Chapter 9

The Role of Endoscopy in Celiac Disease and its Complications: Advances in Imaging Techniques and Computerization

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Abstract

Endoscopy, for many reasons, is an important technique in the diagnosis of celiac disease (CD), since it is currently the most widely used method for performing duodenal biopsies. On the other hand, certain changes in the duodenal mucosa must warn the endoscopist of a possible celiac disease. This is relevant, since it is well-known that most of the people who have this disease remain undiagnosed.

With the development of endoscopy, different markers can be used to predict the existence of villous atrophy, but a high level of suspicion is required. The correct application of guidelines performing biopsies for a celiac disease diagnosis, especially if there is a sufficient number of samples, is important to reach diagnosis. Besides, due to the fact that the spectrum of health problems related to celiac disease is quite wide, their possible association must be taken into account and the performance of duodenal biopsies must be encouraged.

This last decade's technological achievements have greatly facilitated the study of the small intestine via endoscopy. Even if these advanced techniques are generally unnecessary in most cases, there are some of them in which the video capsule and/or enteroscopy allow to achieve a diagnosis, especially in refractory celiac disease cases. Other cutting-edge techniques, such as digital chromoendoscopy, optical coherence tomography and confocal endomicroscopy could be useful to predict the existence of villous atrophy and some of them could even help the endoscopist recognize lesser degrees of celiac disease. The relevance of these techniques in daily practice remains to be dilucidated.

1. Endoscopic Findings in Celiac Disease

Endoscopy is, for several reasons, an important technique in the diagnosis of CD since it is currently the most widely used method for making duodenal biopsies. Furthermore, there are changes in the duodenal mucosa that can lead to the suspicion of CD, which may allow diagnosis in cases in which this condition has not been found. This would be relevant as it is well known that most people with CD remain undiagnosed.

The development of endoscopy allowed the description of different markers associated with CD that predict the presence of villous atrophy associated with CD. For the detection of this disease, especially when test results do not indicate the study of possible CD, a high index of suspicion by the endoscopist is required.

This last decade's technological advances have meant that the small intestine is no longer unaccesible for endoscopy. While these advanced techniques are usually not necessary in most cases, there are situations in which the video capsule and/or enteroscopy make it possible to reach a diagnosis, especially in cases of refractory CD.

Numerous authors have described endoscopic findings in the duodenum and have linked them to the presence of villous atrophy in duodenal biopsies, which could theoretically allow the prediction of CD. Those most frequently cited are reduction in the folds in the second portion of the duodenum, scalloped folds, mosaic pattern of the mucosa, nodularity of the mucosa and visualization of submucosal vessels.

The features and definition of each one of these changes in the mucosa are detailed below.

1.1. Decrease in duodenal folds (Figures 1 and 2)

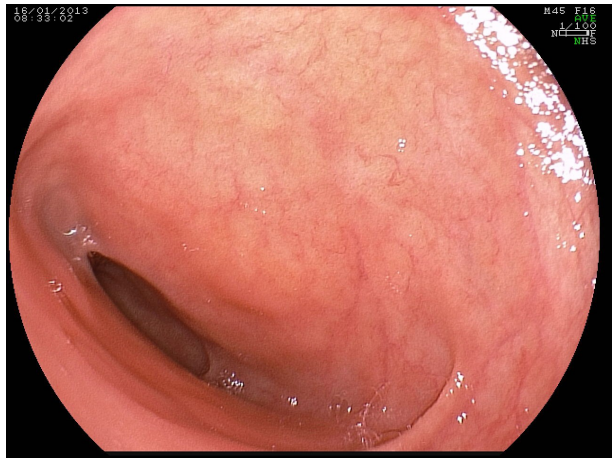


Figure 1. Duodenal mucosa with virtual disappearance of folds.

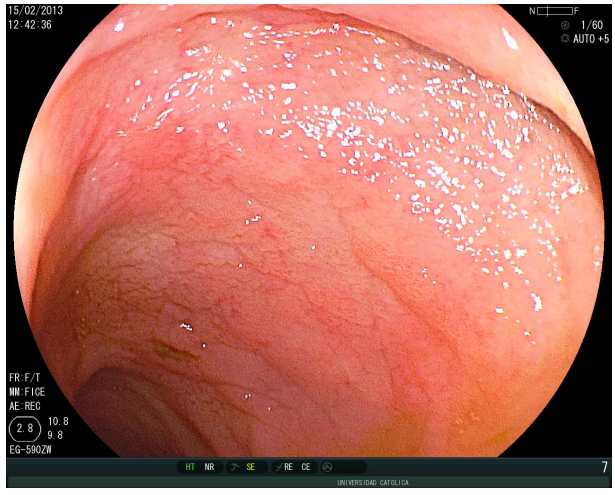


Figure 2. Duodenal mucosa with reduction of folds and granularity (a small ulcer can be observed in connection with the taking of a recent biopsy).

The loss of duodenal folds was first described in the nineteen seventies by Nicollet and Tully in radiological small intestine studies made using barium,[1] their endoscopic description being first published 1988 by Brochi et al.[2], who described loss of folds in the duodenum, defining it as the finding of only three folds in the second duodenal portion, with maximum insufflation. Evaluated in celiac disease patients, it described a sensitivity of 88% and specificity of 83%.

Subsequent studies, which defined this finding more subjectively as an obvious alteration found while performing an inspection, showed a sensitivity of 73% and a specificity of 97%.[3]

1.2. Mosaic pattern and scalloped folds (Figures 3- 6)

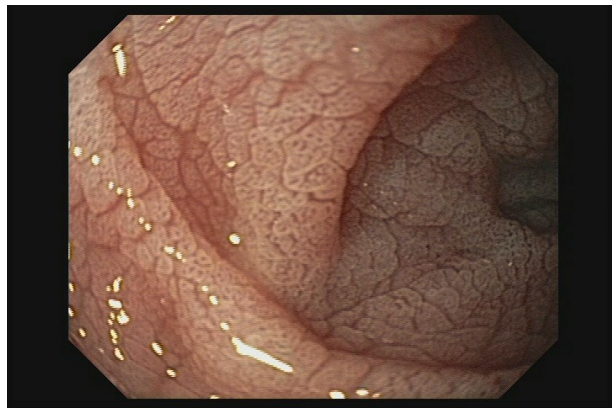


Figure 3. Duodenal mucosa with mosaic pattern (tenuous pattern).

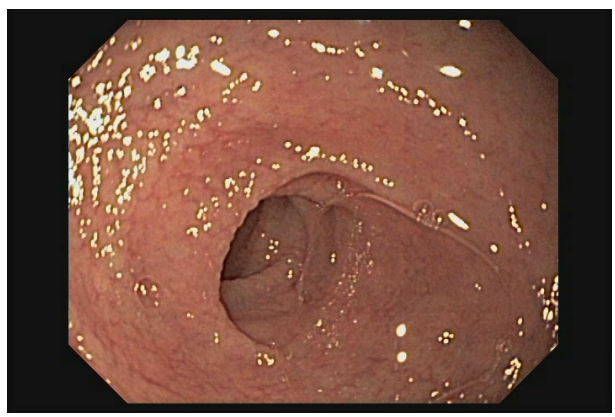


Figure 4. Duodenal mucosa with evident mosaic pattern.

