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ORIGINAL ARTICLE



Duodenal mast cells and eosinophils in children with celiac disease: occurrence and distribution pattern*

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ABSTRACT

Objective: The aim of this study was to characterize duodenal mast cell (MC) and eosinophil (EO) numbers, their distribution within the lamina propria and possible impact on disease severity of paediatric celiac patients compared to children without celiac disease (CD).

Methods: We analysed duodenal samples of 215 children (109 CD, 106 controls) who underwent esophagogastroduodenoscopy from 2010 to 2018. After immunohistochemical staining, average MC and EO counts were histologically examined in ten high-power-fields. Additionally, cell-distribution within the lamina propria was analysed. Possible influence of relevant clinical parameters was evaluated.

Statistics: Student's *t*-test, Mann-Whitney *U*-test, Chi-square-test, ANOVA, significance-level <.05. Trial registration-number: DRKS00024669.

Results: MC-density was higher in CD-patients compared to the control-group (23.7 (±12.1)/HPF versus 19.7 (±9.1)/HPF; *p* = .008), varying in number interindividually. Eosinophils were also increased in the duodenum of celiac patients (23.3 (±9.3)/HPF versus 12.2 (±6.3)/HPF; *p* = <.001). MCs were distributed more often homogenously in all parts of CD lamina propria (44 biopsies (40.4%), residing more distant from the intestinal lumen in controls (0 biopsies with homogenous distribution-pattern (0%); *p* = <.001). Regarding EOs no polarity was observable. Atopic diseases did not occur significantly more often in patients with elevated EO-counts.

Conclusion: MC- and EO-numbers were increased in the duodenum of CD-patients and MCs showed a different distribution-pattern in the lamina propria of celiac patients. These findings support the concept that both cell-types contribute to disease-pathogenesis. However, functional studies highlighting both cell-types' and their mediators' role regarding mucosal alterations during the course of the inflammatory process in celiac patients are needed.

Trial registration number and URL: DRKS00024669; https://www.drks.de/drks_web/

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Introduction

Celiac disease (CD) is an autoimmune disease triggered by dietary gluten in genetically susceptible individuals resulting in malabsorption, caused by intestinal inflammatory cell infiltration and mucosal remodelling [1]. Patients are diagnosed by specific autoantibodies and in uncertain cases with conclusive histopathological evaluation after esophagogastroduodenoscopy [2], although omitting biopsy in children has become possible under defined conditions according to the ESPGHAN/GPGE-guidelines [3,4]. Adaptive and innate immune responses promote disease-pathophysiology [5], and contribution of bystander immune-elements (neutrophils, eosinophils (EOs) or mast cells (MCs)) seems likely [6].

MCs, key players for host-defence, operating at the interface of innate and adaptive immunity [7], have multiple membrane-bound receptors and either newly synthesized or

granule-stored mediators [8], fulfilling regulatory, secretory and immunological functions for gastrointestinal homeostasis, which explains their involvement in gastrointestinal diseases [9]. EOs store biologically active mediators in their granules, interact with other immune cells (i.e., MCs [10]), maintain homeostasis of the mucosal epithelial-barrier [11], and are potentially associated with functional gastrointestinal disorders [10].

Both cell-types' interaction may impair intestinal epithelial-barrier function [12,13], by direct mucosal cell-injury [14] and increasing mucosal permeability in colonic [13,15,16], and small intestinal mucosa [17].

Studies concerning MCs in CD demonstrate conflicting results, either decreased or increased MC-numbers, and opposing cell-numbers under gluten-free diet [18–23]. EO-numbers [18,21,24,25] and -mediators have been shown to

be elevated in CD [26,27], in association with persistent symptoms despite gluten-free diet [28].

Only few studies with smaller sample sizes evaluated both cell-types in (mostly adult) CD-patients [18,21,27]. Therefore, we compared both cell-types' count, their distribution in duodenal lamina propria and association to Marsh-score in CD-patients to controls in a larger paediatric patient-cohort.

Patients, materials, and methods

Study design

This is a retrospective case-control study.

Ethics approval

The study was approved by the ethics committee of the Ruhr-University Bochum (registration number: 20-6814-BR, approved 2020-01-09). The study is registered in the national register for clinical studies (Deutsches Register Klinischer Studien, DRKS, ID: DRKS00024669).

