Endoscopic evaluation of celiac disease severity and its correlation with histopathological aspects of the duodenal mucosa

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Background and study aims: Celiac disease (CD) is a chronic systemic autoimmune disorder affecting genetically predisposed individuals, triggered and maintained by the ingestion of gluten. Triggered and maintained by the ingestion of gluten, celiac disease is a chronic systemic autoimmune disorder affecting genetically predisposed individuals. Persistent related inflammation of the duodenal mucosa causes atrophy architecture detectable on esophagogastroduodenoscopy (EGD) and histopathology. We investigated the association between endoscopic features and histopathological findings (Marsh) for duodenal mucosa in celiac disease patients and propose an endoscopic classification of severity.
Patients and methods: Between January 2000 and March 2010, an electronic database containing 34,540 EDGs of patients aged >14 years was searched for cases of CD. Out of 109 cases, 85 met the inclusion criteria: conventional EGD combined with chromoendoscopy, zoom and biopsy. EGD types 0, I and II corresponds to Marsh grades 0, 1 and 2, respectively, while EGD type III corresponds to Marsh grade 3 and 4.
Results: Five patients (5.8%) were EGD I but not Marsh grade 1; 25 patients (29.4%) were EGD II, 4 of whom (16%) were classified as Marsh grade 2; and 55 patients (64.7%) were EGD III, 51 (92.7%) of whom were classified as Marsh grades 3 and 4. The Spearman correlation coefficient (r = 0.33) revealed a significant association between the methods (*P* = 0.002).
Conclusions: Changes in the duodenal mucosa detected on EGD were significantly and positively associated with histopathologic findings. The use of chromoendoscopy in addition to conventional EGD enhances changes in the duodenal mucosa and permits diagnosis of CD, even in routine examinations. The proposed endoscopic classification is practical and easily reproducible and provides valuable information regarding disease extension.

Introduction
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Celiac disease (CD) is a chronic systemic autoimmune disorder affecting genetically predisposed individuals, triggered and maintained by the ingestion of gluten. Gluten is a protein found in wheat, rye, barley (malt) and oats. Originally described by Gee [1], it can induce a strong immune response in sensitive patients which is both cellular (T cells) and humoral (B cells). The disease is characterized by a variable combination of the haplotypes HLA DQ2 and/or HLA DQ8, enteropathy and the production of autoantibodies, including tissue anti-transglutaminase antibodies (anti-tTG), anti-endomysial antibodies (EMA) and deamidated gliadin peptide antibodies (DPG) [2]. Dicke [3] demonstrated an association between CD and certain types of cereals and was the first to propose a gluten-free diet (GFD).

In recent years, advances in endoscopic technology have greatly improved the accuracy of macroscopic evaluations of duodenal villous patterns. Techniques such as chromoendoscopy, Fujinon intelligent chromoendoscopy, narrow band imaging, optical coherence tomography, water immersion, confocal laser endomicroscopy, high-resolution magnification endoscopy, capsule endoscopy and I-scan technology have been described. These tools may be used in combination with histology, increasing the diagnostic power of endoscopy in patients with CD [4].

Advances in diagnostic and screening methods have contributed to an apparent increase in the incidence of CD. Most patients (>80%) diagnosed with CD upon screening are asymptomatic or oligosymptomatic. In addition to gastrointestinal changes, CD patients may present neurological problems, dermatitis herpetiformis, anemia, early
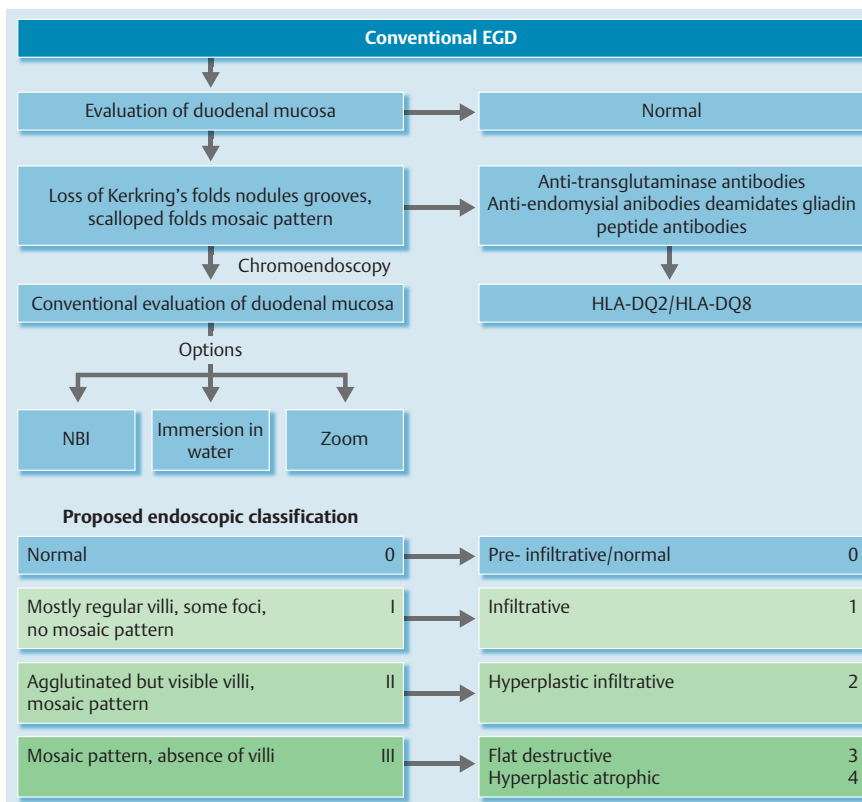
Fig. 1 Algorithm proposed by the author.

osteoporosis, hypoproteinemia, hypokalemia, and raised liver enzyme concentrations [5].

