Primary eosinophilic gastroenteritis and colitis (EGE/EC) is a rare entity. For clinical and practical reasons, it is distinguished from the better known eosinophilic esophagitis (EoE), although both are suspected to be caused by Th2-mediated food hypersensitivity.

Identifying patients with EGE can be demanding. Clinical symptoms like abdominal pain or recurrent diarrhea are unspecific [1–3]. Endoscopic findings are equally uncharacteristic, often mimicking other diseases [4–6].

Diagnosing EGE/EC therefore strongly relies on histopathologic evaluation of mucosal biopsies. But unlike in the esophagus, where diagnostic criteria for EoE are widely agreed upon [7, 8], there are no such criteria for the ileum or colon. In normal pediatric mucosa, a wide range between 1 and 52 eosinophils per microscopic high power field (HPF) has been reported. Within this range, some observers saw decreasing eosinophil numbers from the cecum to the sigmoid [9, 10], while others showed an increase from the left colon to the rectosigmoid [11]. Values for adults are roughly within the same range, with superimposed seasonal undulations [12]. For North America, a regional increase from north to south has been reported [11, 13], while in Asia there seems to be no difference according to geographic region, race, or sex [14].

Considering this variance, it is not surprising that there is no consensus about the threshold above which the diagnosis of “eosinophilic ileitis/colitis” can be made. Some authors suggest a value as low as at 6 eosinophils per HPF [15], some 15 to 20 [2, 16], some 30 [17], and some 50 [18]. Recently, a differentiated approach was proposed, with upper limits...
locations each (terminal ileum, hepatic flexure, rectum)
scoopist (F.H.). Two biopsies were taken from 3 standardized
biopsies. All patients were examined by one single endo-
scopic examination using the same macrogol-based solution
within <50 Kilometers from the hospital and prepared for
colonoscopy using the same macrogol-based solution
(Moviprep, Norgine GmbH, Marburg, Germany).

2. Methods

2.1. Patients. We retrospectively examined the histopatho-
logic biopsies of 521 consecutive adult patients who under-
went diagnostic ileocolonoscopy in our department from
January to December 2017. 84 of them had mucosal biopsies
taken because of chronic abdominal pain, recurrent diarrhea,
or both. Out of these patients, 10 aroused the clinical or
endoscopic suspicion of an elevated eosinophil count in
the lower GIT or showed elevated eosinophils in routine
endoscopic suspicion of an elevated eosinophil count in
the histopathologic findings. Possible confounders like the
variability in microscopic HPF size, selection of fields near
or far from lymphoid follicles, and differing criteria for
including cells in the count had been discussed [20].

To test these hypotheses, we had the same standard-
dized mucosal biopsies from 10 patients examined by six
independent specialists in pathology who were blinded for
each other's results. Eosinophils were counted according to
a standardized protocol, and results were normalized for the
HPF areas of the microscopes used.

2.2. Biopsies. All patients were examined by one single endo-
scopist (F.H.). Two biopsies were taken from 3 standardized
locations each (terminal ileum, hepatic flexure, rectum)
using 2.3 mm calipers (MTW Wolfgang Haag KG, Germany)
through flexible colonoscopes (Fujinon EC600, Fuji Corp,
Japan). Probes were fixated in 4% buffered formaldehyde
(R. Langenbrinck GmbH, Emmendingen, Germany) and
brought to the pathologist's laboratory. They were then
embedded in 10% paraffin wax (Tissue Tek, Sakura Finetek
Europe B.V., Netherlands), cut to 4 μm slices (Microtome
SM2000R/SM2010R, Leica, Germany), and underwent stan-
dard H&E staining (Hämalaun Mayer and Hämatoxylin Gill
III, Dr. K. Hollborn & Söhne GmbH & Co KG, Germany;
Erythrosin, Carl Roth GmbH + Co. KG, Germany) in
an automated slide stainer and coverslipper (TCA 44-720,
MEDITE GmbH, Germany). After routine histopathologic
assessment, they were archived.

