Endoscopy International Open

Food intolerance in patients with functional abdominal pain – evaluation through endoscopic confocal laser endomicroscopy

Thomas Frieling, Besmir Gjini, Ilka Melchior, Philipp Euler, Christian Kreyssel, Sigrid Kalde, Britta Krummen, Ralf Kiesslich, Bernhard Hemmerlein.

Affiliations below.

DOI: 10.1055/a-1978-6753


Conflict of Interest: The authors declare that they have no conflict of interest.

Abstract:

Background and study aims Gastrointestinal symptoms assumed 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 duodenal lumen was detected 10 minutes following intravenously application of fluorescein and 10 minutes after duodenal food challenge (DFC).

Results 67.6% of the patients 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. 23.5% of the patients showed spontaneous leakage of fluorescein suggesting leaky gut syndrome. Histology of duodenal biopsies and mast cell function were normal. Overall, 69.5% improved by the food exclusion therapy and 13% were symptom free according to eCLE.

Conclusion 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 an individual dietary therapy with clinically benefit for the patients.

Corresponding Author:
MD Thomas Frieling, HELIOS Klinikum Krefeld, Medizinische Klinik II, 47805 Krefeld, Lutherplatz40, 47805 Krefeld, Germany, thomas.frieling@helios-gesundheit.de

Affiliations:
Thomas Frieling, HELIOS Klinikum Krefeld, Medizinische Klinik II, Krefeld, Germany
Besmir Gjini, HELIOS Klinikum Krefeld, Medizinische Klinik II, Krefeld, Germany
Ilka Melchior, HELIOS Klinikum Krefeld, Medizinische Klinik II, Krefeld, Germany
[...]
Bernhard Hemmerlein, Helios Klinikum Krefeld, Institute of Pathology, Krefeld, Germany

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Food intolerance in patients with functional abdominal pain – evaluation through endoscopic confocal laser endomicroscopy


Institution
Dept. of Internal Medicine and Gastroenterology, Helios Clinic Krefeld, Germany
*Dept. of Internal Medicine and Gastroenterology, Helios Clinic Wiesbaden, Germany
**Dept. of Pathology, Helios Clinic Krefeld, Germany

Corresponding author
Prof. Dr. med. Thomas Frieling
Department of Internal Medicine and Gastroenterology
Helios Clinic Krefeld
47805 Krefeld, Lutherplatz 40, Germany
Phon. 0049-(0)2151-322707
FAX: 0049-(0)2151-322078
E-mail: thomas.frieling@helios-gesundheit.de
Abstract

Background and study aims Gastrointestinal symptoms assumed 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 duodenal lumen was detected 10 minutes following intravenously application of fluorescein and 10 minutes after duodenal food challenge (DFC).

Results 67.6% of the patients 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. 23.5% of the patients showed spontaneous leakage of fluorescein suggesting leaky gut syndrome. Histology of duodenal biopsies and mast cell function were normal. Overall, 69.5% improved by the food exclusion therapy and 13% were symptom free according to eCLE.

Conclusion 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 an individual dietary therapy with clinically benefit for the patients.

Key words
Endoscopic confocal laser endomicroscopy (eCLE), adverse reaction to food, food intolerance, functional abdominal pain, irritable bowel syndrome (IBS)
Introduction

Gastrointestinal symptoms assumed 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.

Material 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 01/2021 to 06/2022. 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, MR enteroclysis, doppler sonography of the visceral vessels, 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), IgE, autoimmune (antinuclear antibodies, (ANA) and celiac disease (IgA anti-transglutaminase antibodies), respectively. Stool analysis consisted of calprotectin as a marker for gastrointestinal inflammation that was below 50ug/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 fibre) and/or boiled potatoes (648 kcal, 16.2 g protein, 0.9 g fat, 153 g carbohydrate, 1.8 g fibre) and water or coffee without milk and sugar ad libidum for 3 days. eCLE was performed after an eight hour fasting period. Standardized eCLE (Cellvizio System, Mauna Kea Technologies, 9 rue d Enghien, 75010 Paris, France) was applied as previously reported [6, 7]. eCLE was performed after an eight hour fasting period. The investigations were performed during conventional upper GI endoscopy under propofol sedation. Spontaneous transfer of fluorescein into duodenal lumen was detected 10 minutes following intravenously application of fluorescein and 10 minutes after duodenal food challenge (DFC). Local food challenge was performed always by the same sequential application of 5 different main food allergens diluted in 30 ml water (280-310 mosm/l) to the duodenal mucosa. These were 1.5g dry bio-yeast, 31.5g dry egg, 1.5g bio-milk, and 3g soy flour and 3g 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 of 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 were no intra- or inter-observer 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. The images of the eCLE findings before and after food challenge were documented and interpreted by two independent observers. After food challenge, six duodenal biopsies were taken to analyse 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 mm² tissue in each patient. The patients received a food exclusion dietary advice focussed 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, 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 in material and methods revealed no evidence for organic diseases and no organic correlate that could explain their abdominal pain. 9 patients showed elevation of IgE, 1 patient together with elevation of IgG4 and 3 patients had positive marker for Hashimoto thyreoiditis. All patients reported their complaints to be independent of their bowel habit. The patients fulfilled, therefore, the diagnostic criterion for unspecified functional bowel disorder according to the Rom IV classification (9, 10) or to the irritable bowel syndrome (IBS) according to the German guideline (11). 73.5% of the patients (n=25, 20 female, 48.4 ± 15.9 years) reported that their abdominal pain was triggered by food (FI+), whereas 9 patients (7 female, 41.0 ± 11.3 years) did not notice food intolerance (FI-).