Persistent CD-related inflammation of the duodenal mucosa causes atrophy and deformation of the villous architecture detectable on esophagogastroduodenoscopy (EGD). Classic endoscopic markers include loss of Kerckring's folds, scalloped folds, grooves, mosaic patterns, visible submucosal vessels and micronodules [6,7]. These markers are readily identified on conventional EGD combined with chromoendoscopy, using methyl blue or indigo carmine instilled on the duodenal mucosa. With this technique, markers are identified with 91 % accuracy, compared to 9 % without chromoendoscopy ($P < 0.001$) [8]. If available, devices with zoom features (e.g. Olympus GIF-Q160Z, capable of 115 X magnification) should be used [9, 10]. At this level of magnification, villous atrophy can be identified in detail with 90.7 % sensitivity and 63 % specificity [11] and the extension of the affected tissue can be quantified.

CD may be diagnosed based on clinical suspicion or during routine evaluations of patients with dyspepsia, gastroesophageal reflux disease and peptic disease. The method may also be used in follow-up of CD patients receiving GFD [12]. Early diagnosis is very important. Lifelong GFD use is currently the only safe and effective therapy, although alternative therapies can help reduce the need for dieting. Studies have shown that 2 % to 5 % of patients with CD develop refractory disease, a severe complication associated with intestinal lymphoma and very poor prognosis [13]. In this study, we investigated the association between endoscopic features of the duodenal mucosa and histopathological findings (interpreted according to the Marsh classification) in a cohort of patients with CD and propose an endoscopic classification of severity for this patient population.

Justification for the study

Although the relevance of duodenal mucosal changes on conventional EGD to the diagnosis of CD has been demonstrated in the literature, few endoscopists seem to be aware of the advantage of adopting chromoendoscopy and zoom as a routine diagnostic procedure. In view of the scarcity of literature on the subject, we believe that a detailed endoscopic classification of such changes will help understand the development of CD and standardize findings with regard to both severity and extension. In addition, a comparison between endoscopic and histopathologic findings will allow therapists to evaluate the repercussion of gluten on the intestinal mucosa with greater accuracy and establish a more reliable diagnosis of CD.

Patients and methods
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This was a retrospective, cross-sectional study based on medical records. The design followed PUC/PR guidelines (Pontifícia Universidade Católica do Paraná, Brazil) and was approved by the UNIOESTE/Pr Human Research Ethics Committee and filed under entry #344/2011. Due to the retrospective nature of the study, the researchers were exempted from obtaining informed written consent from the patients.

The medical records and the endoscopic images were reviewed by the same physician. Three modalities of EGD were employed: conventional EGD, conventional EGD combined with chromoendoscopy (indigo carmine), and conventional EGD combined with chromoendoscopy and zoom. Histopathologic data from the time of diagnosis of CD were reviewed at Citopar (Center of Cytology and Pathology, Paraná, Brazil). The biopsy specimens were reviewed by a pathologist with experience in CD.

Patients of both genders aged > 14 years diagnosed with CD according to the criteria of the World Gastroenterology Organiza-
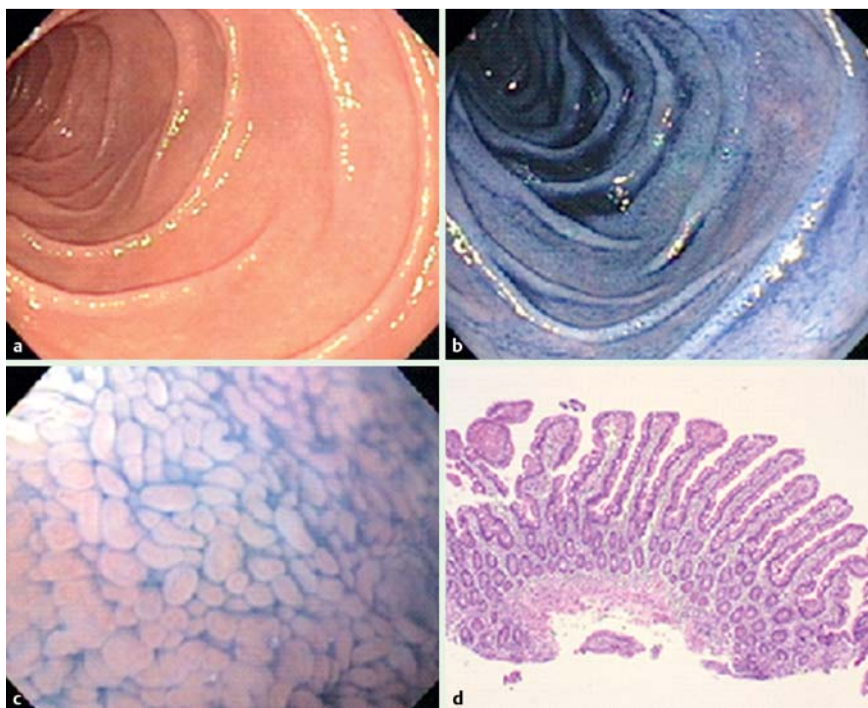
Fig. 2   EGD type 0. Regular, continuous, digitiform and occasionally foliaceous villi, corresponding to Marsh grade 0. **a** Conventional EGD: pink, continuous and regular mucosa. **b** Conventional EGD + chromoendoscopy (0.5 % indigo carmine): continuous and regular mucosa. **c** Conventional EGD + chromoendoscopy + zoom (115x) during follow-up of patient on gluten-free diet: normal, regular villi with no atrophic foci. **d** Slide stained with HE (40x): duodenal mucosa with normal villi. Villus/crypt length ratio 4:1. Example below.

tion [14] were eligible. The diagnosis of CD was based on serum positivity for EMA IgA [15] and/or anti-tTG [16] and on histopathological findings for the intestinal mucosa [17]. Patients with incomplete medical records were excluded.