Patients and controls

Duodenal tissue samples of 109 paediatric patients with confirmed celiac disease, age matched to 109 randomly chosen controls with excluded CD were retrospectively included. From 2010 to 2018, all included patients underwent esophagogastroduodenoscopy (EGD) at the *Department of Pediatric Gastroenterology, St. Josef-Hospital, University Hospital of Pediatrics and Adolescent Medicine, Ruhr-University Bochum*, after their parents had given informed consent. Indication for endoscopic biopsy included typical intestinal and extraintestinal clinical signs and symptoms of CD, such as abdominal pain, malnutrition, diarrhoea, vomiting next to celiac antibody positivity and positive family history or evaluation of other gastrointestinal diseases.

In this retrospective study, inclusion of patients to diagnose CD by taking biopsies during the procedure of an EGD was carried out according to national and international guidelines. These have been updated twice. Therefore, biopsy was always performed in children with corresponding clinical symptoms and positive serology or IgA-deficiency until the guidelines changed in 2012 [2]. In the following years, guidelines recommended to alternatively omit taking biopsies in cases of conclusive symptoms and 10 times increased antibody-titres as well as positive anti-endomysium-IgA titres [3,4]. At the time of biopsy, all CD-patients were consuming gluten. At least, six biopsies were taken from various macroscopically conspicuous duodenal locations, including two of the bulb. All samples were looked at through a stereomicroscope immediately after removal.

After haematoxylin-eosin (HE) staining all duodenal biopsies were evaluated histopathologically (according to Marsh-classification modified by Oberhuber [29]) by an independent pathologist not involved in cell-counting. Patients who were diagnosed solely through serology were not included in the present study. Non-celiac-patients were considered as

controls, mostly being diagnosed with gastrointestinal reflux disease (GERD), gastritis or exclusion of other disease. Three controls' biopsies were excluded because there was not enough duodenal tissue material. Inclusion criteria were age <18 years, a confirmed CD-diagnosis with Marsh-Score of IIIa or higher for CD-patients, no other duodenum-affecting diseases, a histologically normal duodenal mucosa, assessed by an independent pathologist, for controls.

Clinical data

Additional clinical data were collected and analysed (e.g., laboratory results, symptoms, family history, comorbidities (aside from the histological diagnosis after EGD), especially CD-typical diseases and other intestinal diseases, medication) using the hospital's electronic record system and patients' files.

Esophagogastroduodenoscopy

Patients received intravenous analgesedation with propofol, ketamine and midazolam. Biopsy specimens were taken from different parts of the duodenum, stomach, and oesophagus (only duodenal specimens were re-examined in this study) with Olympus endoscopes (until November 2017: GIF Q 160, GIF 160, GIF XP160, since then: GIF XP 190N, GIF HQ 190, GIF H 190), depending on patients' height, weight, and age. Biopsy samples were fixed routinely with 4% buffered formaldehyde and embedded in paraffin.

Immunohistochemistry

The paraffin sections were serially cut at 1–2 µm. For the study's purpose, they were stained immunohistochemically using specific antibodies against MC-tryptase, a mediator stored in their granules [8] and c-kit (CD-117), the receptor for stem-cell-factor (SCF) on their cell-surface [30]. Each patient's slide got a barcode and a positive control to ensure quality of colour-reaction. Antibodies were diluted (tryptase: 1:5000, c-kit 1:500). The antigen-antibody-reactions were accomplished with a Leica Bond III device (automatic staining-machine) which facilitated a standardized and qualitatively equal staining-result. Deparaffinization, antigen-unmasking, staining-process as well as visualising the antigen-antibody reaction with a chromogen and counterstaining were entirely accomplished automatically. Furthermore, HE-staining was performed.

Histological evaluation of the samples

The samples were histologically evaluated using an Olympus CX21 light microscope. MCs and EOs in the duodenal lamina propria, when intact and nuclei visible, were counted out of ten randomly chosen non-overlapping high-power-fields (HPF) at ×400 magnification. Then, an average value was formed in each case. As both cell-types are predominantly located in the intestinal lamina propria [7,31], cells were only counted in this duodenal area. C-kit-positive MCs showed a

