4/mm^2). It is proposed that acute inflammatory states mimicking appendicitis, or a malignant state requiring laparotomy, would sufficiently stimulate the interleukins, chemokines and colony-stimulating factors required for eosinophil development and their recruitment to the GIT (Weller, 1991; Ramirez et al., 2018). International studies did not describe the relationship between eosinophil numbers and the indication for biopsy.

In the study by Collins et al. (2018), the diagnosis of EGID was based on eosinophil values that were twice the normal peak count per HPF. The specific values reported in the study were as follows: 100/HPF (or 178.6/mm^2) in the cecum and ascending colon, 84/HPF (or 150/mm^2) in the transverse and descending colon, and 64/HPF (or 114.3/mm^2) in the rectosigmoid. Our proposed reference ranges are lower in the right colon (138/ mm^2) and the descending colon (90/mm^2). Collins et al. (2018) combined values from the sigmoid and rectum. However, average values appeared to be similar for the sigmoid and rectum (130/mm^2 and 92/mm^2, respectively).

This study had two main limitations. Firstly, our study relied upon the clinical data supplied by the healthcare provider completing the histopathology request forms. Therefore, certain factors, including the patient's allergic history and medication use, might have been omitted from the request form. As we used a retrospective study design, this limitation could not be overcome. Secondly, specimens were retrieved from patients with underlying pathological states, including conditions affecting the lower GIT. As described above, inflammatory states may influence immune responses and eosinophil recruitment into the GIT. However, to mitigate this potential shortfall, we applied strict selection criteria that mirrored the patient recruitment method of several similar international studies (De Brosse et al., 2006; Polydorides et al., 2008; Matsushita

et al., 2015; Turner et al., 2017; Silva et al., 2018). While it would be preferred to limit specimens from healthy volunteers and cancer screening programs only, our study was restricted by budget constraints and the resources available in the South African healthcare system. Restricting the study to healthy volunteers would have resulted in an insufficient number of specimens to maintain the statistical value of the research.

A future study describing the role of the human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS) on GIT eosinophil concentration is recommended, including the influence of antiretroviral medication and CD4 count. As an estimated 7.5 million South Africans live with HIV (UNAIDS, 2020), we have the unique opportunity to analyse the role of HIV on GIT eosinophils in future studies.

Conclusion

The evaluation of eosinophilia in the GIT is imprecise. Reference ranges are not standardised and are not interchangeable between races and geographic regions (Matsushita et al., 2015; Turner et al., 2017). Furthermore, microscope fields are not standardised, and reference ranges displayed as cells per high-power field (rather than cells per square millimeter) may reduce diagnostic accuracy.

Our study is the first of its kind to describe the distribution of eosinophils in the lower GIT of South African adults. We have shown that the eosinophil distribution trend of South Africans is comparable to international findings and that no significant difference exists among genders. Both South African race groups had similar results for most GIT locations. However, compared to international populations, South Africans have far fewer GIT eosinophils. We suggest that geographic region, diet and chronic medical conditions play a significant role in this finding. Our proposed reference ranges provide a guideline for the accurate evaluation of EGIDs in each segment of the lower GIT for our population, which will contribute to the accurate and timeous diagnosis of patients who suffer from EGIDs in future. Our data were limited to the Free State Province's public health sector, and further studies encompassing other provinces and the private sector are warranted.

Acknowledgements

Dr. Daleen Struwig, medical writer/editor, Faculty of Health Sciences, University of the Free State, for technical and editorial preparation of the article.

Conflict of interest

The authors do not have any conflict of interest to declare.

Funding

No funding was obtained for this study.

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Figure legends

Figure 1. Figure 1. High-power light microscopy of a rectal biopsy (haematoxylin and eosin stain; magnification 400X). The green arrows indicate eosinophils.

Figure 2. The eosinophil density per indication for surgery or biopsy (total number of eosinophils per mm^2).

Table 1. Indication for surgery or biopsy per gender and racial group.

Indication	African		Caucasian		Total (n=360)
	Female (n=90)	Male (n=90)	Female (n=90)	Male (n=90)	
	n (%)	n (%)	n (%)	n (%)	n (%)
Suspected appendicitis	29 (32.2)	24 (26.7)	25 (27.8)	15 (16.7)	93 (25.8)
Colonic malignancy	17 (18.9)	17 (18.9)	24 (26.7)	25 (27.8)	83 (23.1)
General surgery	29 (32.2)	27 (30.0)	15 (16.7)	20 (22.2)	91 (25.3)
Non-specific gastrointestinal symptoms	11 (12.2)	17 (18.9)	26 (28.9)	21 (23.3)	75 (20.8)
Extra-colonic malignancy	4 (4.4)	5 (5.6)	0 (0)	9 (10.0)	18 (5.0)

Table 2. The distribution of eosinophils per segment of the gastrointestinal tract (GIT) for all patients (eosinophils/mm^2).

