Eosinophil Counts in the Small Intestine and Colon of Children Without Apparent Gastrointestinal Disease: A Meta-analysis

*Zoltán Kiss, *†Bálint Tél, §Nelli Farkas, †András Garami, ‡Áron Vincze, ‡Judit Bajor, ‡Patrícia Sarlós, †Katalin Márta, *†Adrienn Erős, †Alexandra Mikó, †Zsolt Szakács, †Dániel Pécsi, §Péter Mátrai, †||Péter Hegyi, and *†Gábor Veres

See ''Eosinophilic Gastroenteritis and Colitis: Not Yet Ready for the Big Leagues'' by Zevit and Furuta on page 1.

ABSTRACT

The aim of the current study was to review the available data regarding eosinophil density in healthy tissue specimen originating from lower gastrointestinal segments to support suggested diagnostic cutoffs widely used in clinical practice. A systematic search was performed in 3 different databases. Calculations were made with Comprehensive MetaAnalysis software using random-effects model. Cell number measurements were pooled using the random-effects model and displayed on forest plots. Summary point estimations, 95% confidence intervals (CIs), and 95% prediction intervals (PIs) were calculated. The cumulative mean cell numbers were 8.26 (95% CI 4.71-11.80) with PI of 0-25.32 for the duodenum, 11.52 (95% CI 7.21-15.83) with PI 0-60.64 for the terminal ileum, and 11.10/high-power field (HPF) (95% CI 9.11–13.09) with PI of 0.96 to 21.23 in the large intestine and the rectum (HPF area = 0.2 mm^2). Previous studies included control patients with irritable bowel syndrome and functional gastrointestinal disorders. As mucosal eosinophils have a role in their pathomechanism, those patients should have been excluded. A critical point of interpreting reported data is that HPF is relative to the technical parameters of the microscopes; therefore, it is important to report findings in cell/mm^2. The present meta-analysis does not support the higher (>20) or lower (<10) cutoff values for healthy tissue eosinophil number. In contrast to the esophagus, there is no normal cutoff eosinophil density in the small intestine and the colon. A prospective, multicenter study to establish normal mucosal eosinophil density is clearly needed.

Key Words: cutoff criteria, eosinophilic colitis, eosinophilic gastroenteritis, gastrointestinal, physiological eosinophil density, primary eosinophil cell associated diseases

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What Is Known

- Eosinophil-derived disorders frequently occur in the gastrointestinal tract.
- Consensus diagnostic cutoffs for tissue eosinophil numbers in eosinophil-associated disorders (eosinophilic gastroenteritis and eosinophilic colitis/proctocolitis) are missing.

What Is New

- Normal eosinophil density cutoffs are not supported by high-level evidence.
- To measure the correct eosinophil cell density eosinophil number/high-power field should be replaced by eosinophils/mm^2.
- Larger-scale studies are clearly needed to establish the normal eosinophil density.

Eosinophil cells, first discovered by Paul Ehrlich in 1879, are granulocytes derived from pluripotent myeloid progenitor cells (1). As effectors of the immune system, these cells are present in the peripheral circulation and in various tissues of certain organs (2). Among these sites, the gastrointestinal (GI) tract harbors the highest number of eosinophils in physiological conditions (3). Considerable elevations in cell numbers and activity are observed in several pathologic conditions affecting the GI tract (4). In primary eosinophil cells–associated GI disorders (EGIDs) elevation in tissue eosinophil numbers is suggested to be one of the main elements of the pathomechanism, as underlying causes of

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From the *1st Department of Pediatrics, Semmelweis University, Budapest, the †Institute for Translational Medicine, First Department of Medicine and János Szentágothai Research Center, the ‡Department of Gastroenterology, First Department of Medicine, the §Institute of Bioanalysis, Medical School, University of Pécs, Pécs, and the ||MTA-SZTE Translational Gastroenterology Research Group, Szeged, Hungary.

Address correspondence and reprint requests to Gábor Veres, MD, PhD, DSc, 1st Department of Pediatrics, Semmelweis University, Bókay János str. 53–54. H-1083, Budapest, Hungary (e-mail: veres.gabor@med.semmelweis-univ.hu).

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tissue eosinophilia, such as helminth infection, hypereosinophilic syndrome, or inflammatory bowel disease (IBD), cannot be detected (5,6).