For the study, original glass slides of included patients
were drawn from the archive, anonymized, and sent to the
participating pathologists.

2.3. Pathologists and Microscopes. There are six clinical
institutes of pathology in the state of Brandenburg. Four
of them participated in the study. Mucosal biopsies were
examined by six specialists in pathology with at least ten
year experience in the field (Box 1), according to standardized
counting instructions in German (for English translation see
Box 2). Microscopes are listed in Table 1. Differing areas of
the high power fields (HPF) at 400x magnification were made
comparable by a normalization factor, based on current CAP
and ITBCC recommendations [23].

2.4. Statistics. All data was analyzed using IBM SPSS Statis-
tics 23. If not stated otherwise, eosinophilic numbers are
given as mean +/- standard deviation (SD) out of 5 HPF. For
metrically scaled, non-Gaussian data, Friedman test was used
for more than two paired samples. Cochran Q Test was used
for nominal scaled, paired samples.

3. Results

Eosinophil counts in all biopsies differed between investiga-
gators, up to a factor > 30. Maximum count was 328 per
HPF (mean of 5, hepatic flexure), and minimum count was
0 per HPF (mean of 5, more than one biopsy site). Analyzing
each biopsy site for each pathologist, differences between the investigators were significant for all biopsy sites
(Table 2, Figures 1(a)–1(c)).

Mean eosinophil counts overall differed between the
highest and the lowest counting investigator by a factor of 14.5
(29 vs.2).

Intra-individually, each investigator was concordant to
his own bias, i.e. the one with the highest counts overall had
those highest counts in all but one biopsy, the one with the
lowest count had the lowest count in all but three biopsies
(Table 2).

Independently of the strong interobserver observer
variance, each observer found the highest number of
eosinophils in the hepatic flexure, with values between 73
eosinophils per HPF for the highest counting investigator
(mean of 5 HPF of all patients), and 5 for the lowest. Numbers
in the ileum were intermediate in all investigators with values

Box 1: Institutes of Pathology in Brandenburg that participated in the Study.

University Medical Center Brandenburg
Director: Dr. med. Roland Pauli
Hochstr. 27
14770 Brandenburg an der Havel

Helios Clinic
Director: Prof. Dr. med. Stefan Koch
Pieskower Straße 33
15526 Bad Saarow

Clinical Center Frankfurt
Director: Dr. med. Petra Busch
Müllerroser Chaussee 7
15236 Frankfurt (Oder)

University Medical Center Ruppin
Director: Dr. Frank Lippek
Fehrbelliner Straße 38
16816 Neuruppin

Box 2: microscopes

Leica SM2000R/SM2010R

Erythrosin, Carl Roth GmbH + Co. KG, Germany

Hämalaun Mayer and Hämatoxylin Gill III,
Dr. K. Hollborn & Söhne GmbH & Co KG, Germany

Erythrosin, Carl Roth GmbH + Co. KG, Germany

Tissue Tek, Sakura Finetek Europe B.V., Netherlands

Microtome SM2000R/SM2010R, Leica, Germany

Hämalaun Mayer and Hämatoxylin Gill III,
Dr. K. Hollborn & Söhne GmbH & Co KG, Germany

Erythrosin, Carl Roth GmbH + Co. KG, Germany

An automated slide stainer and coverslipper (TCA 44-720,
MEDITE GmbH, Germany)

Through flexible colonoscopes (Fujinon EC600, Fuji Corp,
Japan)
Intructions for Examination:
(i) The aim of this study is to count the maximum number of eosinophils per HPF in the ileal, colonic, and rectal mucosa.
(ii) To do this, identify the 5 HPF with the highest number of eosinophils in every slide, then count the eosinophils in each!
(iii) HPF must be located > 4 crypts from the next lymph follicle.
(iv) Do not count intravascular and / or degranulated eosinophils.
(v) Do count mucosal eosinophils that can clearly be identified by their granula, even if their nucleus is out of focus or out of the cutting plane.