The scalloped appearance of the folds was first described in 1988 in patients with celiac disease;[4] its proper inspection was described as one performed with maximum insufflation. In pediatric patients it had a sensitivity of 88% and specificity of 87% for the villous atrophy diagnosis.

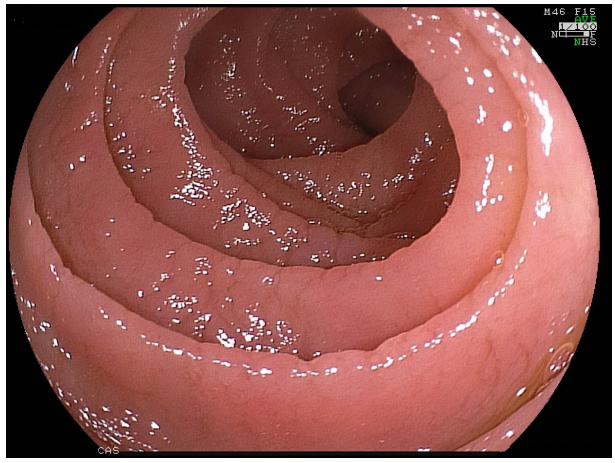


Figure 5. Scalloped appearance of the duodenal mucosa.

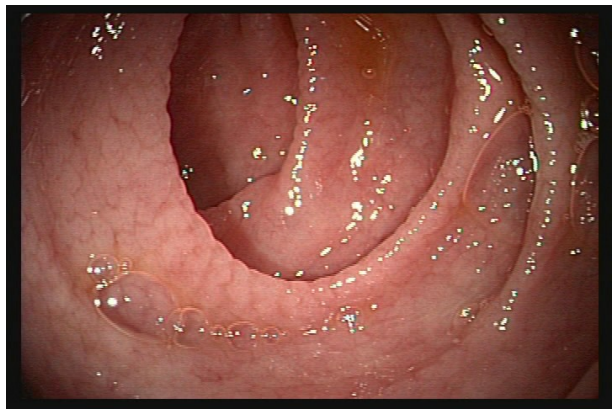


Figure 6. Scalloped appearance of the duodenal mucosa.

Duodenal mucosa grooves that seem to have a mosaic pattern between folds have also been associated with this disease and are probably manifestations of the same process that causes the scalloping of folds as the grooves advance.

Scalloped folds are not specific to celiac disease, and can be observed in patients with immunodeficiency, tropical sprue, giardiasis and eosinophilic gastroenteritis.[6]

1.3. Mucosal Nodularity (Figures 7 and 8)

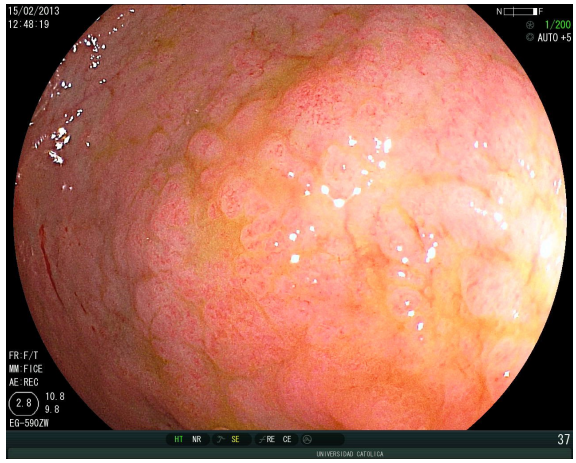


Figure 7. Nodular appearance of the bulb mucosa.

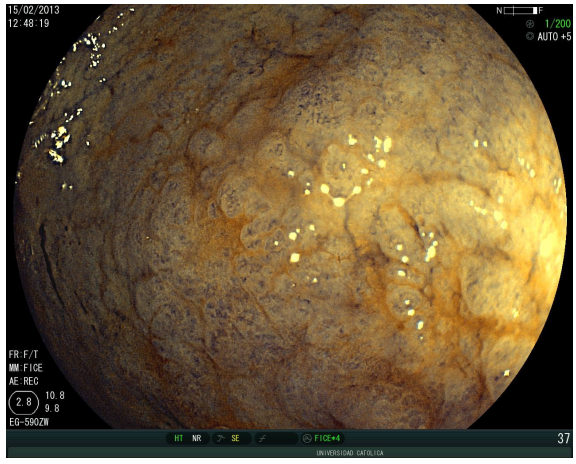


Figure 8. Same image as number 7, using computerized virtual chromoendoscopy with Fuji Intelligent Chromo Endoscopy (FICE).

In the vast majority of celiac patients studied endoscopically, the characteristic findings (mentioned above) were found in the descending duodenum. However Brocchi et al.[7] reported bulb nodularity in a 14-year-old celiac patient and no alterations in the second portion of the duodenum.

1.4. Visualization of submucosal vessels (Figures 9 and 10)

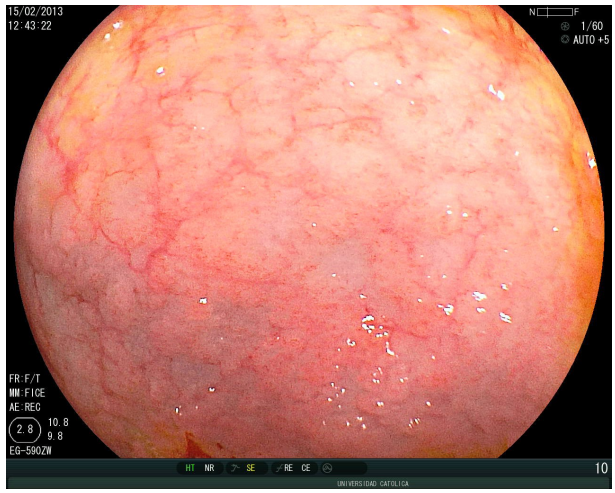


Figure 9. Prominent vessels in the bulb.

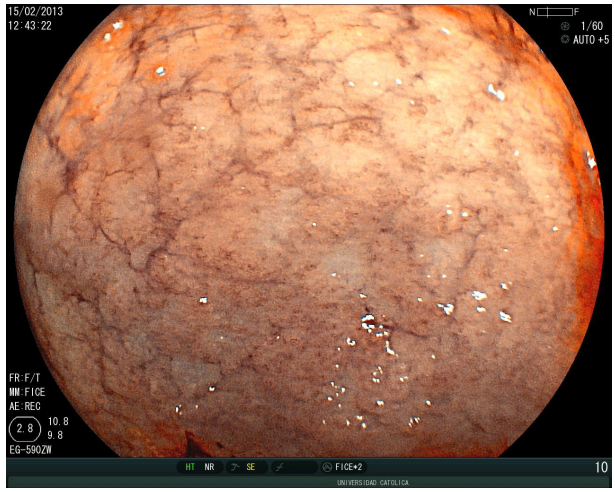


Figure 10. Same image as number 9, using computerized virtual chromoendoscopy with Fuji Intelligent Chromo Endoscopy (FICE).

