Endomicroscopic Diagnosis of Food-induced Allergy-like Reactions

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Introduction

Approximately 4.1% of the world's population is affected by Irritable Bowel Syndrome (IBS) with chronic symptoms of abdominal pain, constipation, cramping, bloating, nausea, and diarrhea.[1][2] The diagnosis of IBS is based on assessment of symptoms, physical examinations, and a limited number of additional tests to rule out conditions that mimic the disorder (e.g., Crohn's disease).

The pathophysiology of IBS remains incompletely elucidated, but most recently altered reactions to food intake have gained prominence as a cause of symptoms and a target for therapy through elimination diets.[3,4] There is an already large body of literature supporting the use of low fermentable oligo-, di- and monosaccharides and polyols (FODMAP) diet as a possible therapeutic approach to alleviating the symptoms of IBS.[5] The underlying mechanism is thought to depend on elimination of poorly absorbable carbohydrates which, through osmotic actions and fermentation, contribute to IBS symptom generation.[3–5] More recent studies identified potential roles for protein elimination as another dietary treatment approach in IBS.[5] While classical IgE-mediated food allergy testing has a low yield in IBS[6,7], recent studies using confocal laser endomicroscopic evaluation in the duodenum found that more than 50% of IBS patients display acute allergy-like local reactions to food protein exposure, despite negative serum food IgE test results.[9–11] Moreover, symptom control was observed with the dietary interventions of eliminating the proteins to which a local mucosal reaction was observed.[9–12] Many of the underlying mechanisms remain to be elucidated, including the cell types involved in triggering the local reaction, the possible involvement of local IgE production or of non-IgE allergic pathways, and the role of pre-existing local low-grade inflammation and increased mucosal permeability.[8,9,12] While these pathophysiological questions are being addressed, confocal laser endomicroscopy (CLE)-based food allergy testing is gaining increasing interest in the management of IBS patients. The current consensus document aims at providing guidance for the use of CLE-based food allergy testing in IBS and may be relevant for other disorders where CLE-based food allergy testing is being applied (i.e. functional dyspepsia (FD), eosinophilic esophagitis (EoE) and inflammatory bowel disease (IBD)).[13–15]

Current diagnostic approach to the role of food in IBS and related disorders

The journey to diagnose IBS-related gastrointestinal symptoms—including any food allergies—is often typified by frequent outpatient visits, inpatient stays, prescriptions, and hospitalizations.[16] These patients may undergo a variety of exams, tests, procedures, and diets (e.g., questionnaires, scans, endoscopy). Many different physicians and specialists, such as nutritionists, allergist, and gastroenterologists, perform these assessments, resulting in multiple (often uncoordinated) visits, care delays, insurance burdens, and out-of-pocket costs to the patient. Furthermore, the patients' perception of their illness, its chronicity, and diagnosis uncertainty—based on symptoms alone—often drives physicians to perform extensive investigations using iterative testing methods.
Food allergy-related symptoms can be treated by an exclusion diet which eliminates the trigger of symptoms, rather than the symptomatic control that is provided by sustained pharmacological treatment. Detection of triggering nutrients constitutes an important step in managing IBS-related symptoms. In the low FODMAP diet approach, this is managed through elimination and gradual reintroduction, requiring an evaluation period of several months with repeat visits to an experienced dietician.[5,17] The alternative approach, based on detection of food IgE antibodies, breath testing, and other biochemical markers, does not contribute to a targeted dietary intervention in the majority of the patients.

Current diagnostic methods for food allergies and intolerances, especially non-IgE mediated allergies, are limited and highly variable (Table 1). Food challenges or exclusion diets are time-consuming—the clinical reaction to the ingested food occurs several hours after ingestion, and a negative response is unknown for days.[17]
There is an urgent need for primary care physicians, gastroenterologists, nutritionists, and allergists to have a diagnostic method for differentiating and identifying food allergens in patients that have tested positive or negative with current food allergies tests, especially those with negative IgE findings. An emerging diagnostic tool is confocal laser endomicroscopy with functional imaging.