The EGID involving the colon and the rectum, eosinophilic colitis (EC) is one of the most frequent causes of lower GI bleeding in the early years of life (7–10). Recently there has been an exponential rise in EC recognition, with an estimated prevalence of 3.5/100,000 (11). EC has overlapping features with extremely early-onset Crohn disease; therefore, it could pose as a differential diagnostic difficulty (12). For this reason, it is of utmost importance to enhance the diagnostic procedure for EC. Eosinophilic gastroenteritis (EGE) is a rare chronic condition of the GI tract. Eosinophilic infiltration of the tissues most commonly occurs in the wall of the stomach, small intestine, and rarely in the colon, rectum, or the esophagus (13).

In all of the EGIDs, confirmation of the diagnosis is primarily based on increased eosinophil cell numbers detected by histological evaluation of biopsy specimen. The pathologic criteria (eosinophil density) for diagnosis are, however, poorly characterized. Although the normal esophagus does not contain any eosinophils, in the case of EoE, the diagnostic criteria have been extensively addressed, and current consensus for diagnosis of disease is $\geq$15 eosinophil/high-power field (HPF) in addition to other clinical criteria. (14).

Up to this date, several publications on various pediatric cohorts discussed the topic of eosinophil cell counts in the lower GI segments with conflicting results (8,15–23). Inconsistency is based on the fact that it is difficult to obtain normal tissue biopsy from healthy children. Even in patients with functional abdominal pain (defined by the Rome IV criteria) and macroscopically normal mucosa (eg, irritable bowel syndrome [IBS]), histology could reveal an increased number of tissue eosinophils. Therefore, previous studies may have yielded a higher number of mucosal eosinophil density data in the control groups if children with functional abdominal pain were included.

In our review, we summarized the relevant publications in a systematic manner, with a meta-analysis. The aim of our study is to quantitatively summarize the available data to facilitate the establishment of normal cutoff values for GI tissue eosinophil density.

METHODS

Data Sources

PubMed, Scopus, Cochrane, and EMBASE databases were screened by complex search criteria constructed by database-specific syntaxes. The search was optimized to find all publications containing numerical data, including publications on healthy tissue characterization, and studies on various GI diseases where a healthy control group was incorporated.

Search, Information Sources, and Study Selection

This study was performed following the principles of the PRISMA statement (24). Databases were screened after database-specific complex search criteria with English language restriction. The search was conducted on April 3, 2017. The exact searching term for Pubmed database was as follows:

("high power field" OR HPF OR "tissue eosinophilia" OR "number of eosinophils" OR "eosinophil count" OR "eosinophil number" OR "number of eosinophil cells" OR "eosinophil cell count" OR "eosinophil cell number" OR "number of eosinophil granulocytes" OR "eosinophil granulocyte count"" OR ""eosinophil granulocyte number") AND (gastrointestinal OR colon OR rectum OR small intestine OR duodenum OR jejunum OR gaster OR Ileum) AND (Humans[Mesh])

Two different reviewers (Z.K. and B.T.) screened the collected articles for eligibility after titles and abstracts. Publications passed this phase if their topic were of interest to the study. Publications about EoE were excluded. Publications on the target GI segments without healthy control groups were excluded. The remaining articles were assessed for eligibility on full-text level by Z.K. and B.T. Disagreements were resolved by consensus.

All studies including healthy patients as the main focus, or as a control group, and presented numerical tissue eosinophil counts were addressed in detail. Studies were eligible for full-text screening if the target population of the study were pediatric patients (under the age of 18) underwent endoscopy and had normal endoscopic findings and histologically normal pathologic results. Patients from the included publications were reported to be free from parasitic or bacterial infections and had no IBD or diagnosis of any systemic disease potentially involving the duodenum, terminal ileum, or the colon. Studies using acceptable histologic methods (preparates from pinch biopsy specimens were fixed, H&E stained and evaluated with optical microscope, cell numbers reported patient by patient, or as cumulative statistics including measures of dispersion), and reported metadata (age, and sex patient by patient or as cumulative statistics including measures of dispersion, means and results of apparent GI disease exclusion, medication status) were analyzed qualitatively. Measurements for whole cell count with absolute values (cell number/mm^2) and cell counts reported in HPF with supporting information for conversion (area of HPF or field number of the microscope eyepiece and magnification) were incorporated into the quantitative analysis. Data given for HPF were transformed to cell/mm^2 (for risk of bias assessment see Supplementary Table 1, Supplemental Digital Content 1, *http://links.lww.com/MPG/B274*).