Overall, eCLE showed spontaneous leakage of fluorescein in 8 patients (23.5%, 50% female) that was not different to the subgroups with or without reported food intolerance (IF+: 14.7%/75%, IF-: 33%/66%). 3 patients (eCLE-, 8.8%, 3 female) who reported food intolerance neither showed spontaneous nor food-induced fluorescein leakage. 23 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 eCLE+, a second eCLE was performed after several weeks to complete the exposure with the remaining food allergens. 2 patients responded to soy and wheat and 1 patient to milk and yeast. Duodenal biopsies collected after food challenge showed normal histology were without evidence for 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/mm², 14-270/mm² and were not significant different between FI+ and FI-, patients with and without spontaneous leakage of fluorescein and patients responding and not responding to food challenge, respectively (table 2). Similar, 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 responding to the food challenge the effect of the dietary therapy was evaluated by a second questionnaire four weeks after eCLE (Table 3a, b). Overall, 69.5% (n=16) of the patients reported about improvement of the pain intensity and reduction of pain frequency. 3 patients (13.0%) were without symptoms and 5 patients (21.7%) reported a reduction of pain frequency < 1x/week. 7 Patients (30.4%) reported to have 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 to evaluate functional abdominal pain associated with food intolerance in patients classified as unspecific functional bowel disorder or irritable bowel syndrome. The response to food challenge was robust and indicated by a clearly visible leakage of i. v. fluoresceine into the duodenal lumen. This occurred always at different locations of the duodenal mucosa. More than two third of our investigated 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 has been reported also 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 the patients. A comparable high rate of eCLE+ in irritable bowel syndrome according to Rom III has been also 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 may be caused by the patient selection.

23% patients showed spontaneous leakage of fluorescein before duodenal food challenge suggesting leaky gut syndrome. This has been also 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 has been also suggested in post-infectious IBS and IBD where 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 pathologic 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 marker 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 intraepithelial lymphocytes (IEL) in eCLE images has been reported in eCLE+ patients following
food exposure, although IELs in histology were not different between CLE+ and eCLE- [6]. In another study [7],
eosinophilic counts were not different between eCLE+ and eCLE- and IELs not different between pre- and post-
exposure in the same patients. However, post exposure IELs were significantly higher in eCLE+ compared to
eCLE-. 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 the leaky gut is unknown but could be mediated by central nervous
(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, we
did neither find an increase of IELs above normal range nor abnormalities in mucosal mast cell morphology and
distribution post exposure. In addition, the number of mast cells was not different between the subgroups and
between eCLE+ and eCLE-.

Interestingly, almost 70% of the patients reported a clinical benefit with reduction of abdominal pain and 13%
were free of symptoms following the food exclusion diary. This is a significant finding because it suggests the
potential benefit of a selective dietary treatment guided by the results of eCLE with food challenge in these
patients. The results of our study open the chance for an individual and tolerable dietary therapy in patients with
adverse reaction to food and abdominal pain. However, the magnitude of 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 that appears to be unlikely to us. We have no
information about the time- or local varying variance of the response to food challenge and cannot rule out this
possibility. So far we know, there are no data in the literature available to proof this assumption. In addition, we
cannot rule out that part of the mucosal response to food challenge was mediated by a non-immune mechanism
such as the 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
making osmotic effects unlikey. 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%/46% of the patients reacted to duodenal challenge with soy/wheat.

In summary, the results of our study indicate that eCLE is a clinically useful tool to evaluate patients with
functional abdominal pain/IBS and adverse reaction to food and to create an individual dietary therapy with
clinically benefit for the patients.
References


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.