Box 2: Instructions for participating pathologists. These instructions follow known procedures in identifying and counting eosinophils in human mucosa [10, 12, 13, 21, 22].
Table 1: Microscopes used by the participating pathologists, area of their high power fields (HPF), and normalization factor [23].

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Microscope</th>
<th>Area HPF (mm²)</th>
<th>Normalization Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Olympus BX50 40x/0.65</td>
<td>0.237</td>
<td>1.000</td>
</tr>
<tr>
<td>2</td>
<td>Olympus BX51 40x/0.75</td>
<td>0.344</td>
<td>0.699</td>
</tr>
<tr>
<td>3</td>
<td>Olympus BX51 40x/0.75</td>
<td>0.344</td>
<td>0.699</td>
</tr>
<tr>
<td>4</td>
<td>Olympus BX51 40x/0.75</td>
<td>0.344</td>
<td>0.699</td>
</tr>
<tr>
<td>5</td>
<td>Olympus BX51 (0.55mm) 40x/0.65</td>
<td>0.237</td>
<td>1.000</td>
</tr>
<tr>
<td>6</td>
<td>Zeiss AxioLab 40x/10/0.65</td>
<td>0.331</td>
<td>0.716</td>
</tr>
</tbody>
</table>

Table 2: Eosinophil counts of each pathologist for each anatomic site of each patient. Values shown are mean ± SD from 5 HPF and normalized (Table 1). * Asterisk: Investigator counted 3 HPF as “>100” each, one as 70 and one as 90. p = “asymptotic significance” - Friedman test p-value.

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Microscope</th>
<th>Area HPF (mm²)</th>
<th>Normalization Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Flexure</td>
<td>4 ±0.3</td>
<td>1 ±0.3</td>
</tr>
<tr>
<td>4</td>
<td>Flexure</td>
<td>5 ±0.4</td>
<td>1 ±0.4</td>
</tr>
<tr>
<td>5</td>
<td>Flexure</td>
<td>6 ±0.5</td>
<td>1 ±0.5</td>
</tr>
<tr>
<td>6</td>
<td>Flexure</td>
<td>7 ±0.6</td>
<td>1 ±0.6</td>
</tr>
</tbody>
</table>

Values shown are mean ± SD from 5 HPF and normalized.
Figure 2: Eosinophil numbers in the ileum, hepatic flexure, and rectum by different investigators (Invest. 1 to 6) examining the same 30 slides according to standardized protocol. Numbers are given as means of 5 HPF from 10 patients = 50 HPF, normalized (Table 2).

Figure 3: Positive and negative diagnoses for eosinophilic enteritis /colitis by different investigators (Invest. 1 to 6) examining the same 30 slides of 10 patients according to a standardized protocol. Thresholds were ileum > 20 eosinophils/HPF, hepatic flexure > 50 eosinophils/HPF, rectum > 20 eosinophils/HPF, normalized (Table 2). Results differed significantly (p < 0.05). Changing the thresholds to different values from the literature [9–13] did not change the overall picture (data not shown).

Figure 4: Virtual incidence numbers of eosinophilic enteritis /colitis in adults calculated for each investigator based on 521 patients scanned for the study. Thresholds for positive diagnosis are > 50 eosinophils per HPF in the hepatic flexure, and > 20 in the ileum or rectum [9–13].

4. Discussion

Eosinophilic diseases of the lower GIT are relatively newly described, incompletely understood, rare, and difficult to detect. Clinical and endoscopic signs are non-specific; histopathologic criteria for mucosal biopsies are lacking. While all authors agree that a certain amount of eosinophils in the ileum and colon is normal, the actual numbers are not known [9–14]. Accordingly, there is no consensus about the limit above which one can safely diagnose an eosinophilic gastroenteritis or enterocolitis. Many authors suggest that the overall morphologic picture together with clinical and endoscopic findings are more important than numeric counts [2,15,16,18,19,21]. This is in sharp contrast to the situation in the esophagus, were a number of 16 or more eosinophils per HPF is considered pathognomonic for EoE [7, 8].