In all cases, when publications containing incomplete data for the purposes the authors were contacted. If data were provided by the authors, we incorporated the publication in our review.

We interpreted data from different anatomical sites without provided covariance within studies as independent subgroup outcomes.

Statistical Methods

For the computations, dimensions were transformed to a common field area (HPF) of 0.2 mm^2. The statistical analysis was completed by means of Comprehensive Meta-analysis Software (Version 3, Biostat, Englewood, NJ) and Stata 11 SE (Stata Corp, College Station, TX). Cell number measurements were pooled using the random-effects model with the DerSimonian-Laird estimation and displayed on forest plots. Summary point estimations, 95% confidence intervals (CIs), and 95% prediction intervals (PIs) were calculated. PIs were truncated at zero. Statistical heterogeneity was tested using the I^2 statistic and the χ^2 test to gain probability-values; $P < 0.05$ was defined to indicate significant heterogeneity. For easier interpretation results are presented in cell number/0.2 mm^2 (area of common HPF), and also in cell number/mm^2.

RESULTS

The study selection of the review process is detailed in Figure 1. The database searches resulted in 1561 articles in total. A total of 1316 publications were reviewed for eligibility after removal of duplicates. Preselection was made by reviewing titles

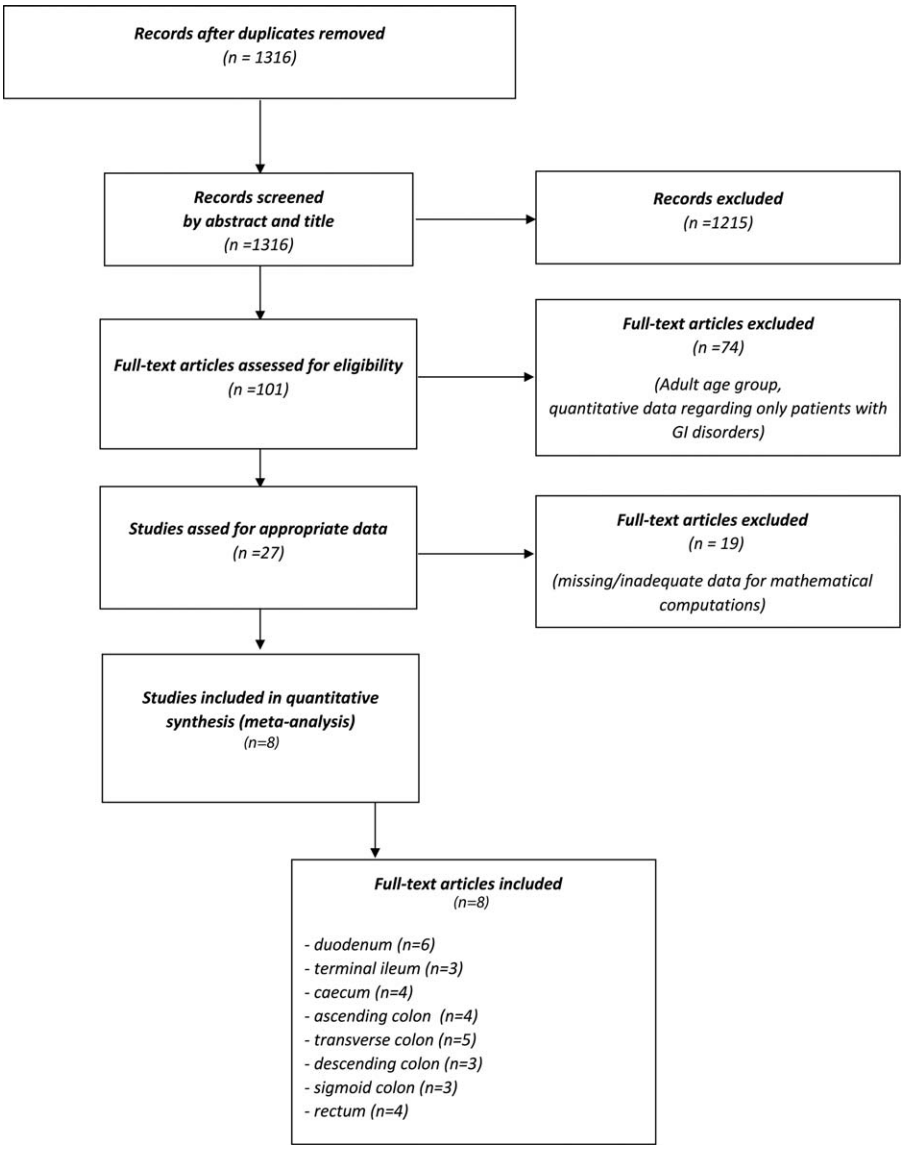

FIGURE 1. Overview of the study selection process.