<table>
<thead>
<tr>
<th>eCLE+</th>
<th>Yeast</th>
<th>Egg</th>
<th>Soy</th>
<th>Milk</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>overall</td>
<td>11.5%</td>
<td>12.0%</td>
<td>50.0%</td>
<td>20.0%</td>
<td>46.1%</td>
</tr>
<tr>
<td>FI+</td>
<td>15.0%</td>
<td>10.5%</td>
<td>41.0%</td>
<td>12.5%</td>
<td>46.0%</td>
</tr>
<tr>
<td>FI-</td>
<td>0%</td>
<td>16.7%</td>
<td>100%</td>
<td>50.0%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Table 2

Number of mast cells in duodenal mucosal biopsies, serum tryptase, N-Methylhistamine 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).

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>FI+</th>
<th>FI-</th>
<th>SL+</th>
<th>SL-</th>
<th>FC+</th>
<th>FC-</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mast cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n/mm²)</td>
<td>99.86 ±</td>
<td>104.95 ±</td>
<td>83.85 ±</td>
<td>133.42 ±</td>
<td>89.18 ±</td>
<td>82.19 ±</td>
<td>168 ±</td>
</tr>
<tr>
<td></td>
<td>57.24</td>
<td>60.54</td>
<td>45.46</td>
<td>54.10</td>
<td>53.83</td>
<td>36.54</td>
<td>144.25</td>
</tr>
<tr>
<td></td>
<td>(14-270)</td>
<td>(14-270)</td>
<td>(20-147)</td>
<td>(70-220)</td>
<td>(14-270)</td>
<td>(14-150)</td>
<td>(66-270)</td>
</tr>
<tr>
<td><strong>Tryptase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ug/l)</td>
<td>3.96 ±</td>
<td>4.13 ±</td>
<td>3.55 ±</td>
<td>3.28 ±</td>
<td>4.19 ±</td>
<td>3.94 ±</td>
<td>4.16 ±</td>
</tr>
<tr>
<td></td>
<td>1.59</td>
<td>1.52</td>
<td>1.79</td>
<td>1.23</td>
<td>1.66</td>
<td>1.47</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>(1.1-7.2)</td>
<td>(1.1-7.2)</td>
<td>(1.8-7.0)</td>
<td>(1.8-4.9)</td>
<td>(1.1-7.2)</td>
<td>(1.8-7.2)</td>
<td>(1.1-7.1)</td>
</tr>
<tr>
<td><strong>N-Methylhistamine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ug/l)</td>
<td>102.7 ±</td>
<td>94.38 ±</td>
<td>119.37 ±</td>
<td>126 ±</td>
<td>94.9 ±</td>
<td>104.20 ±</td>
<td>76.5 ±</td>
</tr>
<tr>
<td></td>
<td>70.47</td>
<td>59.88</td>
<td>92.38</td>
<td>85.32</td>
<td>66.21</td>
<td>72.21</td>
<td>53.0</td>
</tr>
<tr>
<td></td>
<td>(14-250)</td>
<td>(14-226)</td>
<td>(23-250)</td>
<td>(14-233)</td>
<td>(14-250)</td>
<td>(14-250)</td>
<td>(139-114)</td>
</tr>
<tr>
<td><strong>Diamine oxidase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(U/l)</td>
<td>19.43 ±</td>
<td>21.11 ±</td>
<td>14.06 ±</td>
<td>13.13 ±</td>
<td>20.48 ±</td>
<td>19.33 ±</td>
<td>20.35 ±</td>
</tr>
<tr>
<td></td>
<td>18.16</td>
<td>20.18</td>
<td>8.69</td>
<td>7.33 U/l,</td>
<td>19.33</td>
<td>18.42</td>
<td>22.13</td>
</tr>
<tr>
<td></td>
<td>(4.7-88)</td>
<td>(4.7-88)</td>
<td>(8.1-29)</td>
<td>(5.4-20)</td>
<td>(4.7-88)</td>
<td>(5.4-88)</td>
<td>(4.7-36)</td>
</tr>
</tbody>
</table>
Table 3a

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

<table>
<thead>
<tr>
<th>1. Questionnaire</th>
<th>2. Questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Slight</td>
<td>0</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
</tr>
<tr>
<td>Severe</td>
<td>2</td>
</tr>
<tr>
<td>Very severe</td>
<td>0</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>1. Questionnaire</th>
<th>2. Questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1x/week</td>
<td>0</td>
</tr>
<tr>
<td>1x/week</td>
<td>0</td>
</tr>
<tr>
<td>2-3x/week</td>
<td>2</td>
</tr>
<tr>
<td>4-6x/week</td>
<td>1</td>
</tr>
<tr>
<td>Every day</td>
<td>2</td>
</tr>
</tbody>
</table>