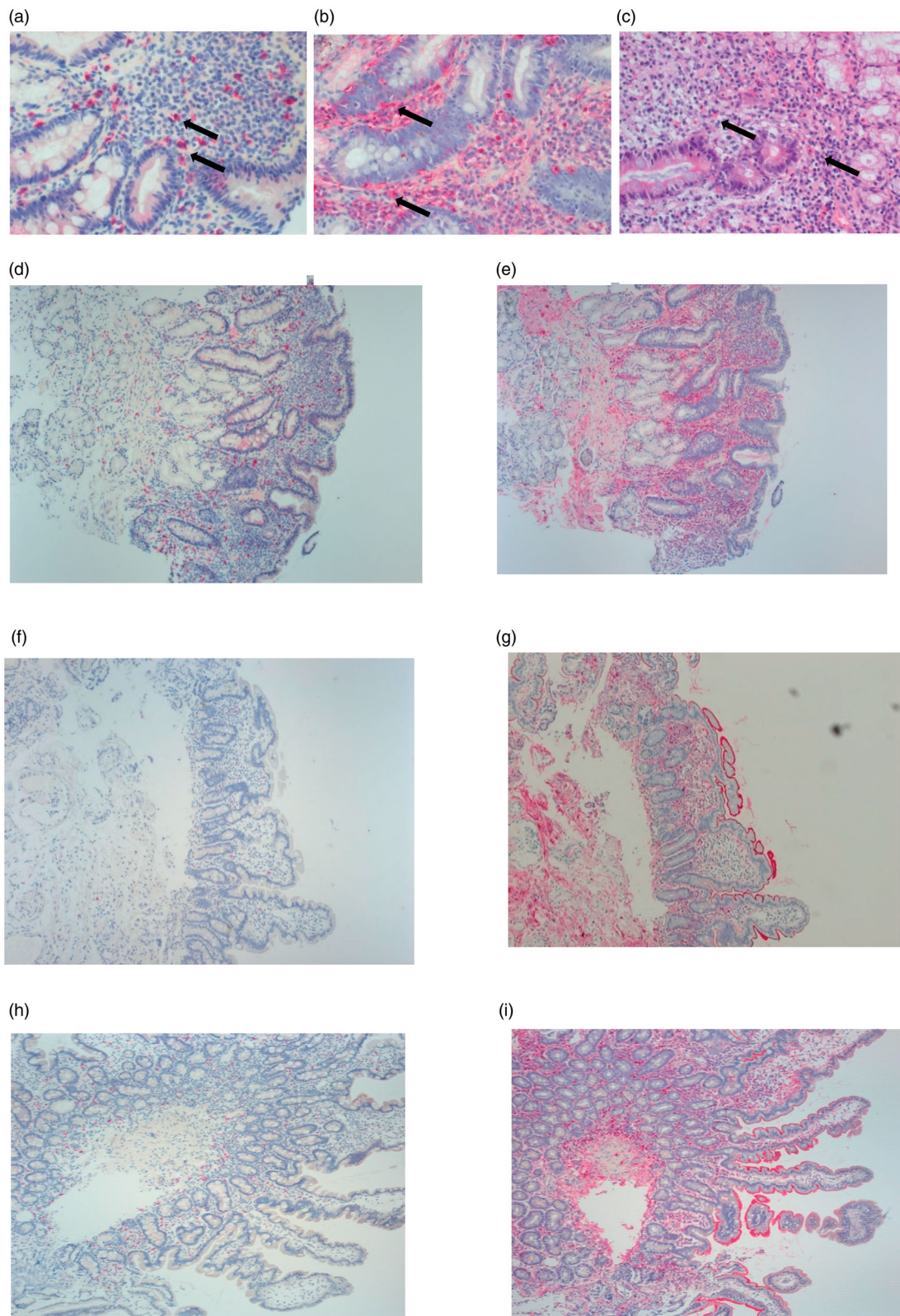


Figure 1. (a) c-kit (CD117) staining, 400 $\times$ magnification, arrows: c-kit-positive mast cells (MCs). (b) Tryptase staining, 400 $\times$ magnification, arrows: tryptase-positive MCs. (c) HE staining, 400 $\times$ magnification, arrows: eosinophils. (d) CD patient, c-kit (CD117)-staining, 100 $\times$ magnification, homogenous distribution. (e) CD patient, tryptase staining, 100 $\times$ magnification, homogenous distribution. (f) Control patient, c-kit (CD117) staining, 100 $\times$ magnification, predominantly middle to lower third. (g) Control patient, tryptase staining, 100 $\times$ magnification, predominantly middle to lower third. (h) Control patient, c-kit (CD117) staining, 100 $\times$ magnification, predominantly lower third. (i) Control patient, tryptase staining, 100 $\times$ magnification, predominantly lower third (the red staining at the brush border may occur as tryptase is bound at the epithelium).

red-pink cell-surface whereas tryptase-positive cells showed red-pink cytoplasm. EOs in HE-staining showed a bilobed nucleus and red-orange cytoplasm (Figure 1). Furthermore, the cell-distribution was examined at $\times 100$ magnification after dividing the lamina propria into thirds (upper third next to the lumen, middle, lower third), and classified as follows: homogeneously in all three layers, predominantly lower third, predominantly middle to lower third (there was no patient with a predominantly upper-third distribution). Representative examples of stained tissue sections are shown in Figure 1(d–i).

