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Food intolerance in patients with functional abdominal pain: Evaluation through endoscopic confocal laser endomicroscopy

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Authors
Besmir Gjini[1], Ilka Melchior[1], Philipp Euler[1], Christian Kreysel[1], Sigrid Kalde[1], Britta Krummen[1], Ralf Kiesslich[2], Bernhard Hemmerlein[3], Thomas Frieling[1]

Institutions
1 Department of Internal Medicine and Gastroenterology, Helios Clinic Krefeld, Germany
2 Department of Internal Medicine and Gastroenterology, Helios Clinic Wiesbaden, Germany
3 Department of Pathology, Helios Clinic Krefeld, Germany

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Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Corresponding author
Prof. Dr. med. Thomas Frieling, Department of Internal Medicine and Gastroenterology , Helios Clinic Krefeld, 47805 Krefeld, Lutherplatz 40, Germany
Fax: +0049-(0)2151-322078
thomas.frieling@helios-gesundheit.de

ABSTRACT

Background and study aims Gastrointestinal symptoms assumed to be caused by food intolerance are reported frequently in the general population. There is a significant difference between self-reported and objective proven food intolerance, as shown by placebo-controlled, double-blind, randomized trials. This discrepancy may be overcome by endoscopic confocal laser endomicroscopy (eCLE).

Patients and methods In an observational study we evaluated 34 patients with functional abdominal pain and adverse reaction to food by eCLE and local duodenal food challenge for the first time. Spontaneous and food-induced transfer of fluorescein into the duodenal lumen was detected 10 minutes after intravenously application of fluorescein and 10 minutes after duodenal food challenge (DFC).

Results Of the patients, 67.6 % responded with a fluorescein leakage into the duodenal lumen. Frequency rank order of food antigens that induced a response were soy (50 %), wheat (46.1 %), milk (20 %), egg (12 %), and yeast (11.5 %), respectively. Of the patients, 23.5 % showed spontaneous leakage of fluorescein, suggesting leaky gut syndrome. Histology of duodenal biopsies and mast cell function were normal. Overall, 69.5 % of patients improved with food exclusion therapy and 13 % were symptom-free according to eCLE.

Conclusions The results of our study indicate that eCLE is a clinically useful tool to evaluate patients with functional abdominal pain and adverse reaction to food and to create individualized dietary therapy with clinical benefit for patients.

Introduction

Gastrointestinal symptoms assumed to be caused by food intolerance are reported frequently in the general population. An estimated one-fifth of the population believe that they have adverse reactions to food [1]. This proportion is even higher in patients with disorders of the gut-brain axis (formerly functional gastrointestinal disorders) and may reach up to 80 % [2]. These symptoms include bloating, abdominal discomfort or pain reported soon after food ingestion. They may occur in different clinical conditions, such as disorders of the gut-brain axis, adverse reaction to food and gluten-related syndromes, which frequently are interrelated [3]. In clinical practice, diagnostic tools to identify food components that trigger gastrointestinal symptoms are limited. They comprise nutrition diary, identification of lactose, fructose, sorbit and histamine intolerance,

food allergies, mast cell disturbances, and elimination diets, respectively. However, there is a significant difference between self-reported and objective proven food intolerance, as shown by placebo-controlled, double-blind randomized trials [4, 5]. This discrepancy may be overcome by endoscopic confocal laser endomicroscopy (eCLE). It has been shown recently that eCLE can provide an objective measure to test immune-mediated reaction to food [6, 7]. During eCLE, duodenal application of specified food via the endoscope channel may induce immediate fluid extravasation through epithelial leaks.