Table 1. Common Diagnostic Methods for Food Allergies and Intolerances

Test	Condition	Population	Duration	Accuracy	Results
Skin Prick Test (SPT) Atopy Patch Tests (APTs)	IgE-related Food Allergy	1-4% Adults[18] 6 % of Children[18]	Weeks	50-60% false positives.[19]	Several studies reported higher incidence of positive skin prick tests in IBS compared to control, but there is a lack of evidence for symptom improvement upon exclusion from the diet.[20–27]
Blood Test (measuring IgE)	IgE-related Food Allergy	1-4% Adults[18] 6 % of Children[18]	Minutes	50-60% false positives. [28]	Not recommended by the National Institutes of Health, due to a lack of scientific evidence and standardization.[18] Reports of higher positivity rate, but no specific association with symptoms or response to exclusion diet in IBS.[22,23,27,29]
Oral Food Challenges Elimination Diets (FODMAP, Paleolithic, gluten-free)	Food Intolerance	3% to 28% (depending on the country studied) 10%-15% in Western countries[16]	Week to months	Self-Reported	Not definitive. Clinical reaction several hours after ingestion, negative response unknown for days. Hard to follow. Risks of nutritional deficiencies. Intended for short-term management only. Lasting effects (diets) to gut microbiota are unknown. [18]
Hydrogen Breath Test	Food Intolerance	European descent 0.05% to 0.2%. With 3-10% in circumpolar populations[30]	Minutes	Unclear for individuals with suspected IBS.[31,32]	Definitive for lactose intolerance with a sensitivity of 88% and a specificity of 85%.[33] *NOTE: Accuracy based on use of evidence-based protocol.*[34]
Confocal Laser Endomicroscopy (CLE)	Food-induced allergy-like reactions in the duodenal mucosa	3% to 28% depending on the country studied and 10%-15% in Western countries.[16]	2-5 minutes of exposure	Over 84% effective in improving symptoms with diet intervention. [34]	Definitive, visually observed changes in intestinal permeability of agent on contact. Current testing detects mucosal changes induced by wheat, yeast, milk, soy, and egg white with a sensitivity of 87% and a specificity of 79%.[11][1]

Using CLE to unravel the role of allergy-like reactions to food in IBS and related disorders

The technique of Confocal Laser Endomicroscopy (CLE)

Confocal Laser Endomicroscopy (CLE) is an established diagnostic application using a fiber optic system. Upon its use during endoscopic procedures, CLE's advanced imaging technology places the power of a confocal microscope at the head of a sub-3mm catheter probe. With a contrasting agent, the endomicroscopy's flexible microscope magnifies—in real time—the patient's internal cellular architecture. This magnification enables the identification of cells and vessels of the mucous membrane lining in the gastrointestinal tract (Figure 2).

Baseline assessment of markers of defective intestinal barrier function

Already at baseline, prior to any nutrient challenge, CLE enables the imaging of dynamic alterations which may be relevant to the process of intestinal allergy-like reactions to food. CLE allows its users to identify markers of intestinal barrier dysfunction at baseline, such as leakage of fluorescein into the lumen, the presence of epithelial cell gaps, and even cell shedding prior to any nutrient exposure.[35,36]

Visualizing the Intestinal Response to Food

CLE enables the imaging of dynamic processes, such as intestinal barrier dysfunction and cell shedding, which constitute positive markers for food allergies.[35] CLE's examination capability is clinically valuable for a better understanding of the intestinal immune pathophysiology. In 2014, Fritscher-Ravens *et al.*, first described the value of endomicroscopy for diagnosing food induced allergy-like reactions when the duodenal reaction to food allergens in IBS patients was observed and quantified. They evaluated the structural/functional changes that occurred in the intestinal mucosa in vivo and noted the response followed a defined sequence indicative of an allergy-like reaction.[9,37]