The medical records (clinical data and EGD images) were retrieved from the electronic database (Sisclínica 2000) of a referral center in Southern Brazil (Gastroclínica Cascavel) covering the period from January 2000 to March 2010. The initial sample was drawn from a universe of 34,540 EGDs of male and female patients older than 14 years of age (with no repetition of EGDs) and consisted of 109 patients presenting classic endoscopic markers [6,7] on conventional EGD with chromoendoscopy (indigo carmine), zoom at 115x (Olympus GIFQ-160Z) and biopsy of intestinal mucosa for histopathological analysis. The inclusion criteria were met by 85 of these 109 patients, of whom 64 (75.30 %) were women (mean age: 35 years) and 21 (24.70 %) were men (average age: 34 years).

The EGD images of the final sample of 85 patients, representing the moment of diagnosis, were selected. The algorithm shown in ◉ Fig. 1 was applied to each case.

The biopsy specimens of the 85 patients were sent to Citopar in the form of slides or paraffin blocks. The original block was used to prepare new slides in order to review each case at the moment of diagnosis and during follow-up on GFD. All specimens were reviewed by the same pathologist and interpreted according to the Marsh classification system [17].

The pathologist was blinded to the result of the EGD. The results were classified according to the algorithm shown in ◉ Fig. 1.

Statistical analysis

Spearman's correlation coefficient was calculated and evaluated for statistical significance at the level of 5 % ($P < 0.05$). Sensitivity, specificity and positive likelihood ratios were estimated to evaluate EGD accuracy, considering the histologic pattern (Marsh) as the gold standard for the evaluation of CD. All analyses were carried out with the software SPSS v.20.

Results

Careful examination of the endoscopic images in the database supported establishment of a macroscopic correspondence between the severity of atrophy on EGD and Marsh grade (1992) (◉ Supplementary Fig. 1). Thus:

▶ EGD type 0 (regular, continuous, digitiform and occasionally foliaceous villi) corresponds to Marsh grade 0 (pre-infiltrative). (◉ Fig. 2 and ◉ Supplementary Fig. 2).

▶ EGD type I (mostly regular villi, with some foci, but no mosaic pattern) corresponds to Marsh grade 1 (infiltrative). (◉ Fig. 3 and ◉ Supplementary Fig. 3).

▶ EGD type II (agglutinated but visible villi, mosaic patterns) corresponds to Marsh grade 2 (hyperplastic infiltrative). (◉ Fig. 4 and ◉ Supplementary Fig. 4).

▶ EGD type III (absence of villi, mosaic patterns) corresponds to Marsh grade 3 (flat, destructive) and 4 (hyperplastic atrophic) (the appearance of the duodenal surface on EGD was the same for the two histologic grades). (◉ Fig. 5 and ◉ Supplementary Fig. 5).

Based on these correspondences, we developed an algorithm associating EGD type and Marsh grade in patients with CD (◉ Fig. 1).

Our 85 patients with CD were diagnosed as follows:

▶ EGD type 0 = None.

▶ EGD type I = 5 patients (5.8 %) of whom 4 (80 %) were Marsh grade 2, and 1 (20 %) was Marsh grade 3.

▶ EGD type II = 25 patients (29.4 %) of whom 4 (16 %) were Marsh grade 2, and 21 (84 %) were Marsh grade 3.

▶ EGD type III = 55 patients (64.7 %) of whom 4 (7.2 %) were Marsh grade 2, 50 (90.9 %) were Marsh grade 3, and 1 (1.8 %) was Marsh grade 4.