Analysis of possible factors influencing intestinal MCs/EOs

After cell-quantification, patients and controls were divided into two subgroups according to their average MC-/EO-number per HPF (≥ 29 MCs/HPF; <29 MCs/HPF; ≥ 19 EOs/HPF; <19 EOs/HPF (mean of controls' cell-counts + one standard deviation)) to evaluate other factors possibly influencing cell-counts in the lamina propria. As MC-counts obtained with both stains were similar, our subgroup-analysis was performed using only c-kit-stained MC-counts.

Statistical analysis

All data were implemented in an inhouse data base (Excel) and statistically analysed using Excel and SPSS (SPSS Inc., Chicago, IL). Continuous variables were expressed as mean $\pm$ standard deviation, discrete variables as percentages. Chi-square test or Fisher's exact test were used for frequencies and dichotomic measures when comparing two or three groups. Student's *t*-test was used for numeric factors, especially for comparing cell counts between CD-patients and controls, and the subgroups. Statistically significant factors and considered relevant were analysed with an ANOVA test for bifactorial variance. Statistical significance was determined at *p* value $<.05$.

Results

Patients/clinical data

109 patients with celiac disease (51 male, 58 female, age range 0.75–17.7 years, mean 8.4 years) and 106 controls with excluded CD but other diseases, mostly GERD or gastritis (50 male, 56 female, age range 0.5–17.25 years, mean 8.5 years) were enrolled in the current study. Only two CD-patients with less than one year of age were included, unfortunately information about age when complementary feeding started is not available.

26 (23.9%) CD-patients were classified as Marsh-score IIIa, 65 (59.6%) as IIIb and 18 (16.5%) as IIIc.

General clinical or epidemiological data were similar between both groups (i.e., weight/height percentiles). However, celiac patients had a more frequent positive family-history of autoimmune diseases (32.1 versus 2.2%) and CD-associated diseases, like diabetes mellitus type 1 and autoimmune thyroid disorders occurred more often (33 versus 9%). Controls suffered twice as often from atopic diseases (35.6 versus 17.4%), and more frequently from pulmonary (31 versus 3.7%) and intestinal diseases (20 versus 9.2%) next to other diseases (14 versus 4.6%). They were more often taking medication, like immunomodulating drugs (13.3 versus 1.8%) (Table 1).

Histological findings

We found significantly higher mast cell-numbers in celiac patients (23.7 (± 12.1)/HPF versus 19.7 (± 9.1)/HPF; *p* = .008 (c-kit-staining) and 25.3 (± 10.4)/HPF versus 20.9 (± 8.1)/HPF; *p* = .001 (tryptase-staining)). In both groups, MC values showed a large interindividual variability. CD-patients had significantly more duodenal eosinophils than controls (23.3 (± 9.3) versus 12.2 (± 6.3)/HPF; *p* = $<.001$) Both cell-counts were broadly overlapping between the groups (Figure 2(a)) (Table 2).

Table 1. Clinical data of the included celiac patients and controls.

Characteristic	Dimension	Controls	Celiac patients	p
N		106	109	
General data				
Male sex	<i>n</i> (%)	50 (47.2%)	51 (46.8%)	.955*
Age at EGD	(<i>n</i>) years, mean ($\pm$ SD)	(106) 8.5 (± 4.2)	(109) 8.4 (± 4.1)	.880 ^T
Laboratory results				
Peripheral eosinophils	(<i>n</i>) mean ($\pm$ SD)	(106) 258.5 (± 265.6)	(109) 265.8 (± 218.1)	.827 ^U
Family history ^A				
Autoimmune diseases	<i>n</i> / <i>N</i> (%)	2/89 (2.2%)	17/53 (32.1%)	.000*
Atopic diseases	<i>n</i> / <i>N</i> (%)	32/90 (35.6%)	20/51 (39.2%)	.665*
Comorbidities				
CD associated diseases ^B	<i>n</i> / <i>N</i> (%)	9/100 (9%)	36/109 (33%)	.000*
Atopic diseases ^C	<i>n</i> / <i>N</i> (%)	36/101 (35.6%)	19/109 (17.4%)	.003*
Gastroesophageal reflux disease	<i>n</i> / <i>N</i> (%)	73/106 (68.9%)	43/109 (39.4%)	.000*
Gastritis	<i>n</i> / <i>N</i> (%)	67/106 (63.2%)	73/109 (67%)	.563*
Other intestinal diseases ^D	<i>n</i> / <i>N</i> (%)	20/100 (20%)	10/109 (9.2%)	.026*
Pulmonary diseases ^E	<i>n</i> / <i>N</i> (%)	31/100 (31%)	4/109 (3.7%)	.000*
Other diseases ^F	<i>n</i> / <i>N</i> (%)	14/100 (14%)	5/109 (4.6%)	.018*
Immunomodulating drugs ^G	<i>n</i> / <i>N</i> (%)	13/98 (13.3%)	2/109 (1.8%)	.002*
Macroscopically inconspicuous patients	<i>n</i> (%)	90 (84.9%)	8 (7.3%)	.000*