The first description of this type of finding was by Stevens and McCarthy in 1976;[8] Jabbari[4] later described the prominence of duodenal submucosal vessels in celiac disease patients. Subsequent studies found for this endoscopic find a sensitivity of 2%, 5% and 14% respectively in patients who were undergoing duodenal biopsy.[9-11] Therefore, this sign seems the least relevant and reliable among those examined.

The first systematic study that globally assessed endoscopic features in celiac disease specifically encompassed 100 patients specifically referred for endoscopy in order to obtain intestinal biopsies.[9] The evaluated endoscopic findings were mosaic pattern, scalloped folds, creases and loss of blood vessel visualization. Among all the evaluated patients, 36 had a severe villous atrophy histopathological diagnosis, of which 39% had atrophic mucosal pattern, 75% loss of folds, 33% scalloped folds and 14% blood vessel visualization. The presence of at least one endoscopic finding had a sensitivity of 94% and a specificity of 92% for the celiac disease diagnosis.

Subsequently, Niveloni et al. demonstrated in a prospective study that endoscopy allowed to correctly determine which patients had celiac disease in 94% of the cases, and that chromoendoscopy with staining allowed a better outlining of the scalloped folds and mosaic pattern, with no impact on diagnosis.[10] The "interobserver" concordance was excellent for the scalloped folds (kappa 0.83) and mosaic pattern (kappa 0.76) findings and regular for loss of folds (kappa 0.41).

In patients who underwent an endoscopic duodenal biopsy, the finding of at least one endoscopic marker has a sensitivity ranging between 77% and 94%, the scalloped folds and the mosaic pattern being the most frequent in different series.[10-11]

As reviewed in other chapters of this book, many different clinical scenarios confront us with patients with suspected celiac disease (Table 1).

Clinical Scenario	**Sensitivity**	**Specificity**
Patients with celiac disease suspicion[10]	94%	99%
Patients with dyspepsia[12]	50%	99%
Patients with no celiac disease[11]	87%	100%
Patients iron deficiency anemia[15]	59 %	92%

Table 1. Endoscopic finding performance to predict villous atrophy in different celiac disease clinical scenarios.

In the differential diagnosis, iron deficiency anemia should be evaluated in the context of possible CD. About 5-12% of patients with iron deficiency anemia have celiac disease endoscopic markers and, when these are found, their sensitivity has been shown to be nearly 60% with a 92%-100% specificity. The most common findings vary according to different publications, no research has been able to show the superiority of one over the other.[12-15] When an upper endoscopy is performed in patients with iron deficiency anemia who have not been previously studied for celiac disease serological markers, the taking duodenal biopsies is recommended.[16] When said markers are negative, taking biopsies is generally not recommended except in very symptomatic patients.[16] There are, however, discrepancies regarding this subject.[17]

One controversial subject is the need for duodenal biopsy in dyspeptic patients (especially in those with the dysmotility type) without endoscopy study findings. There is a marked variability in the published series of studies on the percentage of patients in whom CD symptoms can be confirmed, with a range of 1-19%.[18,19]

The unexpected find of an endoscopic marker in a patient without an *a priori* indication from a duodenal biopsy has also been evaluated and results in this setting have shown mixed concordances between identification of endoscopic markers and pathologic correlation with a sensitivity of 50% and a specificity of 99.6%.[12,20]

Summing up, several studies have shown a high degree of correlation between the above findings and the presence of villous atrophy due to celiac disease. The high specificity of these signs warrants endoscopic biopsies in its presence, therefore, the endoscopist should be actively seek them out in the course of exploring, even in patients not referred for suspected CD.

2. Advanced Diagnostic Techniques: Chromoendoscopy, Magnification, Computerized Virtual Chromoendoscopy with *Fuji Intelligent Chromo Endoscopy (FICE)*, Narrow Band Image (NBI) Optical Coherence Tomography, Confocal Endomicroscopy and Endocytoscopy

In order to improve the endoscopic detection of villous atrophy, various diagnostic methods have been implemented, among them, magnification endoscopy with or without chromoendoscopy, computerized virtual chromoendoscopy obtained with *Fuji* color enhancement technology, Narrow Band Imaging (NBI), optical coherence tomography and ultra-magnification techniques such as endocytoscopy and confocal endomicroscopy.

These technological innovations would be potentially useful to identify atrophy sites with patchy distribution, thus allowing the taking of targeted biopsies in suspicious areas, improving diagnostic efficiency compared with random biopsies. Another potentially relevant use of endoscopic-microscopic techniques is their possible ability to discern milder degrees of histological damage (Marsh 1 and 2).

2.1. Magnification Endoscopy and Chromoendoscopy (Table 2 and Figures 11- 14)

Badreldin et al. included CD patients in treatment, and sought to evaluate this technique not regarding its ability to predict villous atrophy existence, to determine its degree.[22] Concordance between endoscopic and histological according to atrophy degree was fair-good (kappa 0.631), positive and negative predictive value to predict villous atrophy was 83% and 77% respectively.

Magnification endoscopy has been considered in the diagnosis and evaluation of the degree of CD villous atrophy; some classification schemes attempt to characterize the various endoscopic

villi patterns (Table 2). As it is known, this technique can increase image size by pressing a button on the endoscope controls, which is helpful in predicting histological diagnosis. However, regarding CD, the number of studies is small and the results obtained are contradictory.

In a descriptive prospective study, Cammarota et al. studied patients referred for duodenal biopsies, in which magnification endoscopy was used.[21] The evaluation was performed without and with the water immersion technique. Results were excellent, with sensitivity, specificity, positive and negative predictive values for villous atrophy at 95%, 99%, 95% and 99%, respectively, values which did not improve using water technique.

	Technique	Patients (n)	Sensitivity	Specificity
Maurino 1993[9]	CE	100	94%	92%
Dickey 2001[11]	CE	129	77%	-
Cammarota 2004[21]	ME	191	95%	99%
Badreldin 2005[22]	ME	53	77%	63%
Iovino 2010[24]	ME + IC	50	98%	100%
Singh 2010[25]	ME + NBI	21	93%	98%

CE: Conventional endoscopy; ME: Magnification endoscopy; IC: Indigo carmine; NBI Narrow Band Imaging

Table 2. Sensitivity and specificity of conventional and magnification endoscopy in duodenal atrophy diagnosis.

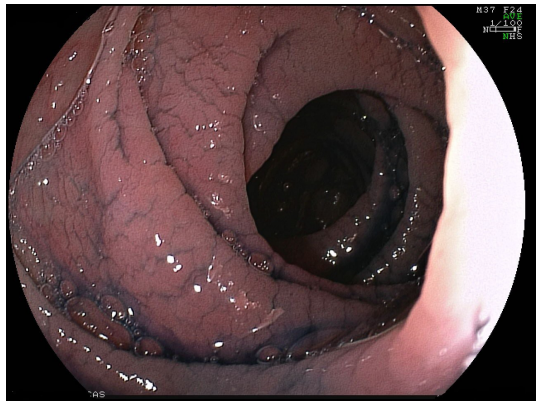


Figure 11. Mosaic pattern, with indigo carmine.