The results of the two diagnostic methods (EGD and histopathology) were expressed as frequencies and percentages. As shown in ◉ Table 1, the estimated Spearman correlation coefficient was r = 0.33 ($P = 0.002$), indicating a significant and positive association between the methods. However, despite the significant P value,
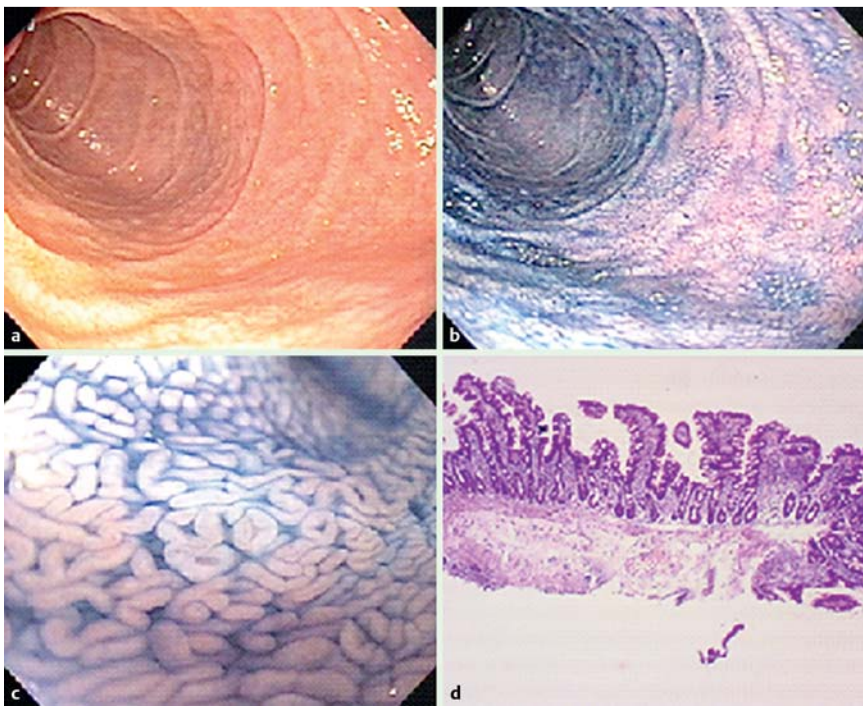
Fig. 3 EGD type I. Mostly regular villi, with some foci, but no mosaic pattern, corresponding to Marsh grade 1. **a** Conventional EGD: continuous and regular mucosa with minor atrophic foci but no mosaic patterns. **b** Conventional EGD + chromoendoscopy (0.5 % indigo carmine): minor atrophic foci, no mosaic patterns. **c** Conventional EGD + chromoendoscopy + zoom (115x): minor atrophic foci, no mosaic patterns. **d** Slide stained with HE (40x): duodenal mucosa with some shortened villi minor atrophic foci (epithelial lymphocytosis). Example below.
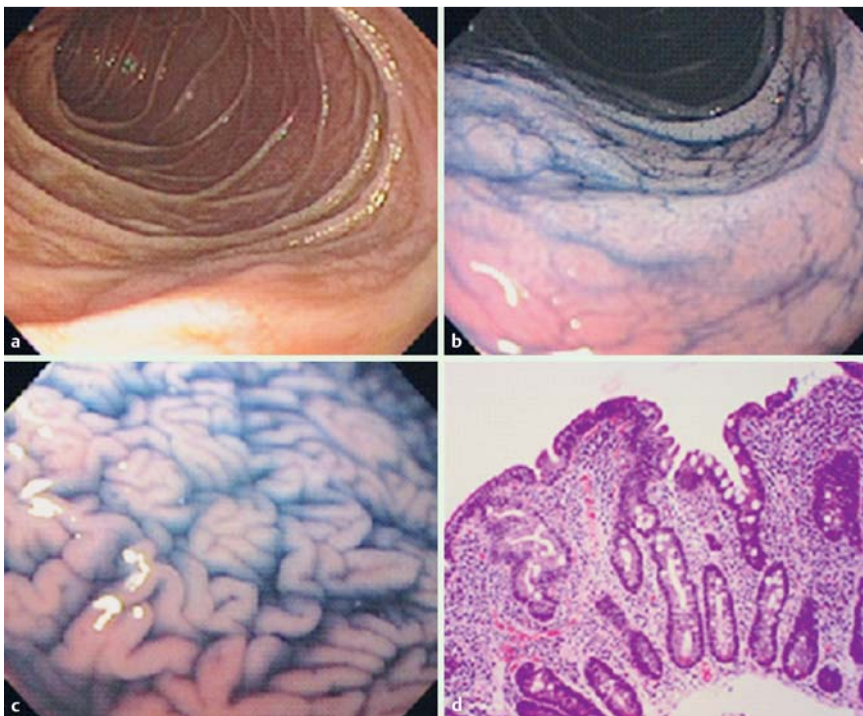

Fig. 4 EGD type II. Agglutinated but visible villi and mosaic patterns, corresponding to Marsh grade 2. **a** Conventional EGD: continuous and regular mucosa with minor atrophic foci but no mosaic patterns. **b** Conventional EGD + chromoendoscopy (0.5 % indigo carmine): atrophic foci and mosaic patterns. **c** Conventional EGD + chromoendoscopy + zoom (115x): atrophic foci and mosaic patterns. **d** Slide stained with HE (40x): duodenal mucosa with poorly distinguishable villi, crypt hyperplasia and lymphocytosis. Example below.

the coefficient was not high. EGD sensitivity was 69.9 % in relation to Marsh classification, with a specificity of 66.7 % and a positive likelihood ratio of 2.1.

Discussion
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The incidence of CD in the United States has increased at least four-fold over the past decades. Studies on the general population estimate an incidence of 0.8 % to 1.0 % [15]. On the other hand, many individuals with CD remain undiagnosed: the figure

was 97 % in a study by [18] and, more recently, 95 % (Olmsted County), 90 % (Wyoming) and 89 % (Maryland) in a study based on a national health and nutrition survey [19].