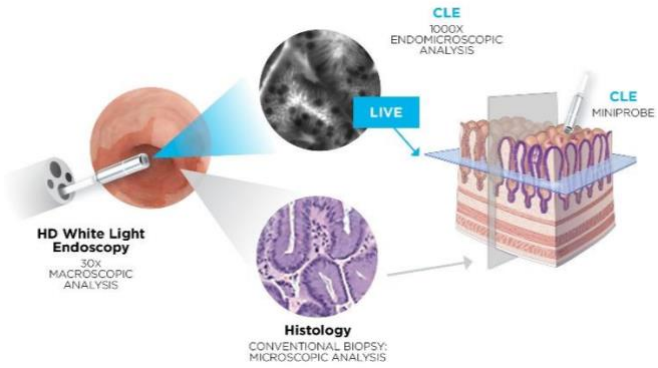


Figure 1: CLE Probe in Contact with Tissue

In a subsequent study using CLE on IBS patients with negative serum IgE for food allergens, Fritcher-Ravens et al. found wheat was the predominant trigger.[8] These findings supported what has been commonly accepted—food allergens trigger an immune system response in the gut resulting in intestinal low-grade inflammation.[9] Furthermore, IBS patients exposed to specific foods showed changes in intestinal permeability. A change in cellular structure can be seen as layer(s) of epithelial cells break up and are shed, forming gaps and inducing an immediate increase in duodenal mucosal fluid permeability. As a result, the contrasting agent floods into the lumen, widening the space between the villi, and changing the appearance from black to white.[8] This response to food antigens is clearly visible with CLE and follows a defined pathophysiology sequence within 2-5 minutes of exposure to the food allergen (Figure 3).[11]

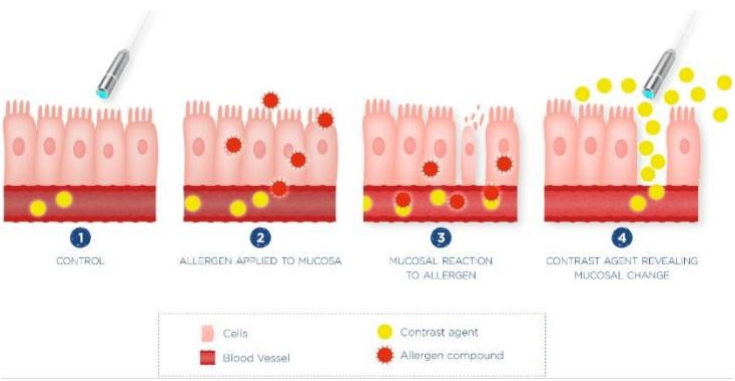


Figure 2: Cell Structural Changes with Food Challenge

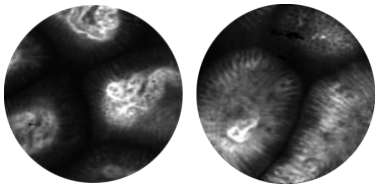
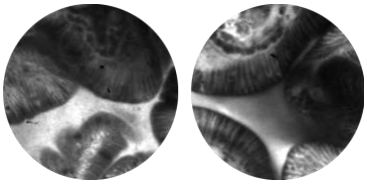
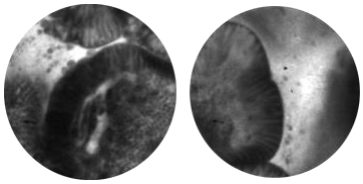
The observations of Fritscher-Ravens et al. have been confirmed in follow-up studies in patients with IBS, FD, EoE and IBD.[10,11,13–15]

CLE Criteria for Positive Food Reaction

The high-resolution visualization of the reaction to applied food on the mucosa is characterized and interpreted using the CLE Criteria for Positive Food Reaction (Table 2). Any observed positive

reaction (CLE+) indicates an immune reaction to the applied food nutrient. Note that based on current knowledge both criteria for contrasting agent leak and cell shedding must be visible to validate a CLE positive reaction.