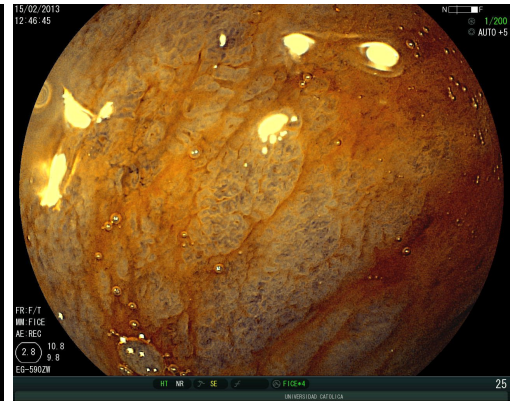


Figure 12. Bulb mosaic pattern, shown with computerized virtual chromoendoscopy using Fuji *color enhancement technology (*FICE*).*

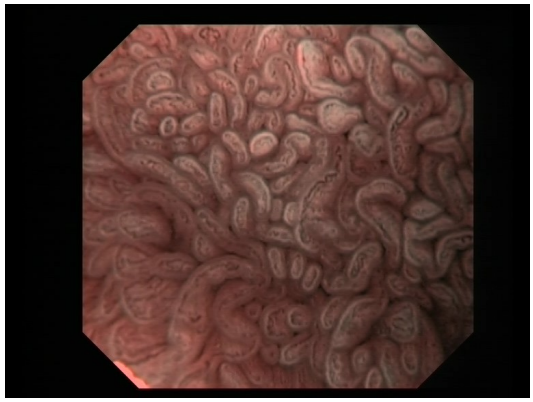


Figure 13. Normal duodenal mucosa view obtained using narrow band imaging and magnification.

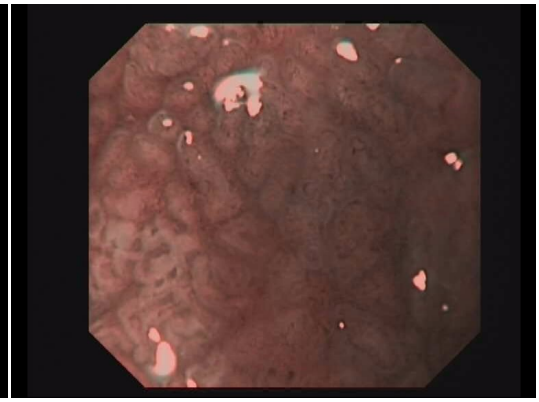


Figure 14. Duodenal mucosa with partial to total villous atrophy view obtained with narrow band imaging and magnification.

In a study of 12 CD patients, which compared the use of magnification associated with 3% acetic acid versus conventional endoscopy, sensitivity was higher for the combined technique compared to the standard (100% versus 58%).[23] Furthermore, magnification endoscopy identified patchy areas of villous atrophy in 5 patients, while conventional endoscopy did not identify them in any case.

Another recent study evaluated the usefulness of magnification endoscopy (*Fujinon*, Omiya, Japan EG 490 ZW) associated with indigo carmine staining to recognize duodenal villi pattern changes in patients with difficult CD diagnosis.[24] This was defined as a lack of concordance between diagnostic tests or as a result of beginning the study at a stage in which gluten had already been removed from the diet. In the control group, 100% of cases were diagnosed accurately; in the group of CD patients the accuracy was of 97%. However, in the group of difficult diagnosis, sensitivity was only 67%.

A system has been proposed for classifying villous atrophy using magnification endoscopic associated to narrow band imaging.[25] Twenty one patients (3 with CD and 18 controls) were studied and a simple classification was used:

1) Normal pattern (normal villi with finger-like projections),

2) Atrophy pattern (cerebroid/shortened villi or their absence).

The sensitivity and specificity required to correctly distinguish the presence or absence of villi were of 93.3% and 97.8%, respectively; furthermore, the sensitivity and specificity required to differentiate partial or total atrophy were of 83.3% and 100%, respectively.

Magnification endoscopy provides high-quality images, and the results of available studies are promising; however, large, well-designed studies aiming at confirming that it is more effective than conventional endoscopic examination are necessary.

2.2. Optical coherence tomography (Table 3)

Author, year	OTC	CEM	Endocystoscopy	Patients (n)	Sensitivity	Specificity
Leong, 2008[34]	-	Yes	-	31	94%	92%
Masci, 2009[28]	Yes	-	-	134	82%	100%
Venkatesh, 2010[32]	-	Yes	-	19	100%	80%
Günther, 2010[33]	-	Yes	-	60	74%	100%
Matysiak-Budnik, 2010[37]	-	-	Yes	23	83%	100%

TOC: Optical coherence tomography, EMC: Confocal endomicroscopy.

Table 3. Sensitivity and specificity of optical coherence tomography, confocal endomicroscopy and endocytoscopy in duodenal atrophy diagnosis.

Optical coherence tomography is an imaging technique that allows the histological study of tissue inserting a probe through the working channel of the endoscope *in vivo* and *in situ*, which has led to the term *optical biopsy*.[26] This technique was first demonstrated in 1991 with an axial resolution of ~30 µm. With every generation the technique has progressed to a higher resolution; in 2001 optical coherence tomography achieved submicron resolution due to the introduction of wide-band light sources (emitting wavelengths over a range of ~100 nm); currently there exists ultra-high resolution equipment. At present, the optical coherence tomography is widely accepted, providing a penetration of a 2-3mm depth with axial and lateral resolution of a micrometric range (1 to 3 microns).

In 2006, Masci et al., presented a preliminary report on the usefulness of optical coherence tomography (*Pentax*; *Lightlab Imaging*, Westford, Massachusetts, USA) in CD.[27] They included 18 CD patients and 22 controls, optical coherence tomography was performed in all cases and biopsies were simultaneously taken. Subsequently, the images and histological findings were evaluated in the blind by an independent gastroenterologist with experience in optical coherence tomography, who was not informed of the clinical data and the endoscopic appearance of the duodenal mucosa, and also by an anatomopathologist. There was 100% concordance between optical coherence tomography and histology in determining the villous morphology in both groups.

In a more recent study by the same group, 134 pediatric patients were prospectively included, 67 with serological CD suspicion (group 1) and 67 with negative histology for atrophy (group 2).[28] In all cases, an optical coherence tomography was also performed in the second duodenal portion;

biopsies were taken in the area where the optical coherence tomography had been performed. Three patterns were considered: Pattern 1 with no atrophy, pattern 2 with mild atrophy and pattern 3 with marked atrophy. OCT Concordance with histology was of 100%, 94% and 92% respectively for patterns 1, 2 and 3. Sensitivity and specificity were of 82% and 100% respectively. In the control group, there was a 100% concordance between optical coherence tomography and histology.

According to these results, optical coherence tomography appears to be a promising method to correctly identify villous atrophy and can be of help in the selection of intestinal biopsy patients.