p-Values in bold indicate statistical significance. ^AFirst- or second-grade relative suffering from the disease; ^Be.g., diabetes mellitus type 1, trisomy 21, autoimmune thyroid disorders, vitiligo, dermatitis herpetiformis Duhring; ^Ce.g., bronchial asthma, atopic dermatitis, hypersensitization; ^De.g., fructose-/lactose malabsorption; ^Ee.g., chronic cough, recurrent obstructive bronchitis; ^Fe.g., profound developmental disorders, rare diseases; ^Ge.g., (inhalative/topical) corticoids, mast cell stabilizers. *Chi-square-test; ^TT-test; ^UMann-Whitney *U* test.

Moreover, MCs were distributed significantly more often homogeneously in celiac lamina propria compared to controls in both staining-methods (44 biopsies (40.4%) versus 0 biopsies (0%); $p = <.001$). Regarding eosinophils no specific distribution-pattern was observable.

Correlations

MC- and EO-counts were moderately correlating in the duodenal tissue of both patient-groups ($r = 0.55$, $p = <.001$ (CD) versus 0.5 , $p = <.001$ (controls)) (Figure 2(b)). Moreover, MC-number/HPF and number of peripheral eosinophils/ μ l showed a slightly positive correlation in CD-patients ($r = 0.362$, $p = <.001$) as well as in controls ($r = 0.21$, $p = <.05$).

Number of peripheral and duodenal EOs slightly correlated as well ($r = 0.278$, $p = .003$ (CD)) and ($r = 0.301$, $p = .002$ (controls)). However, mast cell-count and number of intraepithelial lymphocytes (as assessed in the previous histological

evaluation) did not correlate in celiac patients ($r = 0.026$, $p = .784$). The extent of elevated serological antibody-titres was not associated with number of cells, neither for MCs nor for EOs.

Analysis of subgroups with higher and lower MC or EO counts

About 67.8% of our celiac patients had <29 MCs/HPF, whereas 68.8% had ≥ 19 EOs/HPF. Epidemiological data (including gender and age) did not differ between the subgroups [Table 1 (Supplement)]. However, CD-patients with increased duodenal MC- and EO-counts had significantly more peripheral eosinophils/ μ l than those with lower cell-counts, being the only difference between both ($336.6 (\pm 249.9)$ versus $232.3 (\pm 192.4)/\mu$ l, $p = .021$).

This was not observable in controls. Furthermore, controls with ≥ 29 MCs/HPF were taking immunomodulating drugs (mainly inhalative/topic corticosteroids), mostly because of