Possible explanations for this discrepancy are a lack of clinicopathologic data supporting any particular threshold in the lower GIT, anatomic [9, 10], seasonal [12], genetic or geographic [11,13] variations in eosinophils, and variations in counting methodology like microscopic field size or criteria of eosinophil counting [20]. Our own suspicion was that numeric eosinophil counts may be observer-dependent.

In this study, we therefore standardized the sampling, processing and examining of 30 slides from ileal, colonic and rectal biopsies from ten Caucasian patients living in the proximity of our center. We then showed the exact same glass
slides to six experienced specialists in pathology and asked them to count the eosinophils according to a standardized protocol without knowing the results of each other. Results were normalized to HPF sizes. With this setup, we are confident to have ruled out any influence by the preparation, the patient, the geography, the endoscopist, the endoscopes used, the calipers, biopsy size and number, the topography of the sampling site, the staining method, and the microscopic field sizes. Still, results were strikingly differing.

When discussing this phenomenon with the participating pathologists and gastroenterologists, we did not find an explanation for it right now. One possibility is that the spotty distribution of eosinophils in the lower GIT leads to highly varying concentrations in different HPFs. The error then occurs as early as in the overview, where fields of interest are identified. So, after optical magnification of 400x, every pathologist counts five different regions of the same slide. Further studies, possibly on multiphider microscopes, may be needed to explain these discrepancies. Until then, we can only describe it as extremely high interobserver variance.

Against this background, one may have to rethink some of the facts that are thought to be known about EGE today. Overall prevalence is reported to be about 5 per 100,000 [24, 25], with some observers suggesting it may be higher [17]. One study saw a higher prevalence in male children and female adults [26]: one showed a higher prevalence in Asians [27] and another hinted to a higher incidence in family members of known EGE patients [28]. All of these numbers are derived from histopathologic diagnoses and therefore have to be met with reserve. Extrapolating from our own findings, the true incidence of EGE in our preselected collective could be anywhere between <1 per 100,000 and 1500 per 100,000.

Since we do not know the exact reason for this phenomenon, we cannot offer a solution right now. Significantly increasing the number of biopsies and HPFs could even out the mean values, but that would be impractical in a real-life clinical situation. Another possibility could be a different staining method to identify and automatically count eosinophils in lower magnification. However, H&E staining that we used is standard for examining mucosal biopsies, and no pathologist reported problems with identifying eosinophils in the slides. So new staining and counting methods would not necessarily be suitable for daily pathologic routine but rather of scientific interest.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CAP</td>
<td>College of American Pathologists</td>
</tr>
<tr>
<td>EGID</td>
<td>Eosinophilic gastrointestinal disease(s)</td>
</tr>
<tr>
<td>EoE</td>
<td>Eosinophilic esophagitis</td>
</tr>
<tr>
<td>EGE</td>
<td>Eosinophilic gastroenteritis/colitis</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and eosin staining</td>
</tr>
<tr>
<td>HPF</td>
<td>High power field</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>ITBCC</td>
<td>International Tumor Budding Consensus Conference</td>
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</table>

Data Availability

Original slides are archived in the Department of Pathology. Counting protocols are available from author Anna Franziska Jansen.

Conflicts of Interest

All authors declare that there are no conflicts of interest.

Acknowledgments

Authors Marlis Günther and Roland Pauli were taking part in the study as pathologists. The authors would like to thank the additional pathologists and institutes (see Box 1) who volunteered to examine the slides and Dr. Werner Dammermann for proofreading the manuscript.

References