2.3. Confocal Endomicroscopy (Table 3)

Confocal endomicroscopy is a new imaging technique which allows the observation of cell morphology at the time of endoscopic examination (*in vivo* histology). Confocal microscopy uses a fine laser beam to scan the specimen. Currently, a miniaturized confocal microscope was developed to be integrated into the distal tip of a conventional endoscope (a joint venture between *Pentax*, Japan and *Optiscan*, Australia).[29] This technology allows simultaneous conventional white light endoscopy and confocal microscopy. More importantly, the working channel allows biopsies guided by endomicroscopy and/or immediate and specific endoscopic therapy.

Conventional endoscopes provide an optical magnification of 50x, while confocal endomicroscopy a magnification of 1000x.[30] Therefore, the use of this technology requires that the endoscopist have basic anatomopathologic knowledge of the mucosal to recognize and interpret the findings. With this technique, it is possible to obtain deep images down to the lamina propria, approximately 250µm.[31] Confocal endomicroscopy requires the use of an excitable fluorescent contrast agent which has emission spectra in the blue light range (excitation wavelength of 488nm). The most widely used contrast agent is sodium fluorescein, which is administered intravenously, is not toxic and is distributed throughout the tissues in a few seconds.[30]

The results of confocal endomicroscopy in CD were described in a pediatric trial of 9 patients with suspected CD comparing the findings with paired controls.[32] Endoscopists and anatomopathologists were blinded to the diagnosis. 1384 pictures of the patients were obtained and 5 images per patient were selected and compared with a biopsy sample from the same site. According to the data provided by this study, confocal endomicroscopy sensitivity was 100%, specificity 80% and positive predictive value of 81%, the relatively low specificity was related to the score employed to define the suspected CD diagnosis according to findings according to confocal endomicroscopy. With more stringent criteria, the specificity would have been of 100%.

In a clinical trial in 30 adult patients with CD, including 6 with disease refractory to the gluten-free diet, the sensitivity of confocal endomicroscopy was useful for the detection of increased intraepithelial lymphocytes (81%), but decreased for villous atrophy diagnosis of (74%) and for crypt hyperplasia (52%).[33] In the same study, 30 patients without CD who underwent confocal endomicroscopy and duodenal biopsies showed, in all cases, normal architecture under

endomicroscopy confocal and in histology, resulting in a specificity of 100%. It must be pointed out that for the intraepithelial lymphocyte (semiquantitative) determination is necessary, by means of applying a second contrast staining agent (topical acriflavine).

In the widest study, 31 patients (17 with CD, 14 controls) were evaluated and over 7,000 confocal endomicroscopy images were compared with 326 pairs of biopsy samples.[34] Diagnosis sensitivity for CD was of 94% with a specificity of 92% and good correlation with Marsh scoring system. This study also concludes that by aiming biopsies at microscopically abnormal regions, confocal endomicroscopy may be a promising approach to investigate patients with clinical CD suspicion and with negative biopsies.

According to the results provided by the few published studies, confocal endomicroscopy seems to be a technique with high sensitivity and specificity for the diagnosis of villous atrophy and can also evaluate intraepithelial lymphocytes and crypt features, although the diagnostic yield for this last purpose is not so elevated.

2.4. Endocytoscopy (Table 3)

Endocytoscopy is a form of ultra-high magnification, which allows visualization of the epithelial surface architecture at cellular and subcellular levels, being able to establish cell abnormalities, and other features such as cell density, cell size and organization, shape of the nuclei, staining pattern as well as the nucleus/cytoplasm ratio. This is a microscopy technique where physical contact with the mucosal surface is required for imaging (Figures 15-18).[35]

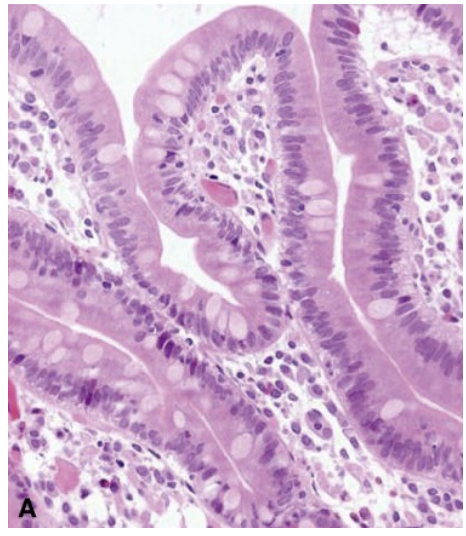


Figure 15. Microscopy image of normal duodenum with hematoxylin eosin staining (reprinted with permission from Elsevier*, reference 38).*

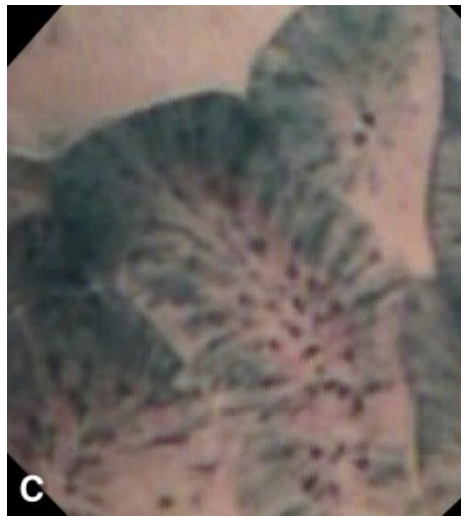


Figure 16. Normal duodenum endocytoscopy image (same case as W) (x450). The mucous layer shows long, thin villi, and epithelium with low stromal/epithelial ratio and normal vellositary capillaries (reproduced with permission from Elsevier*, reference 38).*

The use of a contrast agent for visualizing subcellular entities is necessary. For the proper performance of this technique, the mucosa must be pre-treated with a mucolytic agent, such as N-acetylcysteine after which staining can be performed directly with 0.5%-1% methylene blue or 0.25% toluidine blue.[36]

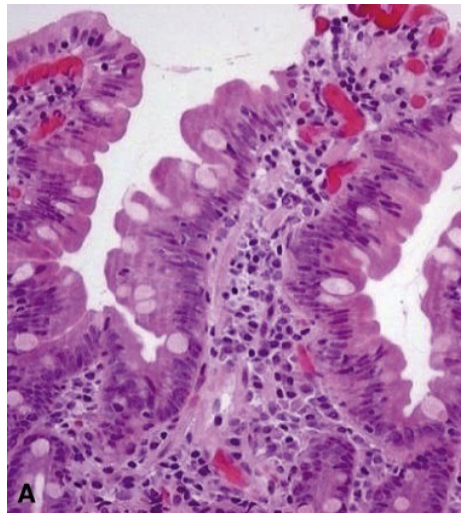


Figure 17. Duodenal mucosa with Marsh III compatible villous atrophy (staining: hematoxylin-eosin) (reproduced with permission from Elsevier*, reference 38).*

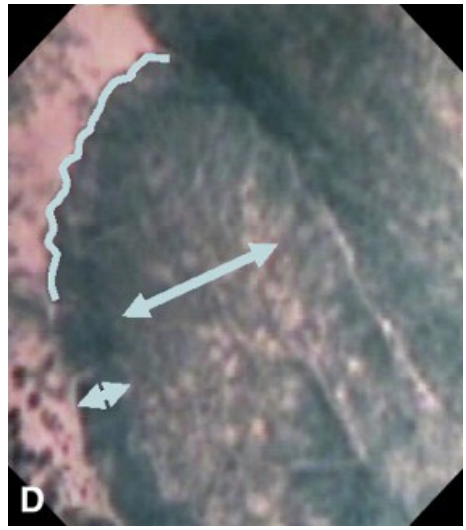


Figure 18. Image endocytoscopy (x450). The mucosa shows irregular atrophic villi, absence of large villi, irregular epithelium (short arrow), with a high stroma/epithelium ratio (long arrow), and absence of vellositary capillaries (reproduced with permission from Elsevier*, reference 38).*

Endocytoscopy is limited by its ability to image only the superficial layer of the mucosa and is therefore not a suitable technique for lesion depth analysis.

