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Chronic urticaria is associated with mast cell infiltration in the gastroduodenal mucosa

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Abstract Chronic urticaria (CU) is characterized by recurrent itching skin eruptions caused by mast cell degranulation. Relapses can be provoked by food intake. The aim of this study was to investigate if the mast cell number in the gastroduodenal mucosa is increased in CU patients, and whether mast cell counting by pathologists is clinically useful. We defined two study groups: 50 disease controls (16 Belgians and 34 Italians) and 43 Belgian CU patients. Mast cells were detected using immunohistochemistry for tryptase and CD117. The mast cell number in the disease controls was 20.2/high-power field (HPF; $133.3/\text{mm}^2$) in the stomach and 32.5/HPF ($209.2/\text{mm}^2$) in the duodenum. There was no difference between Belgian and Italian controls, indicating that dietary habits have no influence on the normal gastroduodenal mast cell number. In CU patients, mast cell numbers were significantly higher: 32.4/HPF ($186.0/\text{mm}^2$) in the stomach ($P<0.0001$) and 44.8/HPF ($246.0/\text{mm}^2$) in the duodenum ($P=0.0002$). CU is thus associated with mast cell infiltration in the gastroduodenal mucosa, even if patients do not have gastrointestinal symptoms. Mast cell counting in gastro-

duodenal biopsies of CU patients can be useful in selecting patients who may respond to a therapy with intestinal mast-cell-stabilizing agents.

Keywords Mast cell · Chronic urticaria · Stomach · Duodenum · Nutrition

Introduction

Urticaria is a very common skin disease characterized by the sudden appearance of smooth, raised, red, pink or white bumps and wheals of varying sizes almost anywhere on the skin (Fig. 1). The lesions usually last for several hours and then disappear without therapy [22]. About 20% of the general population has at least one episode of urticaria during their lifetime. When the disease lasts for more than 6 weeks and the skin eruptions occur at least twice a week, the condition is called chronic urticaria (CU) [15]. The aetiology of CU is unclear in most cases. Epidemiological studies show that it occurs most often in middle-aged women, but it can affect all ages, genders and races. Hidden or overt infections, abdominal disorders, and in some cases, mental strain have been associated with the disease. The skin lesions of CU are caused by vasoactive mediators released through specific or non-specific mast cell degranulation in the skin or elsewhere. CU patients are particularly susceptible to the non-specific histamine-releasing effect of pseudoallergenic substances in various foods and drugs, and the success rate of pseudoallergen-free diets varies between 30 and 50% [5, 27]. It is conceivable that food pseudoallergens induce non-specific mast cell degranulation, rather in the gastrointestinal tract than elsewhere. Activation of many intestinal mast cells may then result in enough histamine release to cause urticaria either directly or indirectly.

Mast cells are involved in numerous inflammatory and immunologic processes. They are part of our innate defence against bacterial and parasitic infections, and they play a role in the induction of the acquired immune response [12]. They store and release various mediators

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Fig. 1 Bumps and wheals of varying sizes in a patient with chronic urticaria

including histamine, tryptase, fibroblast growth factor-2, vascular endothelial growth factor and nerve growth factor. Histamine causes blood vessels to widen and leak fluid into the surrounding tissue, thereby producing swelling. Histamine also binds on the H1 receptors of neurons, which results in an itching sensation.

Another substance released upon mast cell degranulation is β -tryptase, which is a mast-cell-specific product. In humans, there is a constitutive low-grade release of α -tryptase from mast cells. Therefore, the determination of baseline serum tryptase, which measures both α - and β -tryptase, may be used to estimate the mast cell load. In CU patients, baseline levels of histamine and tryptase are higher than in normals [4, 14]. This may result either from mast cell activation or from a higher number of mast cells in the skin or elsewhere. Haas et al. [13] report an increase in mast cells in lesional and non-lesional skin of CU patients with classical histochemical staining methods. However, recent studies that have used immunohistochemical techniques with specific monoclonal antibodies to mast cell tryptase have not confirmed these findings [6, 21].