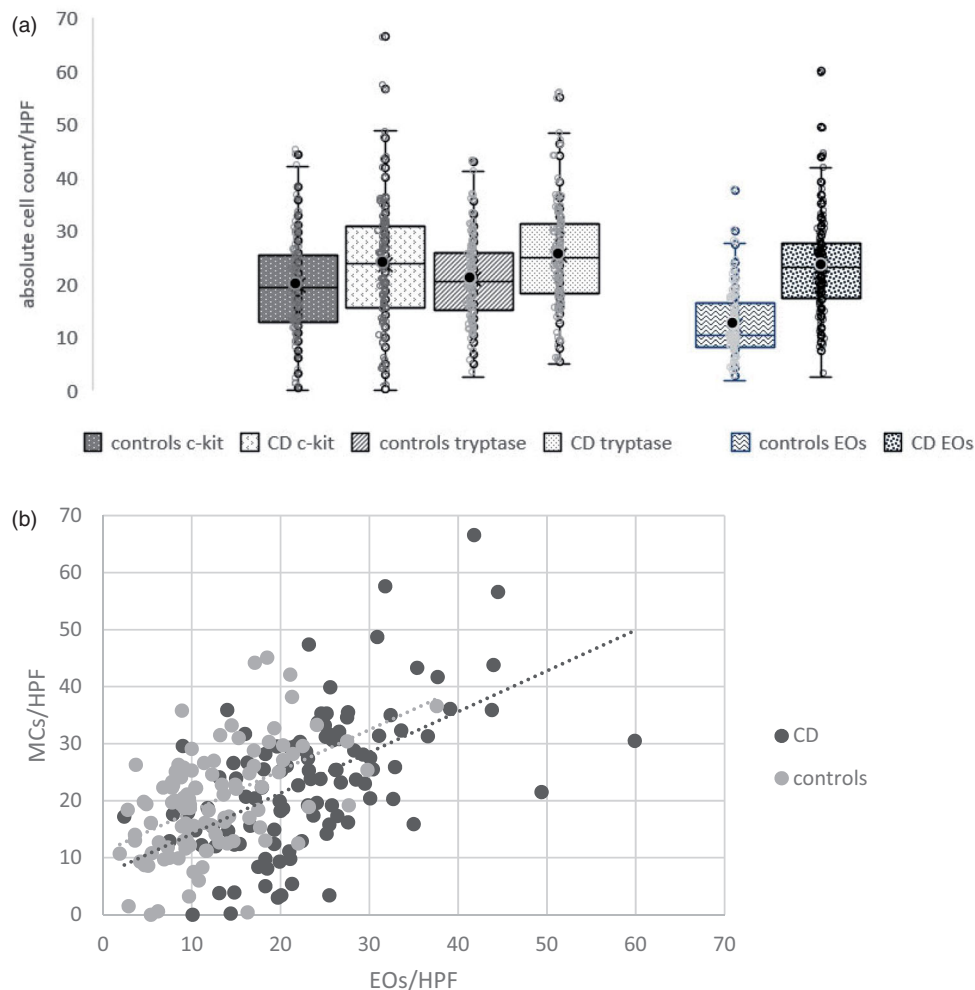


Figure 2. (a) Absolute mast cell (MC) (c-kit-, tryptase-staining) and eosinophil (EO) count (HE-staining); controls compared to celiac patients (abscissa). All patients are included, each dot represents one patient. Data are presented as number of MC/EO per high-power field (HPF) (ordinate). C-kit-staining: mean (black dots) $\pm$ SD: c-kit-staining: $19.7 (\pm 9.1)$ versus $23.7 (\pm 12.1)$; tryptase staining: $20.9 (\pm 8.1)$ versus $25.3 (\pm 10.4)$; EOs: $12.2 (\pm 6.3)$ versus $23.3 (\pm 9.3)$. Median (thin line): c-kit-staining: 19.35 versus 23.8 , tryptase-staining: 20.55 versus 24.9 ; EOs: 10.3 versus 23.1 . (b) Correlation of EOs/MCs/HPF. Both were moderately correlating in both groups: ($r = 0.55$, $p = <.001$ (CD) versus 0.5 , $p = <.001$ (controls)).

Table 2. Duodenal mast cell (MC) and eosinophil (EO) counts and distribution of MCs in the lamina propria of included celiac patients and controls.

Characteristic	Dimension	Controls	CD	p
n		106	109	
Average MC number ¹	Mean ($\pm$ SD)	18.7 ($\pm$ 5.1)	23.8 ($\pm$ 8.8)	.008^T
Median and range of MC number ¹	n (range)	19.35 (0–60)	23.8 (0–66.6)	
Average MC number ¹	Mean ($\pm$ SD)	20.9 ($\pm$ 8.1)	25.3 ($\pm$ 10.4)	.001^T
Median and range of MC number ¹	n (range)	20.55 (2.6–43.2)	24.9 (4.9–56.1)	
Average EO number	Mean ($\pm$ SD)	11.5 ($\pm$ 4.8)	23.4 ($\pm$ 7.6)	<.001^U
Median and range of EO number	n (range)	10.3 (1.9–66)	23.1 (2.4–77)	
Lamina propria MC distribution				<.001[*]
Homogenous	n (%)	0 (0%)	44 (40.4%)	
Esp. middle to lower third	n (%)	48 (45.3%)	50 (45.9%)	
Esp. lower third	n (%)	58 (54.7%)	15 (13.8%)	
				.002[*]
Homogenous	n (%)	0 (0%)	44 (40.4%)	
Further away from the intestinal lumen	n (%)	106 (100%)	65 (59.6%)	

p Values in bold indicate significant significance. ¹c-kit staining; ²tryptase-staining. ^{*}Chi-square test/Fisher's exact test; ^TT-test; ^UMann–Whitney U test.

obstructive pulmonary diseases, more frequently than those with <29 MCs/HPF. However, these subgroups were too small for further statistical analysis. The used anaesthetics did not differ within the subgroups [Table 1 (Supplement)]. A statistical analysis after exclusion of all patients taking immunomodulating drugs revealed similar results.