and abstracts to exclude the obviously irrelevant search hits. The remaining 27 publications were systematically screened for eligible numerical data. Eight publications were enrolled in the quantitative analysis (22,35–41). Most of the publications reported data regarding multiple GI segments. Six articles reported cell counts in the duodenum on a total of 231 patients. Three articles reported data on the terminal ileum on a total of 47 patients. Four articles reported data on the cecum for 131 patients. Four articles reported on the ascending colon on 140 patients. Five articles reported on transverse colon on 148 patients. Three articles reported on the descending colon on 125 patients. Three articles reported data on the rectum and the sigmoid colon on 42 patients. Anthropometric data for the involved groups are presented in Supplementary Table 2 (Supplemental Digital Content 2, *http://links.lww.com/MPG/B275*) for the small intestinal segments and in Supplementary Table 3 (Supplemental Digital Content 3, *http://links.lww.com/MPG/B276*) for the large intestinal segments and the rectum.

Overall I^2 values in the cumulative analysis of the small intestine, and large intestine (including the rectum) were above 90% in the random effect model calculations. To attenuate the effect of the high heterogeneity to the overall conclusions, we presented the analyses with cumulative means (with 95% CI) and with more relevant PIs.

Results in Cell Number/High-Power Field (HPF Area = 0.2 mm^2)

In the small intestine, the segment subtotals were 8.26 (95% CI 4.71–11.8) with PI of 0 to 20.57 for the duodenum, and 11.52 (95% CI 7.21–15.83) with PI of 0 to 60.64 for the terminal ileum (Fig. 2).

In the large intestine the subtotals of for the segments were the following: 14.12 (95% CI 9.05–19.19) with PI of 0 to 38.64 for the cecum, 13.25 (95% CI 8.65–17.86) with PI of 0 to 35.42 for the ascending colon, 11.52 (95% CI 7.80–15.23) with a PI of 0 to 25.85 for the transverse colon, 10.32 (95% CI 7.22–13,42) with a PI of 0 to 49.10 for the descending colon, 8.80 (95% CI 6.82–10.77) with a

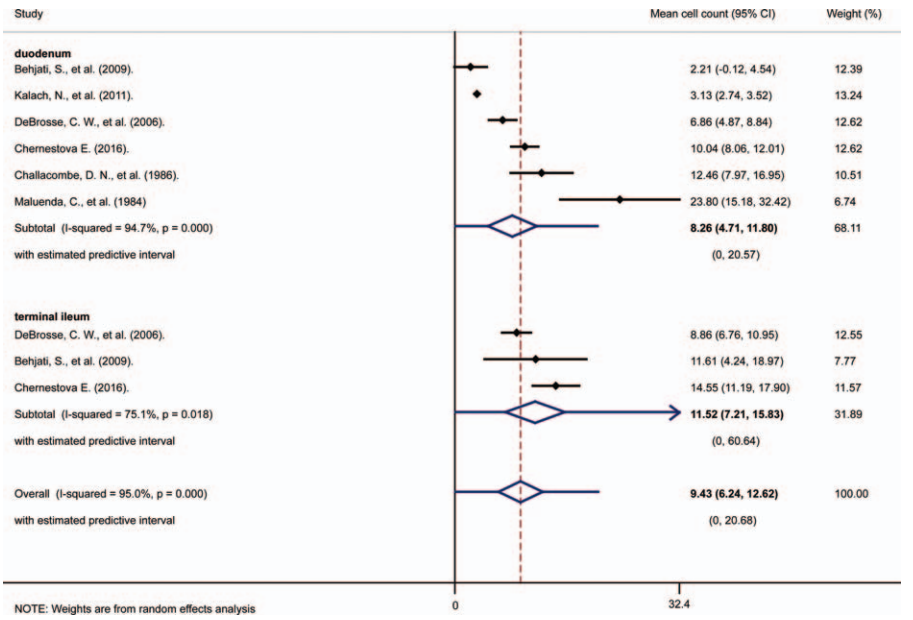

FIGURE 2. Forest plot depicting the analysis of cell counts in the duodenum and terminal ileum (data presented in cell/high-power field (HPF), where the area of the HPF = 0.2 mm^2).