Table 2: CLE Criteria for Positive Food Reaction

Criteria	Positive Reaction (CLE+)	
Control Image *Before food exposure, the physician records a baseline endomicroscopy video for post-food challenge comparison and checks for barrier dysfunction without exposure. This image shows an absence of any leak, cell shedding, breaks, or gaps with a mostly dark lumen compared to bright villi.*	**Contrast Agent Leak** *Gaps formed from exposure to food allergens let the contrasting agent leak into the lumen, changing from bright/white lumen in contrast to dark villus.*	**Cell Shedding** *The intestinal epithelial, representing a thin layer of the villi, breaks up with pronounced and continued shedding of cells.*
	Both criteria must be met for CLE+	
		

Solution

CLE Food Allergy Sensitivities Test (FAST)

Using the high-resolution, real-time imaging capabilities of CLE, physicians can perform a new endomicroscopic diagnostic method to detect—and definitively differentiate—food allergens, including non-triggering nutrients. This standardized procedure, CLE Food Allergy Sensitivities Test (FAST), consists of observing microscopic immune reactions in the duodenal mucosa after the application of food allergens known to be contributors of IBS symptoms (Figure 4). Currently available data mainly refer to patients with IBS symptoms, but ongoing research evaluates other conditions where intestinal food reactions may play a role such as FD, EoE, IBD, and possibly others.[13–15]

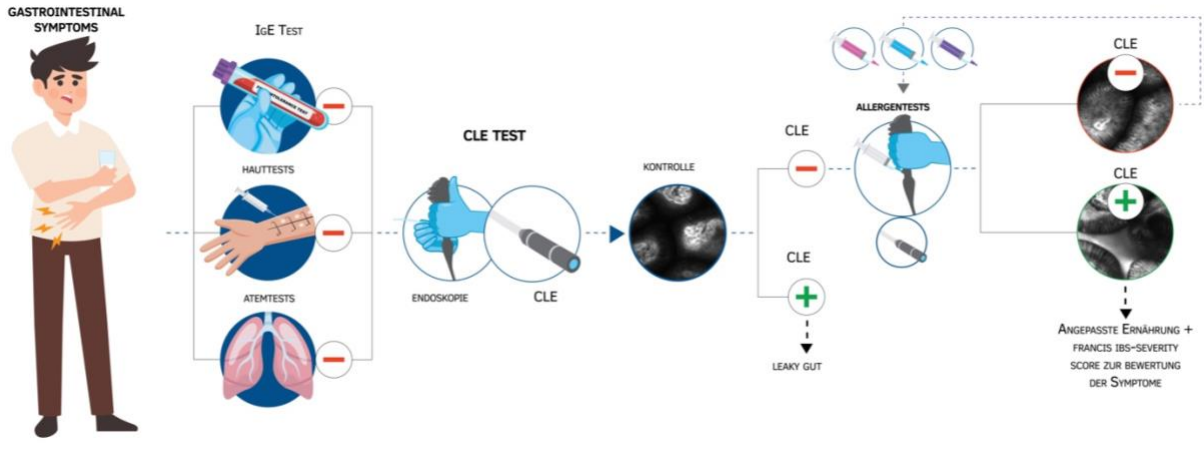


Figure 3: CLE FAST Procedure

Standardized List of the Most Common Food Allergens

The CLE FAST includes a standardized list of the most common allergens (Table 3). Other foods such as gluten, walnut, sesame, crustacean shellfish, fish, and celery can be added when considered useful by the clinician or indicated by the patient as a possible allergen. In the United States, serious food allergy reactions are attributed to eight foods: eggs, milk, peanut, tree nuts, soy, wheat, fish, and crustacean shellfish. (foodallergy.org)

The food concentration amount for the CLE FAST imitates the natural amount a healthy person can consume without showing negative effects/symptoms. Using CLE, the defined amount of allergen is applied directly on the duodenum using the working channel of the endoscope to achieve uniform application, rather than swallowing.

Screening for CLE Eligibility

The following screening tests are recommended for candidates of the CLE FAST procedure:

- IBS identified using Rome IV Criteria (possible future additions: FD, EoE and IBD[13–15])
- No significant improvement of symptoms after 6 to 8 weeks of low FODMAP diet and/or standard first-line medical therapy
- No structural cause of symptoms identified with gastroscopy or colonoscopy;
- Serology, and breath tests for lactose and fructose intolerance;
- Triggering of symptoms by meals or certain food items.