There are two types of endocytoscopy tools, although they are currently not commercially available: one based on probes (*Olympus*, Tokyo, Japan; *Xec-120* and *Xec-300* models) and another based on the endoscope (*Olympus* models *XGIF-Q260EC1* and *XCF-Q260EC1*). The two probe-based models provide a 450x magnification, which means a 300 µm x 300 µm field of view. The endoscopic models use an endocytoscope integrated to the endoscope and provide a 580x magnification.

Applied to CD, endocystoscopy has demonstrated the presence of three different *in vivo* histopathological patterns: normal pattern, subtotal villous atrophy pattern and total duodenal atrophy pattern.[37]

In a clinical trial of 40 patients (32 with known CD and 8 with CD suspicion) 166 endocytoscopy recordings were prospectively obtained and compared with histopathology (Marsh classification).[38] One endocytoscope with a 450x magnification was used; prediction was accurate for moderate to severe atrophy (Marsh III), however it was not reliable in detecting atrophy in its early stages (Marsh I). The use of the endocytoscope with a 1100x magnification provided no additional diagnostic value.

Another recent study, in which an endocytoscope with a 450x magnification was used, encompassed 16 patients with CD diagnosis and 7 controls.[39] In this study, the three above-

mentioned patterns were also identified. Sensitivity and specificity for the villous atrophy diagnosis, calculated by patients, was of 88% and 100% respectively. However, it was not possible to determine the presence of intraepithelial lymphocytes.

Therefore, according to the results of the existing studies, endocytoscopy permits real time visualization of the duodenal mucosa and villous architecture characterization; thus, it can be considered a promising method for in vivo duodenal mucosa evaluation for villous atrophy diagnosis. Nevertheless, it has limitations when it comes to displaying intraepithelial lymphocytes and crypt hyperplasia; therefore, the endocytoscopic early-stage diagnosis of celiac disease is not possible currently.

Technique	Intraepithelial lymphocytes	Crypt Hyperplasia	Vellositary atrophy
Magnification	-	-	+++
CEM	++	+	+++
OCT	No data	No data	+++
Endocytoscopy	-	-	++

CEM: Confocal endomicroscopy; OCT: Optical coherence tomography; (-) Bad; (+) Regular; (++) Good; (+++) Very good.

Table 4. Diagnostic utility of the various techniques for the visualization of intraepithelial lymphocytes, crypt hyperplasia and villous atrophy.

To sum up: new endoscopy techniques allow high-accuracy prediction of villous atrophy, but are less accurate for determining histologic of injury grade (Table 4). Although, in difficult cases or in those without histological confirmation they would be potentially useful for directing biopsies, studies are needed to evaluate their utility and cost-effectiveness in the overall diagnosis and management of CD.

3. Biopsies: How, Where and Whom to Biopsy?

To confirm the CD diagnosis biopsies should be taken from duodenum while the patient consumed a diet containing gluten. It has been established that 4-6 biopsies must be taken to make the diagnosis, including samples from the duodenal bulb.[40]

Formerly, biopsies were obtained by peroral suction techniques (Watson´s and Crosby's capsules, and multipurpose tube). Several studies demonstrated that duodenal endoscopic biopsy was comparable to that of the capsule to detect vellositary atrophy.[41-45] The recommended biopsy site was the second portion of the duodenum distal to the bulb, due to the presence of Brunner's glands or duodenitis, which may interfere with recognition of vellositary atrophy.[46] Later research

showed that changes attributed to celiac disease may occur in the duodenal bulb[7] and that this may even be the only site with atrophy.[47,48]

The multiple biopsy strategy is suggested to reduce the risk of false negatives, since mucosal damage may be irregularly distributed, a condition known as "patchy villous atrophy." That is why, as stated before, for best results, the recommendation is to take 4-6 biopsies, one or two from the bulb and the rest of the second duodenal portion (Table 5).[49-53]

Subsequent studies showed that by using immersion techniques and magnification endoscopy it is feasible to take directed biopsies;[54,55] in this sense, the technological advances mentioned above (narrow band imaging, computed virtual chromoendoscopy obtained with *Fuji* color enhancement technology *(FICE)*, confocal endomicroscopy) guide us in the taking of endoscopic samples, improving the diagnostic yield. Future studies should confirm the practical utility of such techniques in relation to random sampling.[56,57]

The orientation of the duodenal biopsy is fundamental for an appropriate histopathological study. The uppermost placement of the luminal surface of the biopsy and the blood side surface on filter paper facilitates the correct orientation of the specimen avoiding tangential cutting and allowing accurate diagnosis of vellositary atrophy.[58]

Regarding the issue of whom to biopsy, the concept changed over time. More than two decades ago, biopsy was done only in patients with clear symptoms (diarrhea, weight loss or abdominal distension) or significant laboratory abnormalities (mineral, protein or lipid deficits) or with positive antibodies. In recent years, with the emergence of new, more sensitive antibodies and the spread of the disease towards other specialties, the duodenal biopsy prescription increased continuously. Intestinal biopsy must be performed whenever celiac disease is suspected and before eliminating dietary gluten.[59,60] Although this is mentioned in other chapters, it is necessary to remember those situations in which biopsy should be considered in order to rule out CD: chronic diarrhea (the most common symptom), weight loss, anemia, abdominal bloating. Non-gastrointestinal symptoms/alterations: dermatitis herpetiformis, peripheral neuropathy, reduced bone density, unexplained infertility. Also, folic acid, iron and vitamin B12 deficiencies, reduced serum albumin, hypertransaminasemia with no hepatic origin. In patients at increased risk: first- and second-degree relatives (5-15%), HLA-DQ2 or HLA-DQ8 bearers (10-30%), Down's syndrome (12%), autoimmune thyroid disease (5%), chronic active hepatitis, diabetes mellitus type 1 (5-6%), lymphocytic colitis (15-27%), chronic fatigue syndrome (2%) and irritable bowel syndrome. Biopsy is also indispensable when an incidental finding by an endoscopist detects the suspicious signs described above.

In conclusion, there are many situations that lead to duodenal biopsy in search of celiac disease, and, despite the fact that it is the diagnostic "gold standard", we must not forget the existence of patchy celiac disease; therefore, multiple distal duodenal sampling and duodenal bulb sampling must be performed since this will help avoid underdiagnosis (Table 5).