GIT segment	Lamina propria		Epithelium		Total	
	Median (range)	IQR	Median (range)	IQR	Median (range)	IQR
Right colon (n=120)	24.5 (0–211.1)	13.3–55.1	0 (0–3.1)	0–0	25 (0–214.2)	13.3–55.1
Cecum (n=42)	36.7 (0–211.1)	13.3–62.2	0 (0–3.1)	0–0	37.2 (0–214.2)	13.3–62.2
Ascending colon (n=78)	22.4 (0–152.0)	13.3–47.9	0 (0–1)	0–0	23 (0–152.0)	13.3–48
Left colon (n=120)	14.3 (0–129.5)	5.1–31.6	0 (0–4.1)	0–0	15.3 (0–130.6)	6.1–32.1
Descending colon (n=32)	15.8 (0–89.8)	8.2–40.3	0 (0–3.1)	0–0	15.8 (0–89.8)	8.2–40.3
Sigmoid colon (n=60)	16.3 (0–129.5)	7.1–34.7	0 (0–4.1)	0–0	16.3 (0–130.6)	7.1–36.2
Rectum (n=28)	7.1 (0–88.7)	3.1–23.5	0 (0–3.1)	0–0	7.1 (0–91.8)	3.1–24
Appendix (n=120)	59.2 (1–253)	34.7–92.8	0 (0–5)	0–1	59.2 (1–256)	35.2–92.8

IQR: interquartile range

Table 3. The eosinophil density in different gastrointestinal tract (GIT) segments per racial group (total number of eosinophils per mm^2).

GIT segment	African patients (n=180)		Caucasian patients (n=180)		p-value
	Median	IQR	Median	IQR	
Right colon	20.4 (n=60)	13.8–40.8	31.1 (n=60)	13.3–63.8	0.1473
Cecum	37.7 (n=25)	15.3–51	36.7 (n=17)	13.3–76.5	0.5217
Ascending colon	19.4 (n=35)	13.3–31.6	30.6 (n=43)	13.3–58.1	0.0933
Left colon	11.7 (n=60)	4.1–26.5	16.3 (n=60)	8.2–41.8	0.0424
Descending colon	16.3 (n=13)	5.1–31.6	15.3 (n=19)	11.2–41.8	0.2335
Sigmoid colon	16.8 (n=30)	6.1–26.5	16.3 (n=30)	7.1–46.9	0.7170

Rectum	5.1 (n=17)	2–10.2	17.3 (n=11)	7.1–26.5	0.0987
Appendix	40.8 (n=60)	19.4–79.1	71.9 (n=60)	51–102.5	0.0004

IQR: interquartile range

Table 4. The eosinophil density in different gastrointestinal tract (GIT) segments per gender group (total number of eosinophils per mm^2).

GIT segment	Female patients (n=180)		Male patients (n=180)		p-value
	Median	IQR	Median	IQR	
Right colon	27.0 (n=60)	14.3–54.1	23.5 (n=60)	11.7–56.1	0.7113
Cecum	40.8 (n=21)	17.3–69.4	34.7 (n=21)	11.2–55.1	0.5211
Ascending colon	23.5 (n=39)	14.3–43.9	22.4 (n=39)	12.2–57.1	0.9562
Left colon	14.8 (n=60)	6.6–31.6	16.3 (n=60)	5.1–32.1	0.7152
Descending colon	14.3 (n=20)	8.2–30.1	31.6 (n=12)	12.8–41.8	0.3494
Sigmoid colon	18.4 (n=27)	8.2–63.2	15.3 (n=33)	6.1–30.6	0.2063
Rectum	6.1 (n=13)	2.0–20.4	7.1 (n=15)	4.1–26.5	0.6607
Appendix	60.7 (n=60)	33.7–92.3	58.1 (n=60)	36.2–96.4	0.8625

IQR: interquartile range

Table 5. Proposed 2.5^{th}–97.5^{th} reference ranges for the total number of eosinophils per mm^2 in the lower gastrointestinal tract (GIT) of adults in the Free State Province, South Africa.

GIT segment	All patients	Racial group		Gender group
		African	Caucasian	Male
Right colon	0–138	1–126	0–140	1–137
Cecum	0–129	1–126	0–214	0–214
Ascending colon	0–140	1–152	0–137	1–137
Left colon	0–125	**0–75***	**1–130**	0–92
Descending colon	0–90	0–67	1–90	0–67
Sigmoid	1–130	0–129	1–131	1–118

Rectum	0–92	0–39	0–92	0–92
Appendix	4–194	**1–184**	**4–205**	1–184

*Values presented in bold text indicate where race-specific reference ranges are proposed due to statistically significant differences between the racial groups.