Optional:

- To be considered: IgE serological test, celiac

Table 3: Suggested Food Concentrations for CLE FAST of the Most Common Food Allergens

Nutrient	Amount	Recommended volume of Saline
Wheat Flour	3 g	10 to 30 mL
Dry Yeast	1.5 g	10 to 30 mL
Soy Flour	3 g	10 to 30 mL
Milk Powder	1.5 g	10 to 30 mL
Dry Egg White	1.5 g	10 to 30 mL
Peanut Flour	3 g	10 to 30 mL

Standardized Procedure

Preparation

Supplies: The following items are necessary to perform the CLE FAST procedure:

1 Regular gastroscope or double lumen gastroscope with working channel with an inner diameter of 2.8 mm or larger
1 Cellvizio® Confocal Miniprobe GastroFlex™ or ColoFlex™
2.5 mL contrast agent (Fluorescein concentration 10%)
5 Food samples prepared for application (Table 3)
1 Transparent suction cap (optional)
20 mg Butylscopolamine infusion (optional)

Pre-Test Consultation:

- Up to 7 days before the procedure, provide the patient with a validated symptom severity questionnaire (i.e. IBS-SSS for IBS patients[38]). The pre-test responses will be evaluated after the procedure.
- Two to three days before the CLE FAST, have the patient follow an exclusion diet and eat only hypoallergenic nutritional foods (exclusively rice, potatoes, olive oil, salt).
- Provide the patient information and preparation instructions for standard gastroscopy of the duodenum.

Test Instructions (Day of Procedure)

The steps to conduct the CLE FAST are described below and shown in Figure 5.

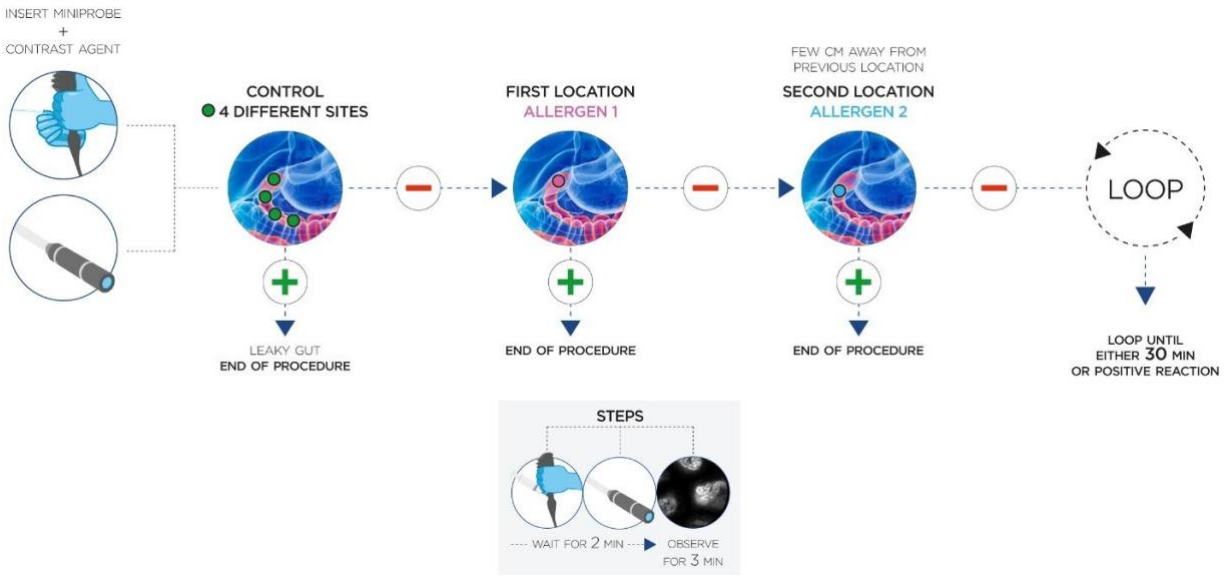


Figure 4: CLE FAST Procedure

1. Perform a standard gastroscopy to observe any sign(s) of abnormal mucosal structural defect that would suggest a known gastrointestinal disease. In the case of any mucosal abnormality observed on endoscopy, the CLE FAST procedure may not be applicable. In the case of mild reflux disease, the test can proceed.
2. Inject 2.5 ml fluorescein 10% contrasting agent intravenously.
3. To establish a baseline, perform endomicroscopy of the duodenum at a minimum of 4 sites (about 20 seconds each) to verify mucosal integrity (i.e., no contrast agent leakage into the lumen) prior to any provocation.
   Some leakage of the contrast agent in the lumen at one of the 4 sites may be normal; however, the test can proceed. For patients showing excessive leaks, testing should be suspended; the barrier dysfunction test would be considered already positive and no allergen testing should be performed.
   During the baseline and all following CLE measurements, mucosal areas where bile is visible or that exhibit bleeding and other visible indications of inflammation should be avoided to get a true baseline and no false positives.