In the normal human gastrointestinal tract, mast cells are mainly located in the mucosal lamina propria. In the submucosa, mast cell density is lower, while the muscularis propria and serosa contain only negligible quantities of mast cells. Immunohistochemical data on gastroduodenal mast cell numbers in healthy subjects are however very scarce. The mast cell number in the digestive tract has been studied in mast-cell-related diseases such as systemic mastocytosis, and in other conditions like coeliac disease. Results are contradictory with either the reported increase or decrease [1, 7, 8, 10, 20, 24]. At this moment, little is known about mast cell numbers in the gastroduodenal mucosa in CU. The aim of this study is threefold: (1) to determine by immunohistochemical techniques the mast cell number in the normal gastroduodenal mucosa, (2) to investigate whether different dietary habits influence the mast cell number in normal subjects and (3) to determine whether the number of mast cells is increased in the gastroduodenal mucosa of CU patients because this could be of importance for the therapy for CU.

Materials and methods

Patients

The patient group consisted of 43 Belgian subjects (M/F ratio 15:28, age range 16–77 years, median age 45 years) with CU referred to the Department of Allergy and Clinical Immunology in our hospital. CU was defined as urticarial lesions appearing at least twice a week for more than 6 weeks. In 42 out of 43 patients, the baseline serum tryptase level was determined with the UniCAP-Tryptase fluoro-immunoassay (Pharmacia-Upjohn, Sweden). In 1 patient, tryptase was not measured because of an administrative error. A gastroduodenoscopy was performed prospectively as part of the investigative procedure in all patients, whether gastrointestinal symptoms were present ($n=30$) or not ($n=13$). One patient had been treated for *Helicobacter pylori* (Hp) infection in the months preceding the endoscopy. Biopsies were taken from the gastric corpus, the antrum and/or the duodenum. A urea breath test was included in the diagnostic work up after the start of the study and was performed in 25 out of 43 patients. All patients were questioned for the presence of rhinitis, asthma and food allergic or pseudoallergic symptoms. They were also asked to report on intolerance reactions to food pseudoallergens such as additives, histamine-releasing agents and alcoholic drinks. They were prospectively investigated by skin prick tests (SPT) with a screening panel including pollen of grasses, trees and weeds, with house dust mite, moulds, cat, dog and with food allergens in patients with a positive history. Specific IgE determinations (CAP system, Pharmacia-Upjohn) for respiratory and/or food allergens were performed in patients with a negative SPT. Patients with a positive history and at least one positive SPT or CAP-test were considered as “atopic”, whereas the other patients were designated as “non-atopic”.

Disease controls

A control group of 16 Belgian and 34 Italian (Sardinian) subjects (M/F 17:33, age range 6–72 years, median age 45 years) without urticaria underwent upper endoscopy for abdominal pain, pyrosis, weight loss or anaemia. Biopsies were taken from the gastric corpus, the antrum and/or the duodenum where appropriate. All disease controls were tested for coeliac disease: serology was negative in all cases except for 1 Italian subject having a positive anti-gliadin but negative anti-endomysium Ab test.

Biopsy evaluation

All biopsy specimens were fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Haematoxylin–eosin (HE) stained sections were examined for routine diagnosis using the Sydney system for gastric biopsies [9].

Immunohistochemical detection of tryptase and CD117 (c-kit) was chosen as the preferred method for identification of mast cells. For tryptase detection, a 1:100 diluted monoclonal mouse–antihuman mast cell tryptase antibody solution (DakoCytomation, clone AA1) was applied for 30 min at room temperature after proteolytic enzyme digestion with trypsin. The reaction was completed by adding avidin–biotin–peroxidase complex and 3,3'-diaminobenzidine (DAB) chromogen. CD117 was detected with a 1:250 diluted polyclonal rabbit–antihuman antibody solution (DakoCytomation, code no. A4502). Antigen retrieval was by boiling the dewaxed paraffin sections in 0.01 M Tris–EDTA solution (pH 9) for 30 min. The reaction was completed by addition of peroxidase-labelled anti-rabbit EnVision + Reagent (DakoCytomation) and DAB chromogen. Sections were faintly counterstained with Harris haematoxylin. Mast cells were counted by two pathologists (F.M. and C.W.) on a Zeiss light microscope. Only cells with a visible nucleus and unequivocal cytoplasmic or membranous staining were considered as mast cells. Differential diagnosis with Cajal cells was by cell shape (rounded instead of elongated) and by location. Counting was done only in vertically oriented tissue pieces. Mast cells were counted in five high-power fields (HPF; surface area of 1 HPF at magnification $\times 400 = 0.229 \text{ mm}^2$) and also in 5- μm grids composed of 100 small squares (surface area of 1- μm grid at magnification $\times 400 = 0.090 \text{ mm}^2$), enabling us to express the results as mean mast cell number per millimeter squared. The optical field of the microscope was positioned at the superficial part of the mucosa, so that as much lamina propria as possible was included.