Patients and methods

In an observational study we evaluated patients with functional abdominal pain who presented at the Department of Internal Medicine and Gastroenterology of the Helios Clinic Krefeld from January 2021 to June 2022. The study was approved by the local ethic commission (IRB Fr-21–01). Symptoms were evaluated via a standardized DSFQ symptom questionnaire [8]. All patients received a standardized diagnostic investigation, which was unremarkable. This included upper and lower gastrointestinal endoscopy with biopsies, magnetic resonance enteroclysis, Doppler sonography of the visceral vessels, and breath tests for sugar intolerances and small intestinal bacterial overgrowth (SIBO), respectively. Blood analysis included markers for inflammation/infection (c-reactive protein [CRP], blood sedimentation rate [BSG], white blood cell count), mast cell dysfunction (tryptase, N-methyl-histamine urine excretion), histamine intolerance syndrome (diamine oxidase), immunoglobulin E (IgE), autoimmune (antinuclear antibodies, [ANA] and celiac disease (immunoglobulin A [IgA] anti-transglutaminase antibodies), respectively. Stool analysis consisted of calprotectin as a marker for gastrointestinal inflammation that was below 50 µg/g stool and microbiological analysis without evidence for pathogenic viruses and bacteria. Before eCLE, patients received an allergen-reduced diet consisting of boiled rice (954 kcal, 18.9 g protein, 1.8 g fat, 211.5 g carbohydrate, 1.8 g fiber) and/or boiled potatoes (648 kcal, 16.2 g protein, 0.9 g fat, 153 g carbohydrate, 1.8 g fiber) and water or coffee without milk and sugar ad libidum for 3 days. eCLE was performed after an 8-hour fasting period. Standardized eCLE (Cellvizio System, Mauna Kea Technologies, Paris, France) was applied as previously reported [6, 7]. eCLE was performed after an 8-hour fasting period. The investigations were performed during conventional upper gastrointestinal endoscopy under propofol sedation. Spontaneous transfer of fluorescein into duodenal lumen was detected 10 minutes after intravenous application of fluorescein and 10 minutes after duodenal food challenge (DFC). Local food challenge was performed always by the same sequential application of five different main food allergens diluted in 30 mL water (280–310 mosm/L) to the duodenal mucosa. These were 1.5 g dry bio-yeast, 31.5 g dry egg, 1.5 g bio-milk, and 3 g soy flour and 3 g wheat flour, respectively. Local application of sodium chloride solution 10 % to the duodenal mucosa before food allergen exposure served as a control. The applications into the duodenal lumen were always above the major papilla. The diluted food allergens were applied directly to the duodenal mucosa through the biopsy channel of the endoscope.

At the end of the exposure time to the diluted food allergens, the remaining fluid was withdrawn by suction through the endoscope. There was no indication of aspiration. Positive mucosal reaction following food antigen exposure consisted of evoked leakage of intravenously applied fluorescein into the duodenal lumen as previously described [6, 7]. The mucosal reaction was always visible clearly and scanned at three different sites of the duodenal mucosa by two investigators. There was no intra-observer or interobserver variability. If a reaction to any of the food components took place, further food applications were discontinued. In this case, a second eCLE was performed after several weeks to complete the sequence of the food challenge. Images of eCLE findings before and after food challenge were documented and interpreted by two independent observers. After food challenge, six duodenal biopsies were taken to analyze for mucosal inflammation, intraepithelial lymphocytes (IELs) as well as number, distribution, and morphology of mast cells by standard immunohistochemistry (CD117 and CD 25) and counted per mm^2 tissue in each patient. Patients received food exclusion dietary advice focused on the results of eCLE. Clinical response to the dietary therapy was controlled 4 weeks after eCLE by repeating the symptom questionnaire. Statistical analysis was performed by Chi Quadrat and Mann Whitney U test and data were expressed as mean + SD.

Results

We evaluated 34 patients, 27 female, 46.4 + 15.0 years old. In all patients, diagnostic evaluation as described in detail previously revealed no evidence of organic diseases and no organic correlate that could explain their abdominal pain. Nine patients showed elevation in IgE, one patient together with elevation of IgG4 and three patients had a positive marker for Hashimoto thyroiditis. All patients reported their complaints to be independent of their bowel habits. Therefore, the patients fulfilled the diagnostic criterion for unspecified functional bowel disorder according to the Rom IV classification [9, 10] or for irritable bowel syndrome (IBS) according to German guidelines [11]. Of the patients, 73.5 % (n = 25, 20 female, 48.4 + 15.9 years) reported that their abdominal pain was triggered by food (FI +), whereas nine patients (7 female, 41.0 + 11.3 years) did not notice food intolerance (FI-).

Overall, eCLE showed spontaneous leakage of fluorescein in eight patients (23.5 %, 50 % female) that was not different from the subgroups with or without reported food intolerance (IF + : 14.7 %/75 %, IF-: 33 %/66 %). Three patients (eCLE-, 8,8 %, 3 female) who reported food intolerance had neither spontaneous nor food-induced fluorescein leakage. Twenty-three patients (eCLE + , 67,6 %) responded to the duodenal food challenge (▶ **Table 1**). Frequency rank order of food antigens that induced a response were soy (50 %), wheat (46.1 %), milk (20 %), egg (12 %) and yeast (11.5 %), respectively. In 10 patients with a positive eCLE, a second eCLE was performed after several weeks to complete exposure to the remaining food allergens. Two pa-

▶ **Table 1** Endoscopic confocal laser endomicroscopy with Fluorescein leakage into duodenal lumen following food challenge (eCLE+). FI+: patients reporting food intolerance, FI–: patients reporting no food intolerance.