PI of 0 to 32.49 for the sigmoid colon, and 7.39 (95% CI 4.20–10,59) with a PI of 0 to 22.33 in the rectum (Fig. 3).

Results in Cell Number/mm^2

In the small intestine the segment subtotals were 41.28 (95% CI 23.55–59.02) with PI of 0–102.92 for the duodenum, and 57.59 (95% CI 36.03–79.14) with PI of 0 to 303.30 for the terminal ileum.

70.60 (95% CI 45.24–95.95) with PI of 0 to 193.22 for the cecum, 66.26 (95% CI 43.25–89.28) with PI of 0 to 177.09 for the ascending colon, 57.59 (95% CI 39.01–76.16) with a PI of 0 to 129.31 for the transverse colon, 51.59 (95% CI 36.08–67.10) with a PI of 0 to 245.49 for the descending colon, 44.00 (95% CI 34.12–53.87) with a PI of 0 to 162.43 for the sigmoid colon, and 36.97 (95% CI 21.00–52.94) with a PI of 0 to 111.66 in the rectum (Forest plots in supplementary Figures 1 and 2, Supplemental Digital Content 4–5, *http://links.lww.com/MPG/B277*, *http://links.lww.com/MPG/B278*).

As described previously, publications often included patients with functional GI disorders. These conditions can potentially influence the eosinophil numbers in the affected tissues of the GI tract. Therefore, measurements originating from these patients are likely to introduce bias into the analysis.

DISCUSSION

Our meta-analysis has found, that the eosinophil density in the small intestine and the colon segments vary between 35 and 70 cell/mm^2 (7–14 cell/HPF, where HPF = 0.2 mm^2), but the corresponding PIs range from 0 to 300 cell/mm^2 (0–60 cell /HPF, where HPF = 0.2 mm^2). We decided to present PI values to complement the CI computations. The CI in a random-effects model reflects only on the summary mean value: it gives highly probable values for the overall mean effect size (in our case, mean cell count); however, it does not show what range of cell counts are likely to be seen in other patients, for example, in future studies or in the patients a clinician actually meets in his practice. A PI provides a predicted range for the true mean cell count in an individual study; accordingly it gives a plausible range of cell counts in a new study or population similar to those included in the meta-analysis. The wide PIs indicate that the available data are insufficient to provide high-level evidence for the establishment of normal tissue eosinophil cell density; therefore, at present, it is impossible to precisely diagnose EGIDs affecting the small intestine or the colon (as oppose to EoE) (14,25,26). The low number of available publications, the inconsistency of the applied histologic methods and suboptimal patient involvement criteria are the main reasons accounting for the heterogeneity in the available body of evidence. Therefore, a large-scale multicenter prospective study with rigorous methodology and the exclusion of patients with functional GI disorders is clearly needed to clarify the physiologic density of eosinophils in the small intestine and the colon.

As the diagnosis of EGIDs relies heavily on the counting of mucosal eosinophil cell numbers without reference standards, the observation of abnormal GI eosinophilia remains subjective. Establishing of normal values and distribution could facilitate the formation of an exact diagnostic process for EGIDs.

Processing the available sources in this topic, we have found that significant amount of the relevant publications has some degree of uncertainty in the reported results. We report the factors leading to exclusion of certain publications in the following paragraph, and state our suggestions to facilitate the better comparability of future studies.

In most of the publications, the cell counts are reported in cell number/HPF. The area of an HPF is, however, dependent on the technical parameters of the microscope (on the magnification of the objective lens and the diameter of the ocular) (Fig. 4). If the size of the field of view of the microscope used in the histopathologic evaluation is not clearly stated (or could not be derived from technical data of the microscope documented in the publication) the study is lost for comparison. Due to the diversity of this parameter of commercially available microscopes, the difference of the cell counts observing the same tissue sample could be up to 5-fold. It is an important issue leading to the exclusion of important data sources from the meta-analysis. Therefore, in future studies, we suggest using H&E staining of the histologic slides, and counting