Biopsies were also stained immunohistochemically for Hp in all CU patients and in the disease controls when appropriate. Hp staining was done using a 1:40 diluted polyclonal rabbit–anti-Hp antibody solution (DakoCytomation, code no. B0471) applied for 30 min at room temperature after heat-induced epitope retrieval by boiling the dewaxed paraffin sections in 0.01 M citrate solution (pH 6) for 30 min. The reaction was completed by addition of peroxidase-labelled anti-rabbit EnVision + Reagent (DakoCytomation) and DAB chromogen.

Statistical methods

All statistical analyses were performed using non-parametric tests (Mann–Whitney test and Spearman's rank correlation). *P* values less than 0.05 were considered statistically significant.

Results

Endoscopic findings

Upper endoscopy was normal in all disease controls. In the CU patients, there were no endoscopic abnormalities in 28/43 subjects. Twelve CU patients had signs of reflux oesophagitis, 1 showed gastric mucosal hyperaemia, 1 had a gastric ulcer suspicious for malignancy and 1 had a duodenal ulcer.

Pathological findings

Examination of the HE-stained sections showed normal gastric and duodenal biopsies in all disease controls. Twenty-seven CU patients had stomach and/or duodenal biopsies within normal limits. Otherwise, there were 4 cases of inactive chronic gastritis, 4 cases of active chronic gastritis, 4 cases of reactive gastropathy, 1 with fundic gland polyps, 1 with gastric adenocarcinoma and 2 with benign duodenal ulcers.

Immunohistochemistry for Hp was positive in 3 CU patients, 2 of whom had a positive breath test (no breath test was performed in the third). On the other hand, there were 2 CU patients with a positive breath test but with a negative Hp stain. Thus, there were 5 CU patients with Hp infection (4 of them had active chronic gastritis).

Immunohistochemistry allowed unequivocal identification and counting of mast cells in well-oriented biopsies. The tryptase stain showed granular positivity in the cytoplasm (Fig. 2), while CD117 was present in the cell membrane. Since mast cell numbers obtained by CD117

Fig. 2 **a** Cytoplasmic staining of mast cells in the stomach [immunohistochemistry (IHC) for tryptase, original magnification $\times 400$]. **b** Cytoplasmic staining of mast cells in the duodenum (IHC for tryptase, original magnification $\times 400$)

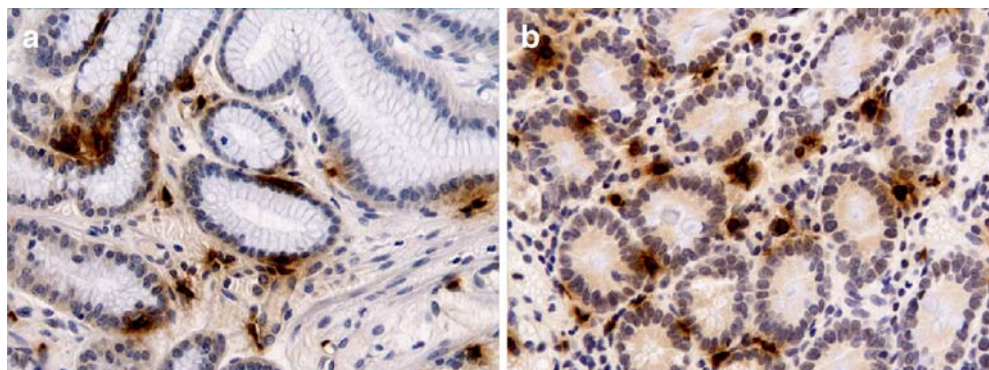


Table 1 Mean mast cell numbers per HPF and per mm² in disease controls and CU patients^a