eCLE+	Yeast	Egg	Soy	Milk	Wheat
overall	11.5%	12.0%	50.0%	20.0%	46.1%
FI+	15.0%	10.5%	41.%	12.5%	46.0%
FI–	0%	16,7%	100%	50.0%	0%

tients responded to soy and wheat and one patient to milk and yeast. Duodenal biopsies collected after food challenge showed normal histology and no evidence of inflammation, mucosal atrophy or increase in IELs. Mucosal mast cells appeared to be normal in morphology and distribution. Average mast cell number in duodenal mucosa was 99.86 + 55.24 /mm^2, 14–270 /mm^2 and there was no significant difference between FI+ and FI–, patients with and without spontaneous leakage of fluorescein and patients responding and not responding to food challenge, respectively (▶ **Table 2**). Similarly, laboratory analysis of mast cell function measured by serum tryptase and N-methylhistamine urine excretion as well as histamine intolerance as measured by diamine oxidase was normal in all patients and subgroups (▶ **Table 2**).

In the 23 patients who responded to the food challenge, the effect of dietary therapy was evaluated with a second questionnaire 4 weeks after eCLE (▶ **Table 3a** and ▶ **Table 3b**). Overall, 69.5% (n = 16) of the patients reported improvement in pain intensity and reduction in pain frequency. Three patients (13.0%) had no symptoms and five patients (21.7%) reported a reduction in pain frequency < 1×/week. Seven Patients (30.4%) reported receiving no benefit from the dietary therapy.

Discussion

To our knowledge, this is the first report describing the application of eCLE in patients with functional abdominal pain. The results of our study show that eCLE is a useful tool for evaluating functional abdominal pain associated with food intolerance in patients classified as having a nonspecific functional bowel disorder or IBS. The response to food challenge was robust and indicated by clearly visible leakage of IV fluoresceine into the duodenal lumen. This occurred always at different locations of the duodenal mucosa. More than two-thirds of the patients reported that their abdominal pain was triggered by food. This high rate of self-reported adverse reaction to food in patients with functional bowel disease also has been reported in the literature [12]. In contrast, the rate of objective proven food intolerance as shown by placebo-controlled, double-blind randomized trials is very low [4, 5]. The findings of our study suggest that eCLE could reduce the gap between subjective feeling and objective measurable adverse reaction to food. In our study, eCLE could detect immune-mediated mucosal reaction with leakage of fluorescein into the duodenal lumen following mucosal food exposure in almost 70% of patients. A comparable high rate of positive eCLE in IBS according to Rom III also has been described in other studies [6, 7]. However, to our knowledge, our findings are the first in patients with functional abdominal pain. Interestingly, soy and wheat were the food allergens that most frequently evoked a mucosal response. With regard to wheat, milk, egg and yeast, this is in line with other studies [6, 7]. However, the high response rate to soy (50%) in our study has not been reported before. The reason for this is unclear but it may be caused by patient selection.

Twenty-three percent of patients showed spontaneous leakage of fluorescein before duodenal food challenge, suggesting leaky gut syndrome. This also has been reported in patients with functional dyspepsia, suggesting loss of mucosal integrity, which could be triggered by stress-induced activation of mast cells [13–15]. A tight junction barrier defect that enhances disease progression also has been suggested in post-infectious IBS and IBD in which the barrier loss induced by infection may be the trigger that drives pathogenesis [16]. A tight junction barrier defect could also explain adverse reaction to different food components. However, further studies are needed to clarify this potential relationship.

In our study, histologic evaluation of duodenal mucosal biopsies after the duodenal food exposure revealed no patho-

▶ **Table 2** Number of mast cells in duodenal mucosal biopsies, serum tryptase, N-Methhylhistamine excretion in urine and serum diamine oxidase overall, in patients with (FI+) and without (FI–) food intolerance, spontaneous (SL+) and no (SL–) i. v. fluorescein leakage into duodenal lumen and positive (FC+) or negative (FC–) food challenge of duodenal mucosa. Mean ± SD, (range).

	Overall	FI+	FI–	SL+	SL–	FC+	FC–
Mast cells (n/mm^2)	99.86 ± 57.24 (14–270)	104.95 ± 60.54 (14–270)	83.85 ± 45.46 (20–147)	133.42 ± 54.10 (70–220)	89.18 ± 53.83 (14–270)	82.19 ± 36.54 (14–150)	168 ± 144.25 (66–270)
Tryptase (ug/l)	3.96 ± 1.59 (1.1–7.2)	4.13 ± 1.52 (1.1–7.2)	3.55 ± 1.79 (1.8–7.0)	3.28 ± 1.23 (1.8–4.9)	4.19 ± 1.66 (1.1–7.2)	3.94 ± 1.47 (1.8–7.2)	4.16 ± 3.00 (1.1–7.1)
N-Methyl-histamine (ug/l)	102.7 ± 70.47 (14–250)	94.38 ± 59.88 (14–226)	119.37 ± 92.38 (23–250)	126 ± 85.32 (14–233)	94.9 ± 66.21 (23–250)	104.20 ± 72.21 (14–250)	76.5 ± 53.0 (l39–114)
Diamine oxidase (U/l)	19.43 ± 18.16 (4.7–88)	21.11 ± 20.18 (4.7–88)	14.06 ± 8.69 (8.1–29)	13.13 ± 7.33 U/l, (5.4–20)	20.48 ± 19.33 (4.7–88)	19.33 ± 18.42 (5.4–88)	20.35 ± 22.13 (4.7–36)