4. Through the working channel of the endoscope, apply 10mL to 30mL of one food allergen onto the duodenal mucosa starting from the most distal part.
   - Leave space between each provocation site.
   - Start with the food allergen that will most likely trigger a reaction. The recommended order is: 1) wheat 2) yeast 3) soy 4) milk 5) egg. The order can be adapted to patient situation.
5. Wait for 2 minutes after application of food before starting imaging and observation.
   Caution:
   During these 2 minutes, avoid touching the mucosa with the probe tip to prevent injuries and fluorescent leakage.
   Switch off the laser during the 2 minutes of waiting time to avoid bleaching artifacts; afterwards switch the laser on again.
   Limit exploration to a few locations (pinpoint and move to the next site).
   Avoid imaging at "6 o'clock position" as bile or fluid with contrast agent may impair the reading.
   Observe the mucosal reaction to the food provocation with the Confocal Laser Endomicroscope (up to 3 minutes per site should be sufficient).
   - If the observed reaction is positive (CLE+), conclude the test.
   - If the observed reaction is negative (CLE-), extract the probe, flush the operating channel with saline, and move to the next site with the next allergen.
6. Repeat steps 4 and 5. Before applying a new allergen, move the endoscope to the new site towards the proximal end of the duodenum.
7. The test should be concluded within 30 minutes after injection of the contrast agent due to the increasing risk of false positives. Eventually, the contrast agent will be visible in the lumen (but with no cell shedding).

Post-Test Observation

- Within 4 to 24 hours of the test, evaluate the general health condition of the patient and rule out any late allergic reaction resulting from the test. Added medical exams could be necessary (e.g., gastroscopy, colonoscopy, abdominal ultrasound).
- Provide the patient a validated symptom severity questionnaire (i.e. IBS-SSS for IBS patients) to be evaluated at the follow-up visit.

Patient's Follow-up Visits

For patients with a positive reaction (CLE+) after provocation:

- Prescribe an elimination diet of the reacted food allergen for 6 months.
- Assess symptoms documented by the patient in the symptom severity questionnaire at baseline and after the exclusion diet.

For patients without any reaction (CLE-), repeat the test with a new set of 5 different foods.
For patients with fluorescence leakage before provocation, either repeat FAST after strict adherence to a hypoallergenic diet or suggest an empirical wheat-free diet.[11] Physicians might also provide symptomatic therapy while discussing further procedures to get final diagnosis.
For continued patient management, follow current guidelines.

Figure 5 shows the typical algorithm used to diagnose food allergies. Standard diagnostic steps include breath testing and serological markers (IgE antibodies). Only a minority of patients with IBS and IgE-related food allergies receive a definitive diagnosis after standard testing.[39]