Disease controls			CU patients		
	per HPF	per mm ²		per HPF	per mm ²
Stomach			Stomach		
All (n=20)	20.2±6.2 [17.4–22.9]	133.3±36.6 [117.3–149.4]	All (n=38)	32.4±9.4 [29.5–35.4]	186.0±46.9 [171.1–200.9]
Belgian (n=15)	20.7±6.5		All, normal ^b (n=20)	30.4±9.6 [26.2–34.6]	173.9±41.0 [155.9–191.9]
Italian (n=5)	18.4±5.3				
Duodenum			Duodenum		
All (n=35)	32.5±9.2 [29.4–35.6]	209.2±61.6 [188.8–229.6]	All (n=36)	44.8±16.9 [39.2–50.3]	246.0±89.2 [216.8–275.1]
Belgian (n=6)	32.0±10.2		All, normal ^b (n=27)	45.2±18.2 [38.4–52.1]	248.6±95.1 [212.7–284.4]
Italian (n=29)	32.6±9.2				

^aMean±standard deviation (95% confidence interval)^bNormal = biopsies histologically normal

stain were comparable to those obtained by tryptase stain (data not shown), all counts were performed on the tryptase stainings. The mean mast cell number for all disease controls together was 20.2/HPF (133.3/mm²) in the stomach and 32.5/HPF (209.2/mm²) in the duodenum (95% confidence intervals for mean mast cell numbers; see Table 1). There was no significant difference between Belgian and Italian controls either in the stomach (Mann–Whitney test, $P=0.49$) or in the duodenum ($P=0.76$).

The mean mast cell number for all CU patients was 32.4/HPF (186.0/mm²) in the stomach and 44.8/HPF (246.0/mm²) in the duodenum. Both in the stomach and in the duodenum, the mean mast cell number was significantly higher in the CU patients than that in the disease controls (Mann–Whitney test, $P<0.0001$ and $P=0.0002$, respectively; Figs. 3 and 4). The difference between CU patients and disease controls was more pronounced in the stomach than in the duodenum (a 61.0% increase in mast cell number in CU patients vs disease controls in the stomach vs a 37.8% increase in the duodenum). The mast cell number in the stomach was not significantly different between

CU patients with (29.4/HPF) and without (32.9/HPF) Hp infection (Mann–Whitney test, $P=0.3574$). When all 20 histologically normal stomach biopsies from CU patients were compared with the control biopsies, the mean mast cell number was still significantly higher in the CU patients (Mann–Whitney test, $P=0.0006$). The same was found when 27 histologically normal duodenum biopsies from CU patients were compared with their corresponding control biopsies ($P=0.0007$).

Relation of mast cell number to atopy, food allergy or pseudoallergy in CU patients

An atopic constitution was demonstrated in 11/43 CU patients. Atopic patients did not differ from non-atopic patients with regard to the gastric or duodenal mast cell numbers ($P=0.8553$ and 0.4126 , respectively).

In 4 of the 11 atopic patients, an IgE-mediated food allergy was detected. In these 4 patients, there was a trend towards higher mast cell numbers in stomach ($P=0.0750$)

Fig. 3 **a** Stomach from control subject (tryptase, original magnification $\times 200$). **b** Stomach from CU patient (tryptase, original magnification $\times 200$)

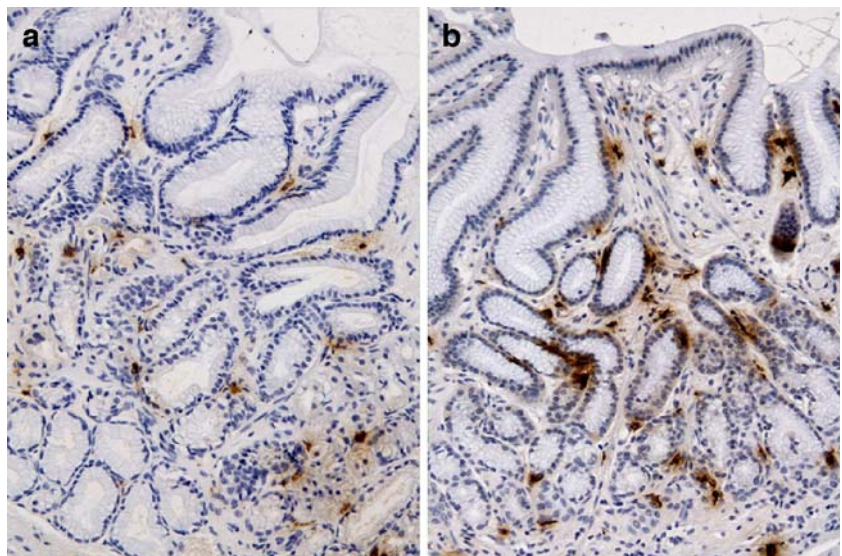
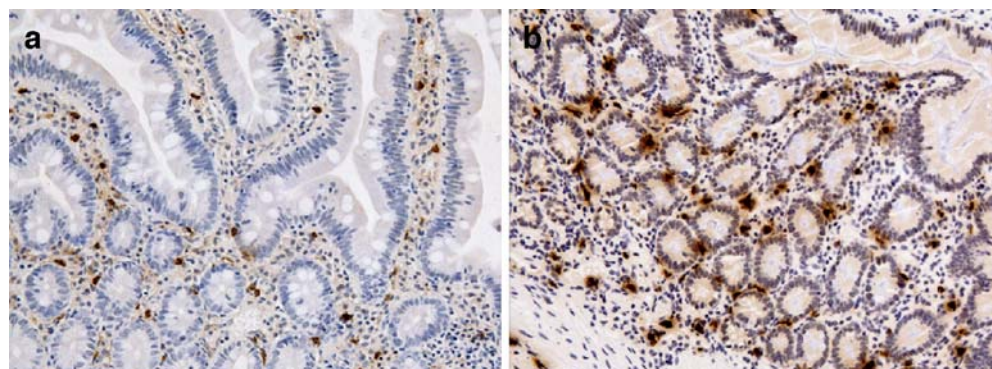