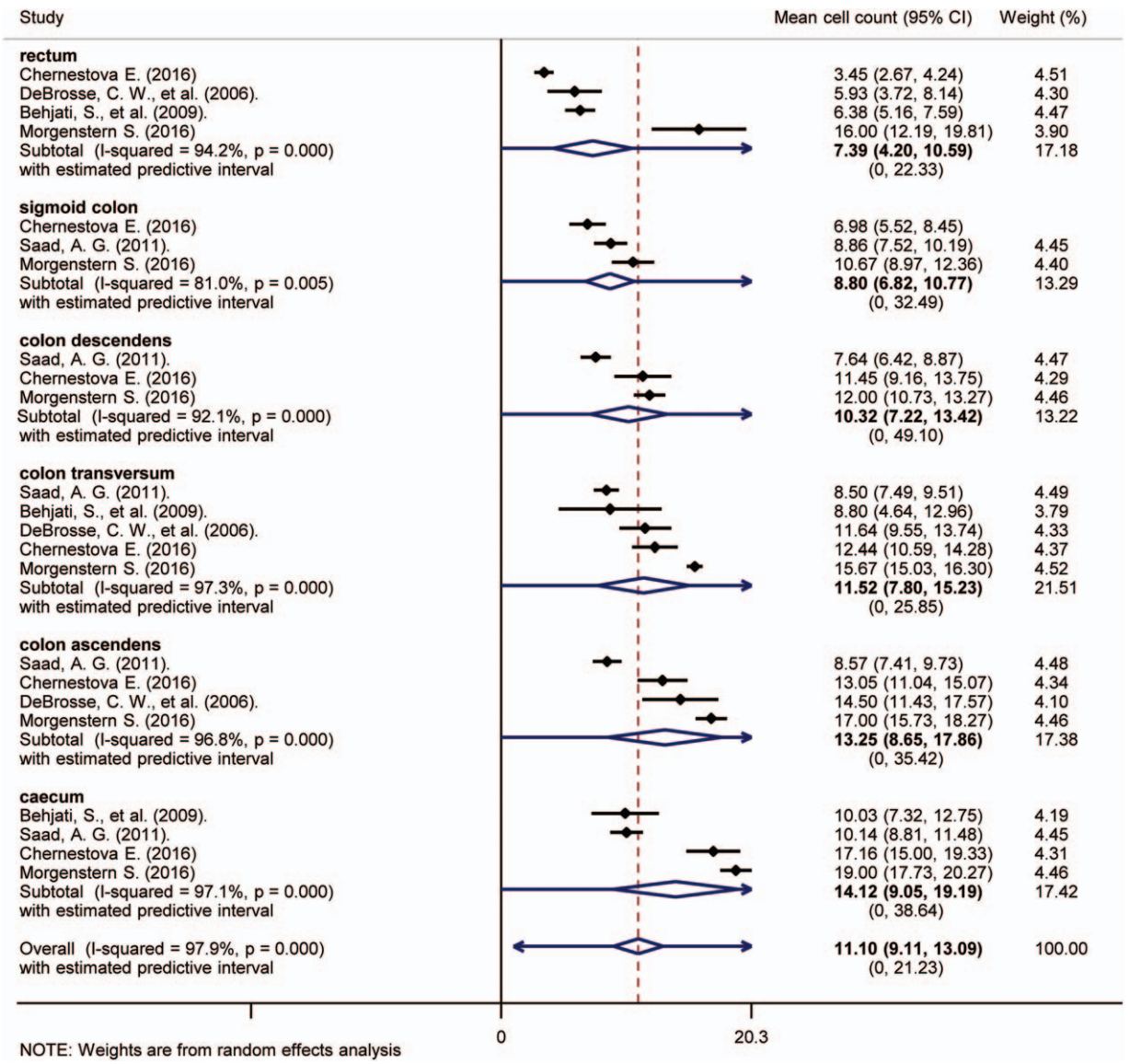

FIGURE 3. Forest plot depicting the analysis of cell counts in the large intestine and the rectum (data presented in cell/high-power field (HPF), where the area of the HPF = 0.2 mm^2).

cell numbers at least in 5 fields of view from the area with peak eosinophil density (identified under lower magnification). We also strongly suggest reporting histopathologic data in cell/mm^2.

In addition to the technical aspects, the second major source of heterogeneity is the possibility of patchy presentation of eosinophils in normal and disease involved tissues in the anatomical subsites (27). Therefore, it would be beneficial to analyze the cell counts in at least 3 samples taken along the same anatomical subsites.