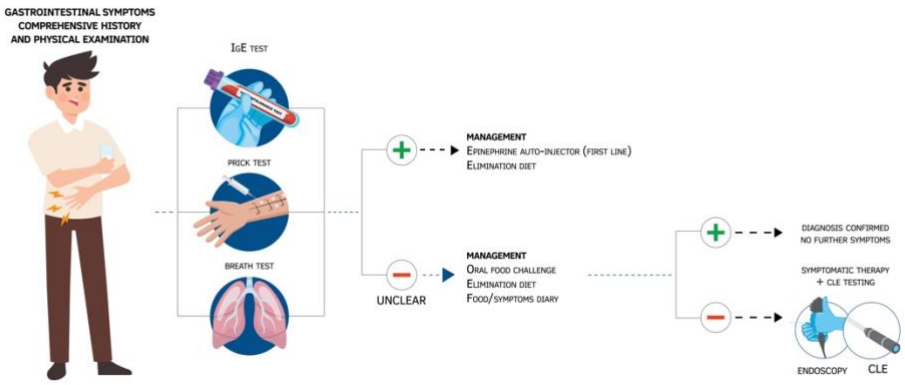


Figure 5: Food Allergy Diagnosis Algorithm

Published Clinical results

Recent studies using this standardized CLE food testing procedure found IBS patients report improved symptoms—and in some cases cessation—after their personalized exclusion diet. Of all CLE+ patients tested, 68.4% had symptom scores improved by 80% or more.[8] The average improvement of symptom scores for all CLE+ patients was 70% after 3 months and 73% after 6 months. A recent abstract suggests the response could go as high as >80% following the diet based on IBS-SSS scores.[11]

A study published by Bojarski *et al.*, indicates that after 6 months of follow-up during which the patient followed an exclusion diet based on CLE FAST results, the sensitivity of CLE for the detection of food allergy was 83,1%. The study also suggested, based on the results, that a Gluten-Free Diet (GFD) should be performed before the CLE FAST test. However, a proportion of patients who benefited from GFD and that have not been detected by CLE may actually have benefited from reduced FODMAP intake rather than reduced gluten intake. A low FODMAP diet prior to the allergy testing may help identify patients with a fructan intolerance, which cannot be assessed with CLE FAST.[10]

Discussion

It is often hypothesized that IBS symptoms involve a low-grade immune activation in the gastrointestinal tract. The CLE procedure with nutrient application allows physicians to clinically observe an allergy-like response after the sequential application of food as part of a standardized diagnostic procedure, CLE FAST. This visualization allows physicians to understand the symptom pattern, identify food-induced allergy-like reactions, and prescribe a tailored exclusion diet. In addition, the CLE FAST procedure is painless, requires no additional preparation for a general endoscopy, and can deliver a conclusive diagnosis on specific food allergens in less than 30 minutes. For physicians, testing can be done with minimal training on CLE image interpretation and performed during standard endoscopy. When integrated as a standard diagnostic tool into the workup/workflow of considering food-driven reactions in IBS and related disorders, CLE FAST

allows physicians to optically diagnose diseased tissue and enables instantaneous treatment decision making in a minimally invasive manner.

However, a number of issues still need to be addressed in future studies. As for all procedures, reproducibility needs to be studied, and healthy control studies are also warranted to better describe specificity. The threshold of leaking to better delineate a “true” positive reaction would benefit from further study. In terms of the clinical application, the choice and optimal sequence of food allergens used in the CLE FAST procedure need to be optimized to extract maximum information from the first procedure. The need for and yield of a second procedure also needs to be addressed. If the majority if patients respond to only a single nutrient class, there is no value in follow-up CLE procedures, but this would be different if many patients respond to two or more allergens. Furthermore, the necessity of a second procedure depends on whether a pathologic result in the first CLE procedure can be functional and thus be resolved over time, or are structural and stable over time. The profile of patients with positive CLE FAST responses also requires in-depth analysis. While the studies to date are focused on IBS, the most prominent functional bowel disorder, it is paradoxical that studies conducted in the proximal duodenum show a high yield of reactivity. Early observations indicate positive responses in FD, EoE and IBD, too.[13–15] Hence, patient case series should be generated to determine whether IBS with overlapping FD, or IBS with a history of symptom triggering early after meals, are able to identify a patient group with a higher rate of positive reactions during the CLE FAST. The stool pattern subtype of IBS patients most likely to show a response also needs analyzing.

In terms of pathophysiology, a large amount of knowledge seeking lies ahead. It has been hypothesized that increased mucosal permeability is a prerequisite for positive CLE FAST responses, but this needs confirmation using state-of-the-art assessments. The cell types involved (mast cells, eosinophils, other inflammatory cells) need clarification, as well as the molecular pathway involved (local IgE production, non-IgE-mediated mast cell activation, IgG-mediated pathways, ….). In the past, submucosal injection of nutrients was shown to induce allergy-like reactions in the rectum of IBS patients.[40] Studies should address whether these submucosal injection-induced acute mucosal flares correspond to the CLE-observed FAST reactions in the duodenum in the same subjects.

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