Author	Patients (n)	Antibodies	HLA	Biopsies	Patchy vellositary atrophy	Bulb only atrophy	Sensitivity
Bonámico, 2004[48]	95	EMA + tTGA +	DQ 2+ DQ 8 +	Bulb (1) Distal duodenum (4)	13/95 (13.7%)	4/95 (4.2%)	_
Ravelli, 2005[49]	112	EMA + tTGA +	110 DQ 2+ DQ8 +	Bulb (1) Duodenum (3) proximal - intermediate - distal)	8/110 (7.2%)	_	_
Hopper, 2007[51]	56	EMA + tTGA +	_	Bulb (1) Proximal duodenum (4) Distal duodenum (4)	10/53 (18.8%)	1/53 (1.8%)	100% (3 biopsies)
Gonzalez, 2010[53]	40	_	_	Bulb (2) Proximal duodenum (4)	5/40 (12.5%)	5/40 (12.5%)	72%

EMA (antiendomisium antibodies), tTGA (Antitransglutaminase antibodies), DQ 2 (HLA-DQ 2 gene), DQ 8 (HLA-8 gene).

Table 5. Performance of biopsies using different protocols.

4. The Role of Capsule Endoscopy in Celiac Disease

The endoscopic capsule has allowed the exploration of the small intestine, which, by its anatomical location and characteristics, has previously been limited and less accessible to traditional endoscopic studies; capsule endoscopy has become a useful diagnostic tool for diseases that affect this segment of the digestive tube.[61-63] Numerous publications show that the capsule's endoscopic ability is superior to imaging techniques traditionally used to detect small intestinal lesions.[64-66] Capsule endoscopy was first used in humans in 1999; in 2001 it was approved for clinical use by the Federal Drug Administration.[67] Capsule endoscopy takes 2 frames per second, has a 8x magnification lens and has an optical dome in close contact with the mucosa allowing a very good evaluation of the villous pattern. The main indication for this study is gastrointestinal bleeding of obscure origin, though there are numerous studies that seek to understand the capsule's value in other small intestinal pathologies.[68]

Serological CD markers, such as endomysial antibodies and anti-transglutaminase, have shown a very good performance, with positive and negative predictive values of near 96%. However, the objectification of villous atrophy identified by means of a histopathological study in duodenal samples are the diagnostic standard.[50,69]

Capsule endoscopy in the context of CD has been the subject of a growing interest to investigate its use; there are several possible scenarios for its use, each of them will be discussed below.

4.1. CD Diagnosis (Table 6)

Publication	n	Sensitivity %	Specificity %	NPV %	PPV %
Petroniene, 2005[74]	10	70	100	77	100
Hopper, 2007[75]	21	85	100	89	100
Rondonotti, 2007[76]	32	87	90	71	96
Biagi, 2006[77]	26	90	63	77	100
Maiden, 2009[78]	19	67	100	60	100
Lidums, 2011[79]	22	93	100	89	100
Total	130	82	92.1	77.1	99

Table 6. Summary of sensitivity and specificity studies and NPV and PPV for capsule endoscopy in celiac disease.

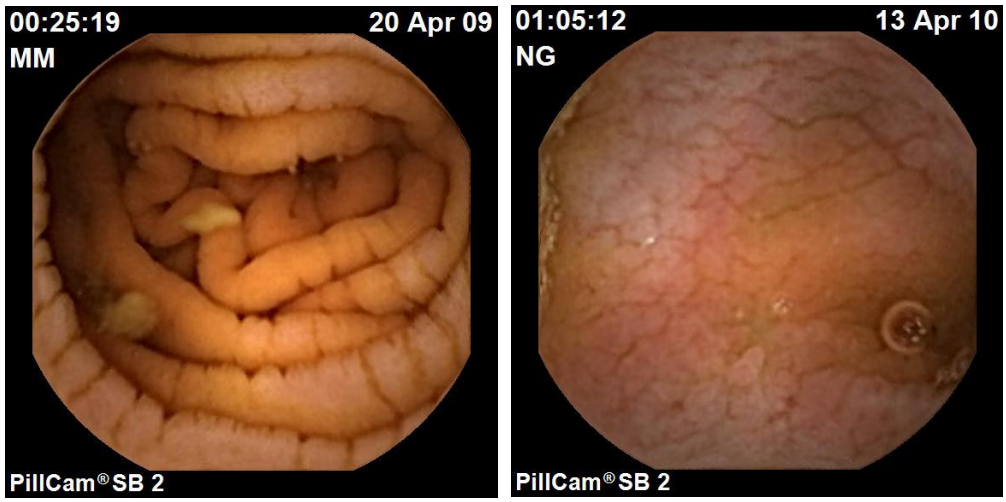
As mentioned earlier, the determination of villous atrophy is a central event in CD diagnosis. Endoscopic methods have made progress regarding image quality, since they are able to distinguish alterations that suggest CD and allow the endoscopist to decide on whether to take biopsies according to certain findings. Capsule endoscopy, by having an 8x magnification and an optical dome which allows a direct view of the mucosa, helps to distinguish alterations which have a high correlation with a CD diagnosis , previously referred to in this chapter.[70]

The findings of capsule endoscopy show good correlation with serological and histological diagnosis, but there are inter-observer variations that may limit this method in terms of reliability and reproducibility. A study made on a cohort of CD patients evaluated the utility of capsule endoscopy in patients with equivocal CD diagnosis (defined as the presence of villous atrophy with negative or inconclusive antibodies with Marsh 1 or 2 histological changes), compared with the diagnostic yield of capsule endoscopy in a cohort of patients with a confirmed CD diagnosis and persistent symptoms. Authors found in the first group of patients a diagnostic utility of 28% (9/32) in the atrophy and negative marker subgroup and of 7% (2/30) in the patients with mild subgroup histological findings.[71,72]

In a retrospective series of 8 patients evaluated using capsule endoscopy for suspected CD, but with non-diagnostic biopsy or with the impossibility of performing an endoscopy, the characteristic capsule endoscopy findings were followed by the initiation of a gluten-free diet; improvement of symptoms and/or serological markers was demonstrated in 7 of the 8 patients.[73]

Overall published studies in this area deal with a limited number of patients and have a high degree of diagnostic suspicion and show an average sensitivity of 82%, a specificity of 92% and positive and negative predictive values of 99% and 77% respectively (Table 6).[74-79]

4.2. Evaluation of the Extent of CD Damage



Capsule endoscopy, by allowing a complete evaluation of the small intestine, can help determine whether the extent of mucosal involvement is limited to the duodenum, if it reaches the jejunum or if it involves the entire small intestine and can also identify areas or patches of involvement with atrophy which can explain or support the diagnosis. The clinical implications of the extension are not yet well defined, there is controversy between different studies, and some suggest that there is a correlation between the severity or intensity of symptoms of CD and the extension in the mucosa, while Murray's publication does not support this view.[80] In a publication by Barret et al., a positive correlation between CD extent and albumin levels was found (Figures 19-20).[73,80-82]

4.3. Evaluation in Patients with Refractory CD or a Poor Response to Gluten-Free Diet (Table 7)

Author	Country	n	Tumors found
Maiden 2009[85]	UK	19	No
Kurien 2013[86]	UK	69	2
Daum 2007[84]	Germany	14 (7 type I,7 type II)	1 T-cell Lymphoma
Barret 2012[73]	France	37 (11 type I y 26 type II)	2 T-cell Lymphoma

Table 7. Utility of capsule endoscopy in patients with refractory CD or with no response to gluten-free diet.