▶ **Table 3a** Efficacy of dietary therapy on pain intensity as illustrated by the results of the first and second symptom questionnaire [8], n = 23 patients.

1. Questionnaire	2. Questionnaire				
	None	Slight	Moderate	Severe	Very severe
None	0	0	0	0	0
Slight	0	0	0	0	0
Moderate	1	1	3	0	0
Severe	2	4	3	3	0
Very severe	0	3	2	0	1

▶ **Table 3b** Efficacy of the dietary therapy on pain frequency as illustrated by the results of the first and second symptom questionnaire [8], n = 23 patients.

1. Questionnaire	2. Questionnaire				
	<1×/week	1×/week	2–3×/week	4–6×/week	Every day
<1×/week	0	0	0	0	0
1×/week	0	0	0	0	0
2–3×/week	2	0	2	0	0
4–6×/week	1	0	0	2	0
Every day	2	4	5	2	3

logic findings, such as mucosal inflammation, increased intraepithelial lymphocytes or mucosal mast cells. In addition, morphology and distribution of mucosal mast cells appeared to be unremarkable and serologic markers for mast cell dysfunction, histamine intolerance syndrome or autoimmune diseases were within normal range. Subtle activation of mucosal immune cells following duodenal exposure to food allergens has been reported in IBS [6, 7] and functional dyspepsia [13, 14]. In IBS [6, 7], a significant increase in IELs in eCLE images has been reported in eCLE-positive patients following food exposure, although IELs in histology were not different between CLE-positive and eCLE-negative patients [6]. In another study [7], eosinophilic counts were not different between eCLE-positive and eCLE-negative patients and IELs did not differ before and after exposure in the same patients. However, post-exposure IELs were significantly higher in eCLE-positive patients compared to eCLE-negative patients. In functional dyspepsia [13, 14], significantly higher epithelial gap density compared to controls has been described. This corresponded to impaired mucosal integrity, as shown by reduced transepithelial electrical resistance, increased number of epithelial cells undergoing pyroptosis, and altered duodenal expression of claudin-1 and interleukin-6. The trigger for leaky gut is unknown but could be mediated by the central nervous system (e. g. stress) as well as luminal factors such as food, acid, bile acids, and microbiota.

In our study, we did not evaluate the number of mucosal immune cells before and after food challenge. Therefore, a potential mucosal immune reaction evoked by food allergens remains to be proven. However, neither did we find an increase in IELs above normal range nor abnormalities in mucosal mast cell morphology and distribution after exposure. In addition, the number of mast cells was not different between the subgroups or between eCLE-positive and eCLE-negative patients.

Interestingly, almost 70 % of patients reported a clinical benefit with reduction of abdominal pain and 13 % were free of symptoms following use of a food exclusion diary. This is a significant finding because it suggests the potential benefit of selective dietary treatment guided by results of eCLE with food challenge in these patients. The results of our study suggest an opportunity for use of individual and tolerable dietary therapy in patients with adverse reactions to food and abdominal pain. However, the magnitude of the placebo effects remains to be proven.

We did not apply the food allergens to the duodenal mucosa in a randomized order and the applications were always above the major papilla. This may have caused a bias, but that appears unlikely to us. We have no information about the timing or local variance in response to the food challenge and cannot rule out this possibility. As far as we know, there are no data in the literature available to prove this assumption. In addition, we cannot rule out that part of the mucosal response to the food challenge was mediated by a non-immune mechanism such as local release of nitric oxide-mediated vasodilation by soy, wheat or the other food allergens [17, 18]. However, we did not see a response to local application of sodium chloride solution 10 % to the duodenal mucosa, which makes osmotic effects unlikely. In addition, if this effect of soy or wheat would have been the main mechanism for the mucosal reaction, we would expect such a response in a greater number of patients. However, this

was not the case because only 50 % and 46 % of patients reacted to duodenal challenge with soy and wheat, respectively.

Conclusions

In summary, the results of our study indicate that eCLE is a clinically useful tool for evaluating patients with functional abdominal pain/IBS and adverse reaction to food and to create individualized dietary therapy that may be clinically beneficial for patients.

Competing interests

The authors declare that they have no conflict of interest.

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