The large number of undiagnosed cases of CD may be explained by variations in clinical presentation, which is not restricted to patients with gastrointestinal symptoms. Thus, CD may affect patients with several atypical extraintestinal or oligosymptomatic disorders accompanied by abdominal pain, anemia, weight loss, neurological changes, hypoproteinemia, hypokalemia and high liver enzyme concentrations [20]. The delay in diagnosing CD is sometimes the result of the physician's lack of familiarity with
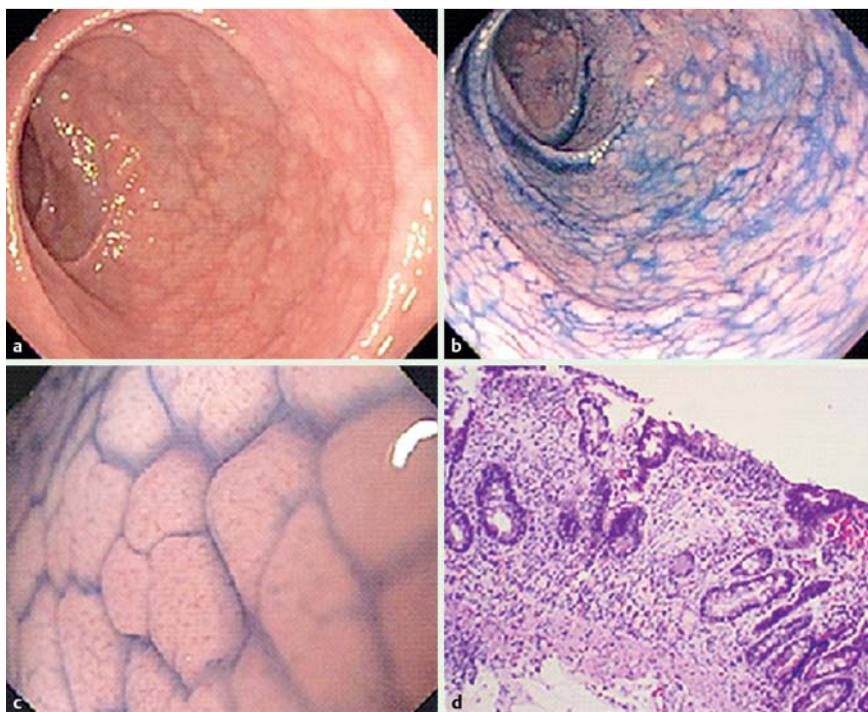
Fig. 5 EGD type III. Mosaic patterns and absence of villi, corresponding to Marsh grades 3 and 4. **a** Conventional EGD: severe loss of Kerkring's folds, nodules and fissures. **b** Conventional EGD + chromoendoscopy (0.5 % indigo carmine): fissures, nodules, mosaic patterns, absence of villi. **c** Conventional EGD + chromoendoscopy + zoom (115x): flat, mosaic pattern, absence of villi. **d** Slide stained with HE (40x): severely atrophied duodenal mucosa without visible villi. Example below.

clinical manifestations [21]. In fact, CD is often diagnosed casually during routine EGD. Relatively little attention is given to this disorder in the United States, despite an incidence comparable to that of Europe [22]. However, failure to clinically detect CD exposes CD patients to the risk of developing severe gluten-related disorders, including autoimmune diseases (thyroiditis, hepatitis, herpetiform dermatitis and alopecia) [23], osteoporosis, anemia, neurologic changes and intestinal lymphoma [24]. The prevalence of undiagnosed cases may be reduced by careful endoscopic examination of the duodenum combined with chromoendoscopy, as proposed in this study, since most patients with dyspeptic symptoms are routinely submitted to EGD.

According to the National Health Council (Conselho Nacional de Saúde, 2012), the global incidence of CD is estimated at 1 %, but no official estimates are available for Brazil yet. A study published in 2005 by the Federal University of São Paulo (Unifesp) found an incidence of 1/214 for a sample of adult blood donors in São Paulo [25,26]. In addition to atypical clinical manifestations, groups at risk for CD such as families with a history of Down syndrome [27,28], Turners syndrome [29] and type I diabetes [30,31], should be given more attention.

The combination of EGD and chromoendoscopy/zoom was first used to evaluate villous atrophy of the duodenal mucosa when Badreldin et al. [11] assigned z-scores corresponding to 4 patterns of villous architecture (from normal to absent villi) to 58 patients. The procedure minimizes disagreement between EGD + chromoendoscopy/zoom and histopathology. [32] submitted 27 untreated CD patients to EGD with zoom, using an Olympus GIF-Q160Z device (as in the present study). The authors instilled acetic acid on the intestinal villi and classified their findings into three patterns: normal areas, areas with partial atrophy, and areas with complete atrophy (100 % sensitivity compared to conventional EGD), thereby potentially reducing the need for blind biopsies. In a study comparing EGD + chromoendoscopy/zoom images of 36 patients to histopathologic findings, [33] classified the observed villous patterns as normal, partially atrophied or

Table 1 Number and percentage of patients with celiac disease diagnosed according to esophagogastroduodenoscopic (EGD) type vs. Marsh's histopathological classification (Marsh, 1992) based on a cohort of 85 patients from a Brazilian referral center (Gastroclínica Cascavel). January 2000 to March 2010.

EGD type	Marsh grade			Total n
	2	**3**	**4**	
I	4	1	0	5
	4.7 %	1.2 %	0 %	
II	4	21	0	25
	4.7 %	24.7 %	0 %	
III	4	50	1	55
	4.7 %	58.8 %	1.2 %	
Total	12	72	1	85

The Spearman correlation coefficient (r = 0.33) shows a significant association between the methods (*P* = 0.002).

completely atrophied, and found the Spearman correlation coefficient (0.92) to be statistically significant (*P* < 0.001).

The studies described above inspired us to develop the endoscopic classification described in the current study. Following a careful review, the magnified images of the duodenal mucosa were correlated with Marsh's schematic illustrations [17]. The classification was centered on the mosaic pattern characterizing moderately advanced forms of duodenal atrophy. Interestingly, when the villous architecture is under recovery, the flat mosaic pattern reverts to a mosaic pattern with visible villi. Eventually the mosaic pattern disappears, leaving only minor foci, followed by complete recovery (○ **Fig. 2**, ○ **Fig. 3**, ○ **Fig. 4** and ○ **Fig. 5**). Thus, the stages of atrophy observed when CD develops are observed in reversed order as the condition resolves. Based on these observations, EGD type 0 (normal), type I (minor atrophic foci) and type II (mosaic pattern, visible villi) were found to be equivalent to Marsh grades 0, 1 and 2, respectively, while EGD type III (mosaic pattern, villi not visible) was found to be equivalent to Marsh grades 3 (destructive) and 4 (hyperplastic atrophic) (the

appearance of the duodenal surface on EGD is the same for Marsh grades 3 and 4). In view of these correspondences, an algorithm was developed associating endoscopic features and histopathological (Marsh) findings (● **Fig. 1**).