Table 6. Comparison of eosinophil density (eosinophils/mm2) in the colon of Japanese* and South African adult patients included in the study.

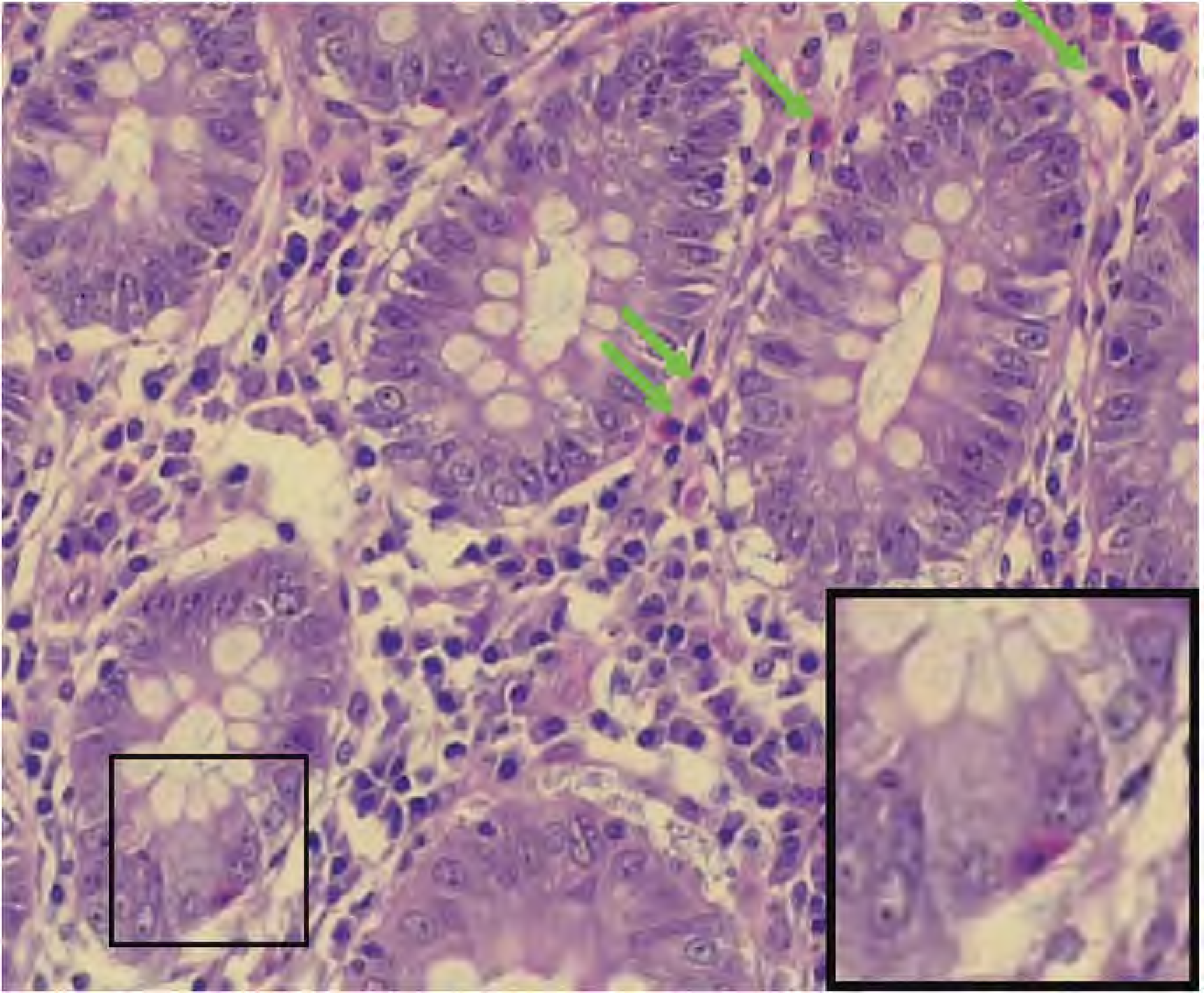
Part of colon	**Japanese (n=25)***	**South African**	
		African (n=90)	**Caucasian (n=90)**
	Median (range)	**Median (range)**	**Median (range)**
Right colon	43.8 (16.9–56.5)	27.0 (1–152)	23.5 (0–214.2)
Left colon	6.3 (0–24.4)	14.8 (0–128.5)	16.3 (0–130.6)