Fig. 4 **a** Duodenum from control subject (tryptase, original magnification $\times 200$). **b** Duodenum from CU patient (tryptase, original magnification $\times 200$)



than in the non-food allergic patients, but statistical significance was not reached probably because of the low number of food allergic patients.

Fourteen CU patients had a history of food pseudoallergen-induced aggravation of their disease. In these 14 patients, we surprisingly found a lower stomach mast cell number than in those without food pseudoallergy ($P=0.0252$).

Correlation of mast cell number with baseline tryptase levels in CU patients

Baseline tryptase levels were normal ($\leq 11.5 \mu\text{g/l}$) in 38 out of 42 CU patients. The mean value for all patients was $7.2 \mu\text{g/l}$ (range $2.3\text{--}21.9 \mu\text{g/l}$). There was no correlation between the gastric or duodenal mast cell number and the baseline tryptase levels (P values for the Spearman's rank correlation were 0.8998 in the stomach and 0.3982 in the duodenum).

Discussion

In this study, we investigated whether CU, a mast-cell-related disorder which is often aggravated by the intake of pseudoallergen-containing foods, might be associated with mast cell infiltration in the gastrointestinal mucosa. Classically, mast cells have been identified by metachromatic stains such as Giemsa or toluidine blue. We chose to detect them by immunohistochemistry for tryptase and CD117, as these methods are more sensitive and specific [16]. Tryptase is present in the cytoplasmic granules of the mast cells, and CD117 is located in the cell membrane. Positive staining results for tryptase may be more specific for mast cells [25]. Moreover, other authors have found that the results of tryptase and CD117 stains (with antigen retrieval) show a highly significant correlation, so that staining for mast cell tryptase alone apparently is sufficient [20]. Thus, we eventually chose to count mast cells in the tryptase stains. Unfortunately, there are only few authors mentioning normal values obtained by immunohistochemical methods, and their results are variable [7, 20]. We were therefore compelled to determine first the normal values in a disease control group. As there are no data available on the potential effect of different dietary habits on gastroin-

testinal mast cell number, we have included in the disease control group both Belgian and Italian (Sardinian) subjects. Italian dietary habits differ from the Belgian by the use of olive oil “extra vergine”, more seafood, more fresh vegetables, more wine, less beer and less food additives.

In the disease control group, we found a mean mast cell number of $20.2/\text{HPF}$ ($133.3/\text{mm}^2$) for the stomach and $32.5/\text{HPF}$ ($209.2/\text{mm}^2$) for the duodenum. Variability was somewhat larger in the duodenal mucosa; 95% confidence intervals were non-overlapping, however. The larger number in the duodenum may have been caused by selection bias: control subjects often had duodenal biopsies taken to rule out coeliac disease, a condition in which mast cell numbers may be increased [23, 26]. However, this cause is not plausible since all controls had normal duodenal histology, and all but one had normal antigliadin and anti-endomysium Ab tests. Our normal value for the duodenum is comparable to the $204.2/\text{mm}^2$ found by Crivellato et al. [7]. In contrast, Siegert et al. [20] found a higher mast cell number in the normal stomach than we did ($331.9/\text{mm}^2$ in the corpus and $251.9/\text{mm}^2$ in the antrum). This may be due to a different positioning of the optical field of the microscope: we chose to count mast cells predominantly in the superficial part of the mucosa, with the aim of including as much lamina propria as possible. Our way of positioning also tends to minimize possible differences between corpus and antrum biopsies.