The proposed algorithm starts with conventional EGD. If markers for CD are suspected, the patient is submitted to image-enhancing chromoendoscopy for confirmation, even without zoom. If necessary (and available), the duodenum may subsequently be evaluated under magnification in order to determine the stage of atrophy. The use of the algorithm not only provides important data for the pathologist, but potentially increases the confidence of the physician interpreting the EGD results due to the additional information on extension and macroscopic patterns.

In the current study, a significant and positive association was observed between the 2 diagnostic methods (EGD vs. histopathology) based on a cohort of 85 patients (Spearman correlation coefficient 0.33; $P = 0.002$) (● **Table 1**) with a sensitivity of 69.9 %, specificity of 66.7 %, and a positive likelihood ratio of 2.1. EGD combined with chromoendoscopy allowed to evaluate the entire surface of the duodenal mucosa macroscopically and detect changes, from minor foci to complete atrophy. When changes in the villous architecture were continuous (as in severe cases), the association between the methods was stronger. Conversely, in less severe cases (minor foci), the association was weaker. This is most likely because EGD evaluates a larger extension of the duodenal mucosa, providing a macroscopic view.

As shown in this study, advances in endoscopic technology have made it possible to identify patterns of villous architecture during EGD, making diagnosis more accurate. It is therefore reasonable to presume that EGD will come to play an essential role in the diagnosis of CD, rather than simply provide a means of collecting biopsy specimens for histopathology [34]. The proposed endoscopic classification of patterns observed on EGD (combined with chromoendoscopy and zoom) makes it possible to standardize atrophic changes in the duodenal mucosa of patients with CD. It should be stressed that conventional EGD with chromoendoscopy (without zoom) is often sufficient to establish the level of atrophy. The agreement between the methods increases if the pathologist is informed of the extension of the affected mucosa (using the proposed algorithm) and of the EGD type of each biopsy specimen. In this study the endoscopic images and histology where reviewed by only 1 endoscopist and 1 pathologist. Another drawback is the retrospective design, restricting the evaluation to image analysis. A prospective design using this method would probably have yielded higher levels of agreement between endoscopy and pathology.

The classification proposed in this study may be used to increase the agreement between EGD and histopathology and the accuracy of diagnosis and follow-up. In addition, it provides endoscopists with an algorithm which will allow them to make more detailed evaluations (using chromoendoscopy in cases of suspicion) and, over time, reduce the prevalence of undiagnosed CD.

Conclusions
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Our results suggest that: 1) changes in the duodenal mucosa detected on EGD were significantly and positively associated with histopathologic findings; 2) the more severe the atrophy, the stronger the association between the methods; 3i) when conventional EGD images arouse suspicion of CD, chromoendoscopy should be employed for confirmation; and 4) the proposed endoscopic classification is practical and easily reproducible and provides valuable information regarding disease extension.