In this clinical scenario, the main cause for suspicion is the appearance of CD complications such as small intestinal adenocarcinoma, T-cell lymphomas and ulcerative jejunitis. In a retrospective study of 14 patients with refractory CD (including 7 CD type 2 refractory) capsule endoscopy identified 2 patients with T-cell lymphomas (Figure 21).

In a study where 47 patients with a high suspicion of CD complications were evaluated, based on symptoms such as weight loss or abdominal pain, lesions were found in up to 50% of patients by means of capsule endoscopy.[83] In a recent publication on 37 patients with refractory CD, capsule endoscopy had a higher correlation with histology in comparison with conventional endoscopic studies (Table 7).[84-86]

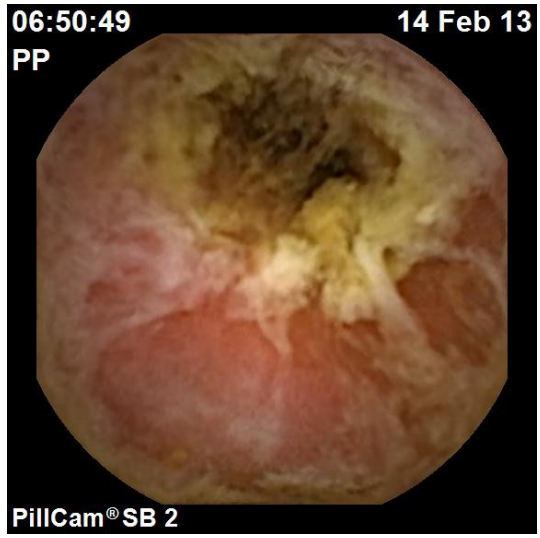


Figure 21. Capsule Image (GIVEN): Ulcerative jejunitis (T lymphoma) in a patient with refractory celiac disease.

4.4. Monitoring Malignancy Development in Patients with Established CD

It is unclear which CD patients ought to be tested and when they ought to have tests made to monitor the development of neoplasias. It is conceivable that patients with long-standing CD or irregular monitoring could benefit from the detection of tumors in early stages.

4.5. Limitations of the Studies by Capsule Endoscopy in Patients with CD

The limitations of capsule endoscopy in the context of CD patients are dictated primarily by variations or inter-observer discrepancies that make this exam operator-dependent if those clinicians conducting the capsule endoscopy evaluation are not familiar with the changes that can be found in CD. Another limitation is the inability to evaluate the entire small intestine.[84]

Published studies show that there is a good correlation with celiac disease diagnosis. However, these have mostly been conducted in patients with high pretest probability, such as patients with suggestive symptoms and/or positive serological markers or contrasted with CD patients with advanced histological stages (Marsh III). In mild villous alteration stages (Marsh I or II) diagnostic

difficulty may be higher. With this in mind, a valuation is being made of the potential usefulness of computerized assessment systems, looking for differences in surface brightness patterns of the mucosa in CD patients compared to healthy ones, or of the spectral analysis of images obtained by capsule endoscopy.[73,87]

Finally, it must be pointed out that capsule endoscopy is, for the time being, a complementary test that can be used in the evaluation of CD patients in the previously discussed scenarios.

5. Push Enteroscopy in Celiac Disease Diagnosis

Just over a decade ago, most widely used endoscopic method for the study of the small intestine was push enteroscopy (length 2000 mm, diameter 9.8 mm). However, the procedure was often frustrating, even though it was possible to use overtubes, by the inability to advance far enough into the small intestine. With the new millennium, capsule endoscopy and double-balloon push enteroscopy were developed (2001).[88]

Double-balloon enteroscopy uses enteroscopes that measure 2000 mm and 8.5 mm (diagnostic) or 9.3 mm (therapeutic) and an overtube 12.2-13.2 mm in diameter, which allows to advance deeper than push enteroscopy.[89] Single balloon enteroscopy single obtains similar results, but spiral enteroscopy, which employs an overtube shaped as the name implies, is not able to penetrate so deeply.[90]

Few studies have evaluated the effectiveness of enteroscopy in the study of celiac disease, and these are not extensive series. A recent systematic review showed that of the existing publications on double-balloon enteroscopy up to 2010 only in 51 (0.4%) of 12,000 explorations the indication was of celiac disease.[91]

The usefulness of enteroscopy in CD would rest, on one hand, on the possibility of taking multiple intestinal biopsies from distal portions to the second portion of the duodenum in patients with clinical suspicion but negative biopsies. In a study (published in abstract form) push enteroscopy was performed on 20 pediatric patients with serological celiac disease suspicion, with biopsies from the bulb, second and fourth portion of the duodenum and proximal jejunum (30 cm from the Angle of Treitz) and distal (60 cm from the Ligament of Treitz).[92] The aim was to map the histological lesion thus evaluate the patchy distribution. Histological celiac injury was found in 90%, 90%, 95%, 90% and 90% respectively at different locations. Bulb involvement was never the exclusive location. In one patient (5%) the diagnosis could only be confirmed by proximal jejunum biopsy.

Another study evaluated the usefulness of push enteroscopy for a confirmatory CD diagnosis in patients with positive serology, but negative biopsies.[93] Out of 31 patients, 23 were positive for anti-gliadin antibodies and enteroscopy with new duodenal and jejunum biopsies did not offer a histological CD diagnosis. However, in 5/8 with antiendomisium, CD was diagnosed from the new biopsies and 3/5 were positive only in the jejunal samples.

A further potential use for enteroscopy, probably the most important, would be the study of refractory celiac disease. Push enteroscopy was useful in patients with refractory CD in one study; out of 8 patients, enteroscopy showed ulcerative jejunitis in five; in 7/8 there was severe duodenal villous atrophy, in all them in the jejune.[94]

In another study, double-balloon enteroscopy and biopsies were performed in 21 patients with a refractory celiac disease indication.[95] In 5 patients (24%) jejunal ulcerations were found whose examination revealed T-cell lymphoma, one of them associated with stenosis. In 3/5 cases the proximal mucosa exhibited Marsh grade III injury. Two patients (9%) had ulcers without lymphoma, which were diagnosed as ulcerative jejunitis. In the 14 (66%) remaining patients, mucosal changes compatible with celiac disease were observed, and were diagnosed as refractory disease. In all of them, duodenal biopsies revealed a Marsh III lesion, but only 8/14 had histological lesions in more distal sections. In two patients with lymphoma, a follow-up double balloon enteroscopy was performed. Based on these studies, enteroscopy should be considered to be a front-line technique in the study of refractory celiac disease by combining imaging and biopsy.

Double-balloon enteroscopy has also been used in patients with malabsorption of unknown origin, and the biopsy procedure allowed a new diagnosis in 33% of cases (Crohn's disease, amyloidosis, and primary intestinal lymphangiectasia).[96]

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