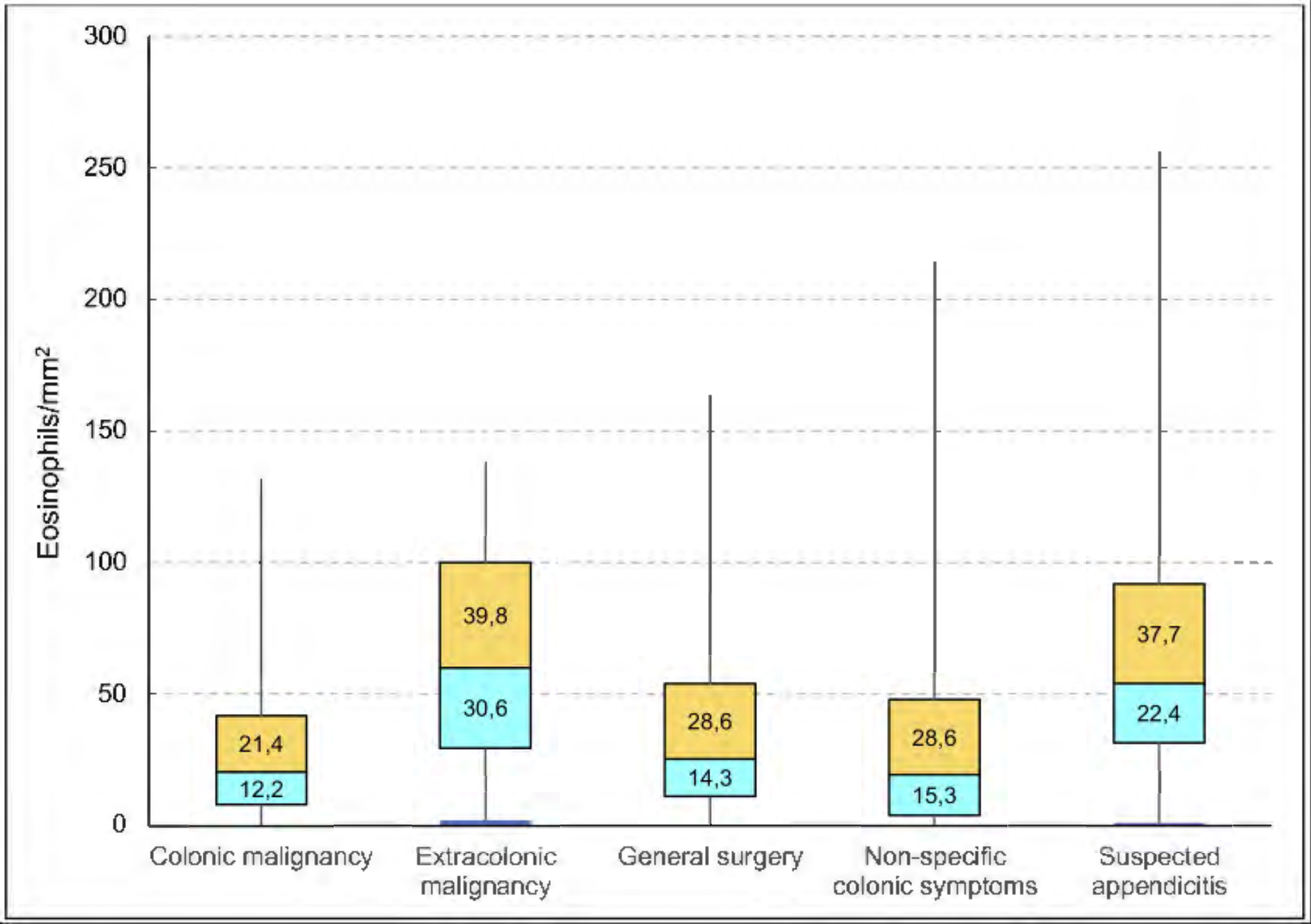
*Matsushita et al., 2015

Table 7. Comparison of colonic eosinophils in the United States of America (USA)* and South African adults (number/mm^2) displayed as medians and ranges

Part of the colon	**Eosinophils in the lamina propria**		**Intraepithelial eosinophils**	
	USA (n=159)*	**South African (n=360)**	**USA (n=159)***	**South African (n=360)**
	Median (range)	**Median (range)**	**Median (range)**	**Median (range)**
Right colon	51 (4–131)	24.5 (0–211.1)	0 (0–8)	0 (0–3.1)
Left colon	25 (0–89)	14.3 (0–129.5)	0 (0–4)	0 (0–4.1)

*Turner et al., 2017.




300
250
200
150
100
50
0
Eosinophils/mm²
21,4
12,2
39,8
30,6
28,6
14,3
28,6
15,3
37,7
22,4
Colonic malignancy
Extracolonic malignancy
General surgery
Non-specific colonic symptoms
Suspected appendicitis