Final considerations

Macroscopic evaluation of the extension of CD-related atrophy obtained with the use of the proposed algorithm is a helpful aid in histopathology. It also alerts endoscopists to the need of chromoscopy following routine EGDs with suspicion of CD. Most cases in the present study were diagnosed with routine EGD, with no specific clinical presentation, (some patients had several previous EGDs, or even surgery for gastroesophageal reflux disease).
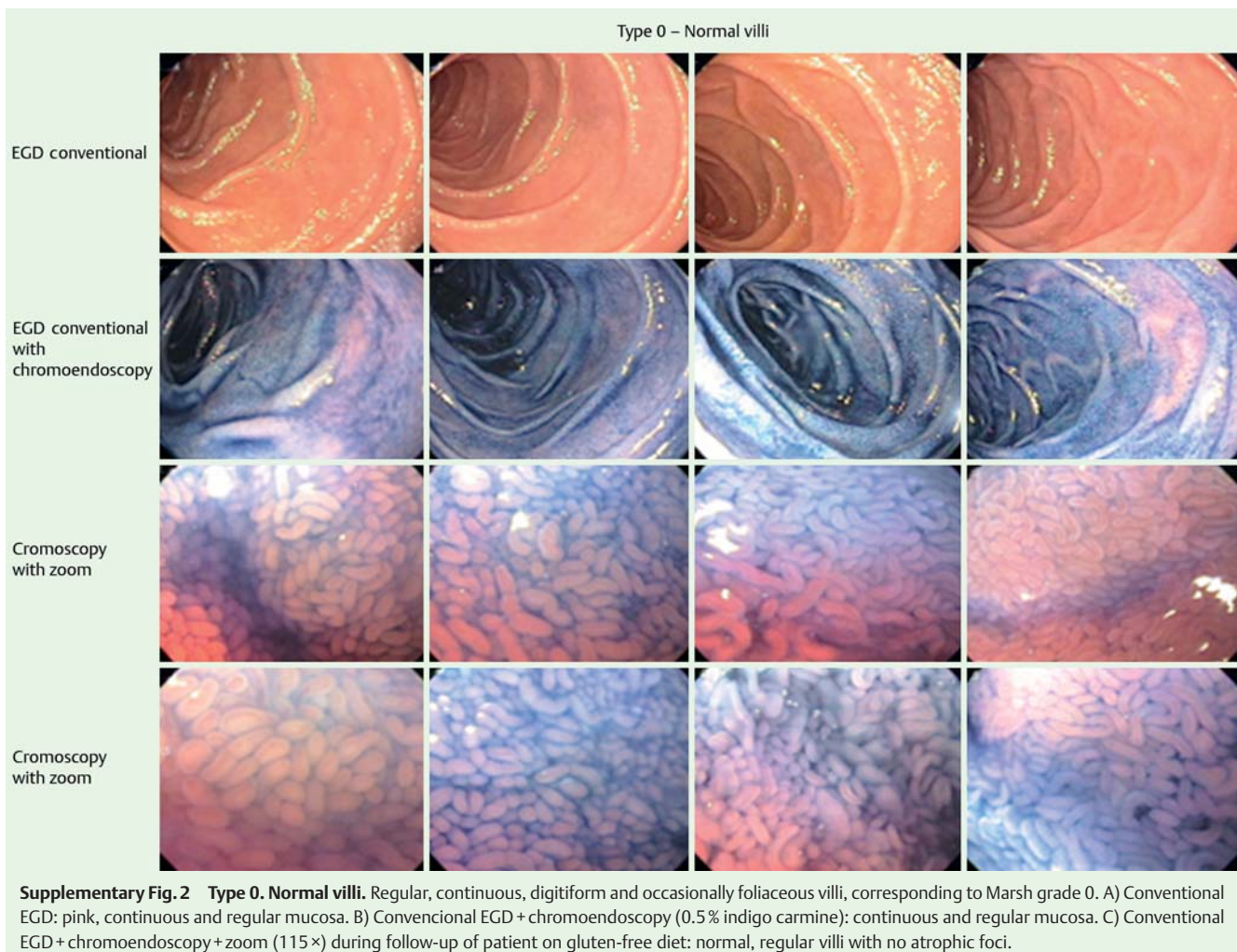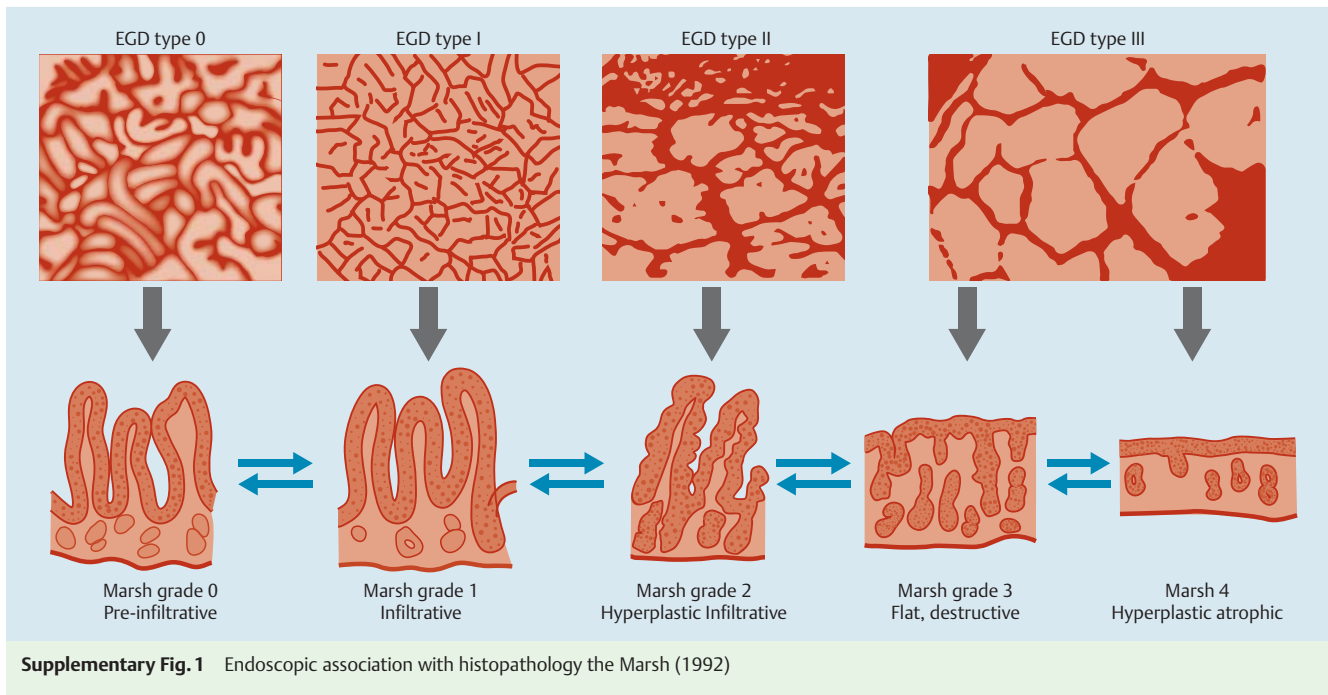
Competing interests: None