The small number of available publications is an important limitation of our analysis. From a clinical point of view, it is important to state that collecting tissue samples from a healthy pediatric population is extremely difficult. In the studies involved in our analysis, pathologists defined histologically normal status of the biopsies. Therefore, it is possible, that normal variants were excluded. This potential bias can only be alleviated by prospective reading of biopsies for eosinophils when no other apparent GI diseases are diagnosed. Accordingly, studies incorporate symptomatic patients with minor health issues, or with diagnoses not involving GI segments. This phenomenon could clearly introduce a bias resulting in overestimation of physiological eosinophil density. In this regard, we would like to highlight that in the past pediatric patients with functional GI diseases such as IBS were included as normal patients for mucosal eosinophil density. It was, however, recently published that neuro-immune interactions are important parts of the pathomechanism of IBS (28). An elevated numbers of eosinophils in blood and biopsy samples of IBS patients were reported in several publications (29–32). This phenomenon is also reported in functional dyspepsia (32,33). In both diseases, the abdominal discomfort and impaired motility are suggested to be in connection with eosinophils. Therefore, as a precautionary principle, we suggest the exclusion of those patients from the future studies.

Previous publications stated that eosinophil numbers have a negative gradient from the cecum to the rectum (34). In consistence with this statement, our results showed a similar tendency of observations along the large intestine (Figs. 2 and 3). It is of note,

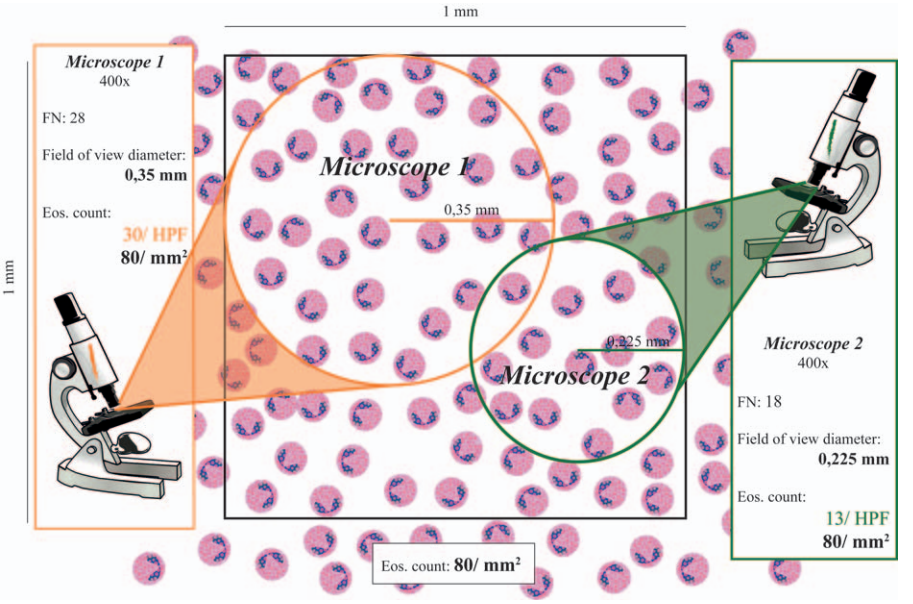

FIGURE 4. Ambiguity of the high-power field (HPF) as dimension for cell density measures.

that specific data for the distinct anatomical regions are sparse and inconsistent. Calculations of covariance are absent in all of the studies; therefore, it is impossible to draw confident conclusions on the correlation between the mean cell counts and the anatomical localization. It is a limitation of our analysis, that we could only interpret these groups of data as independent subgroup outcomes. This unavoidable simplification of the data structure could have introduced computational bias.

Nevertheless, the quantitative analysis of the cell counts yielded unexpected results. The upper limits of the overall PIs for the small and the large intestine were congruent with the generally used criteria for pathologic tissue eosinophil numbers. The data, however, show a large amount of heterogeneity, means, and standard deviation values are rarely overlapping. Considering the relatively small number of data sources, the validity of the PIs as a base for diagnostic cutoff is not supported.

Taken together, after evaluating our systematically collected data in this topic, we were able to conclude, that the currently available body of data is neither abundant nor sufficiently consistent to justify the eosinophil cell count as an evidence-based diagnostic criterion. Based on the available data, diagnosis of EGIDs in the small intestine and colon could not be reinforced with the density of eosinophil cells in the histological samples with lower cell counts. Further large-scale, prospective studies are clearly needed to estimate the number of eosinophil cells in various GI sites under physiological conditions.

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