The severity of duodenal mucosal lesions (Marsh-score) was similar between celiac patients with increased or lower MCs and EOs [Table 2 (Supplement)].

Biopsies of CD-patients with ≥ 29 MCs/HPF were additionally analysed regarding the location of MCs within the duodenal mucosa, resulting in a non-statistically significant tendency to a homogenous cell-distribution. A subgroup analysis comparing biopsies with homogenous MC-distribution within all three layers versus MCs residing more distant from the intestinal lumen showed significantly more CD-patients' biopsies with homogenous MC-distribution ($p = .042$) [Table 2 (Supplement)].

Neither the occurrence of elevated MCs/EOs nor a homogenous distribution-pattern were associated with higher Marsh-scores (data not shown).

Bifactorial analysis of variance [supplement]

Parameters with significant differences considered relevant for MC- /EO-count were further analysed with an ANOVA bifactorial variance analysis, revealing that only the combination of celiac disease and peripheral blood eosinophilia ($p < .01$), but neither atopic diseases nor IgE-elevation, are independent factors with a significant interaction ($p = n.s.$) (Supplement).

Discussion

In our patient-cohort MC- and EO-numbers were higher than recently reported [32], in healthy children as well as CD-patients. Numbers of both cell-types were significantly increased in CD-patients. Cell-counts varied interindividually and two-thirds of CD-patients showed increased EO-values, while two-thirds showed lower MC-values (Figure 2(a)).

MC-quantification in the gut showed conflicting results [19–23,33], probably due to methodological differences (tissue-fixation, staining-techniques, cell-counting-methods). Three studies are in accordance with our results [19,20,33], only one using the tryptase-staining-method: Frossi et al. showed increased MC-numbers in CD-adults, correlating with higher Marsh-scores [33]. The others [19–23] used staining-methods with lower specificity, nowadays not used for MC-staining. Strobel et al. also demonstrated elevated MCs in adult jejunal biopsies in 1983 using astra-blue-/safranin staining, turning to lower values after gluten-free diet [20]. In 1986, Wingren et al. found higher MC-numbers using toluidine-blue-staining in adult CD-patients, highly correlated with the intestinal histamine content [19]. In contrast, Dollberg in 1980 [22] showed opposing results in children using astra-blue-staining [22]. Also, Suranyi et al. found depressed MC-values in children, remaining lower even after introduction of gluten-free diet ($n = 47$) [23]. Patient-groups in these studies were smaller than that in our study-population (e.g., $n = 28$ [33]) and only two were carried out in children [22,23].

We used specific MC-markers, assuming all stained cells were indeed MCs. Some state that 'tryptase-staining underestimates the number of degranulated MCs' because once released tryptase does not stain MCs anymore [34]. Nevertheless, c-kit as second staining-technique gave similar results, demonstrating both methods' validity.

Similar to us, others showed increased EO-numbers (mostly in HE-staining) [18,21,24,25], and -mediators [26,27,35] in CD-patients compared to controls. Lancaster-Smith et al. found increased jejunal EOs using HE-staining in CD-children under gluten-containing diets ($n = 22$) [24]. Similar results in adults were demonstrated by Potter et al. in 2020 who found elevated EOs, however, no correlation with villous-atrophy or clinical symptoms [25]. Significantly higher EO numbers in adult CD patients with persisting symptoms despite gluten-free diet [28] and significantly enhanced extracellular depositions of major basic protein (MBP), an EO-derived cationic protein [26] and activated EOs [35] in adult patients were described.

Only few evaluated both cell-types or their activation in tissue of CD-patients. Marsh et al. found elevated MC- and

EO-levels in CD-adults [18] and increased secretion-rates of ECP/histamine after dietary gliadin-challenge were reported [27]. Kosnai et al. demonstrated decreased MCs, but increased EOs, turning to the opposite following an adequate diet in a paediatric study-population [21].

Various expressed receptors and released mediators facilitate MCs' interaction with other immune-cells and their participation in inflammatory reactions [36]. They reside close to EOs; both communicating *via* their mediators; i.e., IL-5/IL-13 or chemokines and leukotrienes released by MCs to activate and recruit EOs [37,38] but also *via* physical interactions [39]. EO-produced corticotropin-releasing factor (CRF) seems to activate MCs, enhancing epithelial permeability [13,15]. The interaction of both seems to reduce the epithelial-barrier function [12], supporting both cell-types' involvement in CD-pathobiology, where an impaired epithelial-barrier function and enhanced permeability compared to healthy individuals is assumed [40]. We found moderately correlating duodenal MC- and EO-numbers in both patient groups, matching the results of Reed et al. [41] and Mir et al. [37].