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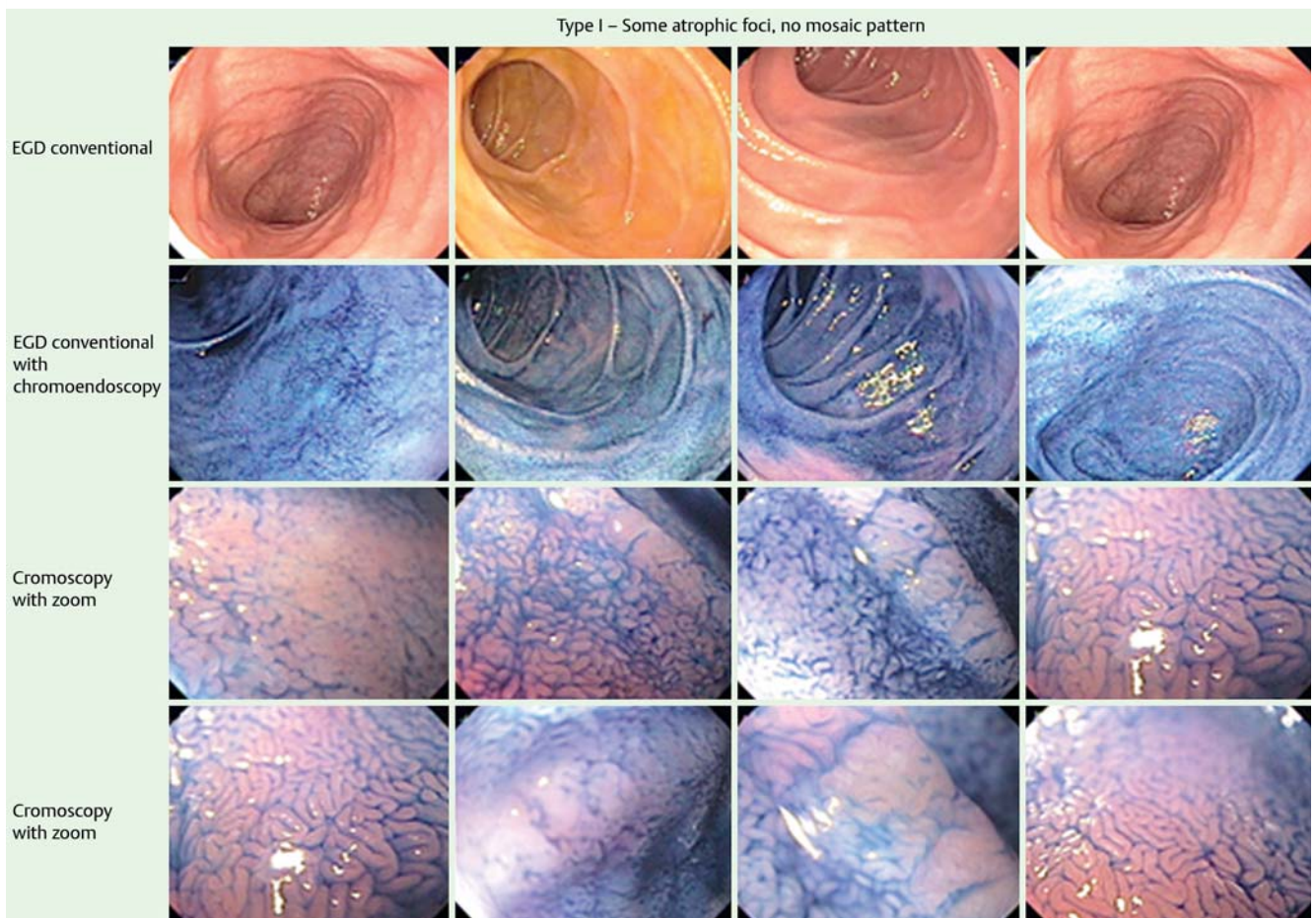
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Supplementary Fig. 1   Endoscopic association with histopathology the Marsh (1992)

Supplementary Fig. 2   Type 0. Normal villi. Regular, continuous, digitiform and occasionally foliaceous villi, corresponding to Marsh grade 0. A) Conventional EGD: pink, continuous and regular mucosa. B) Convencional EGD + chromoendoscopy (0.5 % indigo carmine): continuous and regular mucosa. C) Conventional EGD + chromoendoscopy + zoom (115 ×) during follow-up of patient on gluten-free diet: normal, regular villi with no atrophic foci.

Supplementary Fig. 3 Type I. Some atrophic foci, no mosaic pattern. Mostly regular villi, with some foci, but no mosaic pattern, corresponding to Marsh grade 1. A) Conventional EGD: continuous and regular mucosa with minor atrophic foci but no mosaic patterns. B) Conventional EGD + chromoendoscopy (0.5 % indigo carmine): minor atrophic foci, no mosaic patterns. C) Conventional EGD + chromoendoscopy + zoom (115×): minor atrophic foci, no mosaic patterns.

Supplementary Fig. 4 **Type II. Mosaic pattern with agglutinated villi.** Agglutinated but visible villi and mosaic patterns, corresponding to Marsh grade 2. A) Conventional EGD: continuous and regular mucosa with minor atrophic foci but no mosaic patterns. B) Conventional EGD + chromoendoscopy (0.5 % indigo carmine): atrophic foci and mosaic patterns. C) Conventional EGD + chromoendoscopy + zoom (115 ×): atrophic foci and mosaic patterns.

Supplementary Fig. 5   Type III. Mosaic patterns and absence of villi. Corresponding to Marsh grades 3 and 4. A) Conventional EGD: severe loss of Kerkring's folds, nodules and fissures. B) Conventional EGD + chromoendoscopy (0.5 % indigo carmine): fissures, nodules, mosaic patterns, absence of villi. C) Conventional EGD + chromoendoscopy + zoom (115×): flat, mosaic pattern, absence of villi.