We found that there was no significant difference between Belgian and Italian controls for mean mast cell numbers either in the stomach or the duodenum. Different dietary habits therefore seem to have no influence on the mast cell number in the gastroduodenal mucosa. This result also allowed us to compare the CU patient group (which is exclusively Belgian) with all disease controls (Italian as well as Belgian).

When compared to the disease control group, our CU patients demonstrated a significantly increased mast cell number in the stomach and the duodenum. Liutu et al. [17] found no significant differences in gastric mucosal mast cell numbers between patients with and without CU. However, they only used the less sensitive toluidine blue staining method. Mast cell numbers in the gastroduodenal mucosa have also been determined in mast-cell-related diseases like systemic mastocytosis and urticaria pigmentosa, again with varying results [8, 10, 20].

Gastrointestinal mast cell infiltration in CU may be primary or secondary to concomitant disorders. An increased number of gastrointestinal mast cells has indeed been described in several infectious or non-infectious types of gastritis, with a correlation between the mast cell number and the density of the polynuclear and mononuclear cell infiltrates [3]. In our study, however, there was no relation between the occurrence of a histological abnormality and mast cell counts. Indeed, even CU patients with histologically normal biopsies showed a significantly higher mast cell number in the stomach and the duodenum than disease controls. Hp infection, which is associated with reactive mucosal mast cell infiltration in the stomach [11, 18], is often reported as a possible cause of CU. In our CU patient group, the prevalence of Hp infection was 8% (3/38) with immunostaining and 24% (6/25) with the urea breath test. CU patients with and without Hp infection did not differ in the degree of mast cell infiltration in the stomach.

An increase in the number of intestinal mast cells has also been described in patients with food allergy by some authors [19] but was not confirmed by others [1]. Some reports even mention an increase in intestinal mast cells in atopic subjects with only inhalant allergy [2]. We were able to exclude that atopy and food allergy were the factors that determined intestinal mast cell infiltration in our CU patients. We may conclude that the observed increase in intestinal mast cell numbers in CU patients is directly connected with the disorder itself rather than being part of an inflammatory cell response.

That food allergens are able to cause local degranulation of mast cells has been proven during segmental mucosal challenges [19]. Likewise, one might expect food pseudoallergens to have a similar local effect on mast cells and to cause urticaria by intestinal mast cell degranulation. We therefore expected a higher mast cell number in CU patients with a history of food pseudoallergy than in those without, but we surprisingly found the opposite. This can be explained in different ways. First, the diagnosis of food pseudoallergy is very difficult and the sensitivity of history-taking is low. Ideally, the results of elimination diets should have been taken into account. Second, it could be that there is indeed no relation between mast cell infiltration and food-induced aggravation of CU. Further studies are needed to clarify this.

Determination of baseline tryptase is currently used to estimate the mast cell load. Clearly, elevated levels were found in only 4 out of 42 (10%) of our CU patients. The mean level of 7.2 µg/l in our study is comparable with the level in Hidvegi et al.'s [14] patients and is significantly higher than that in their control group. We did not find a correlation between baseline tryptase levels and intestinal mast cell numbers, which means that peripheral tryptase levels are probably not affected by local mast cell infiltration but predominantly reflect the total body mast cell load.

In conclusion, we showed that CU is associated with gastroduodenal mast cell infiltration. We strongly suppose that there is a causal relation between both phenomena, but

at this stage, it is impossible to establish the exact relationship. CU could indeed be the consequence as well as the cause of the reactive mast cell infiltration. This has still to be investigated. Further studies are also needed to establish whether it is justified to take endoscopic biopsies for tryptase and CD117 stainings in the absence of clear endoscopic lesions in CU patients without gastrointestinal symptoms. The finding of increased mast cell numbers might on the other hand be helpful in directing therapy towards strategies that could prevent intestinal mast cell degranulation. These patients might, for example, benefit more from pseudoallergen-free elimination diets, from H₂-antihistamines or from mast cell stabilizers as disodium-chromoglycate than CU patients with normal mast cell numbers.

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