The MC-distribution-pattern differed significantly between both groups: almost half of CD-patients' biopsies presented MCs localized homogenously over the lamina propria, whereas MCs in control-biopsies resided more distant from the duodenal lumen. Regarding EOs, no polarity was observed.

This underlines the influence of MCs in CD-pathobiology, although functional studies, highlighting the exact processes, are needed. We put to discussion that they reside in upper parts of the lamina propria leading to disruption of epithelial integrity *via* their released mediators and promote CD-development. It further suggests their attraction to intraluminal gluten, the well-known triggering agent of CD [1]. Still, opposed to findings by Frossi et al. [33], neither distribution-pattern or MC-number nor combination of both were associated with higher Marsh-scores, apparently not indicating higher disease-activity. Others also showed neither eosinophil-cationic protein (ECP) secretion-rates [42] nor duodenal eosinophilia [25] correlating with CD-disease-activity. It needs to be clarified whether MCs/EOs are responsible for disease-development or whether their elevation is a secondary phenomenon. To our knowledge, distribution-patterns of both cell-types in the lamina propria of CD-patients have not been looked at before, so the meaning of this finding regarding CD-pathophysiology remains currently unclear.

Although both cell-types are especially associated with allergic inflammation, there is evidence of simultaneous presence also in non-allergic diseases. Eosinophil-numbers are elevated in the late phase of allergic reactions [39], however, we did not always find higher EO-numbers in atopic patients.

Apart from obvious disease-associated parameters, patients and controls showed significant clinical differences (e.g., atopic diseases, comorbidities, and medication). As atopy may increase both cell-types' numbers [39,43] and because of control-patients with increased MC-/EO-populations, we subdivided both groups according to their average cell-number to investigate possible influencing factors. Most

assessed parameters (e.g., age, gender, weight/height percentiles, prevalence of atopic diseases, clinical symptoms, etc.) were not correlated with higher cell-counts. Age discrepancies were eliminated, as MC-numbers usually vary in accordance with age [9].

Considering the entire study-population, atopic diseases occurred significantly more often in controls. However, comparing the subgroups, the prevalence of atopy was equally divided, indicating an independent increase of both cell-types in CD. IgE-levels or IgE-RAST neither differed between CD-patients and controls, nor within the subgroups, so the increase of both cell-types is assumingly not attributed to these factors.

Interestingly, higher peripheral blood eosinophil levels were associated with higher duodenal MC-/EO-numbers in CD-patients compared to those with lower duodenal cell counts, being the only difference between both subgroups.

We compared the serological antibody-titres with cell-number, taking into account the possibility that patients with higher titres could also have higher cell-counts. Twenty-three (21.1%) CD patients were diagnosed before the guidelines changed: all of these patients with elevated serological CD markers received EGD. The other 86 included patients (78.9%) were diagnosed after the update of guidelines. We have diagnosed patients who did not receive EGD because their serological antibody-titres were already sufficiently high for diagnosis in accordance with their clinical symptoms. These patients were not included in the study. However, all patients who received EGD after 2012 were included in the present study and in the examined patient cohort there was overall no association between cell-number and antibody-titres.

It has been shown that MCs next to EOs, both elevated in number, are involved in eosinophilic gastrointestinal disorders (EGID) and that elevation of both cell-types correlates with one another [41]. As we saw this phenomenon also in our study-population, we suggest that CD might show similar characteristics as the yet described EGID. However, the role EOs and MCs play in the pathobiology of these diseases remains to be defined.

A limitation of the study is its retrospective study design, particularly lacking clinical data as we had to draw back on the hospital's electronic record system which was sometimes not entirely complete. We had no information about the recent state-of-the-disease. Moreover, for obvious reasons, we were not able to analyse biopsies from children without indication for EGD. Broad inclusion criteria for performing an EGD and the control group's heterogeneity might be responsible for higher cell-numbers in some control's biopsies, although only control's duodenal samples evaluated normal were examined. Since patients clinically responded to gluten-free diet no control biopsies were available to find out whether and how MC-/EO-counts changed in the course of the disease.

Conclusion

CD-children have significantly higher duodenal lamina propria MC- and EO-numbers than controls. We assume that both

cell-types play a major but not the only role in disease-pathophysiology. Further prospective longitudinal studies will be needed to understand both cell-types' influence on CD-patients' outcome and chronological sequences in which certain mediators (e.g., IL-5, IL-13, ECP) are elevated or depressed during disease-progression and how they promote tissue damage. Analogously to EGID, yet not as high in number, CD shows the phenomenon of increased MCs/EOs, correlating in number with each other, however, this connection will have to be highlighted by further studies.

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