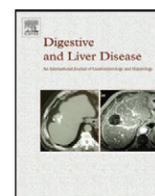




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Coeliac disease: The histology report

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Abstract

To this day intestinal biopsy is justly considered the “gold standard” for the diagnosis of coeliac disease (CD). The aim of the authors in setting up these guidelines was to assist pathologists in formulating a more precise morphological evaluation of a duodenal biopsy in the light of clinical and laboratory data, to prepare histological samples with correctly oriented biopsies and in the differential diagnosis with other pathological entities and complications of the disease. A further intention was to promote the conviction for the need of a close collaborative relationship between different specialists namely the concept of a “multidisciplinary team”.

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1. Introduction

These guidelines are intended as an aid for pathologists, in order to allow a more precise morphological evaluation of duodenal biopsies in the light of clinical and laboratory data. The aim is to arrive at the conviction of the need for a close collaborative relationship between different specialists such as adult or pediatric gastroenterologists, endoscopists, laboratory staff, endoscopy room nurses, pathology laboratory technicians and pathologists. This implies the creation of a “multidisciplinary team” led by a gastroenterologist who,

based on the most recent acquisitions regarding the diagnosis and pathogenesis of coeliac disease, is the only specialist that can make the final diagnosis of coeliac disease. A number of specific points in the diagnostic process will thus be dealt with, considering issues and concerns of differential diagnosis with other similar diseases before the final diagnosis can be reached.

This document is an update of the “guidelines” published by the Italian Group of Digestive Disorders (GIPAD) in 1998 [1].

The document, after a brief historical and epidemiological address, considers the following points:

- Clinical and laboratory aspects
- The methodological approach to duodenal biopsies
- Aspects of normal and pathological duodenal mucosa
- Diagnosis
- Differential diagnosis
- Complications that can be confirmed histologically.

List of abbreviations: CD, coeliac disease; tTGA, antitransglutaminase antibodies; EMA, antiendomysial antibodies; AGA, antigliadin antibodies; IEL, intra epithelial lymphocytes.

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2. Brief history

The first descriptions of coeliac disease can be found in the first century A.D. when the physician Celsus introduced the Latin term “coeliac” to indicate a diarrhea-like disease. Later, in 250 A.D., Areteo Cappadocia described the clinical signs of a prolonged intestinal disease that was very difficult to treat, using the Greek word *koiliakos* to identify “those who suffer in their intestines”. In 1856, Francis Adams translated this Greek word into English, coining the term “coeliac”. A few years later, in 1888, Samuel Gee described the detailed symptoms of this condition both in adults and in children, predicting that the only treatment consisted of an appropriate diet, with few items derived from flour. Only halfway through the twentieth century, however, did it become clear that coeliac disease occurs in some individuals following the ingestion of wheat proteins, which damage the intestinal mucosa. The systematic description of the histopathological alterations of coeliac disease (CD) is mainly due to the work of Marsh [2,3]. Today we know that CD is a chronic, immune-mediated disease occurring in genetically predisposed individuals due to an intolerance to gluten-containing foods and, in particular, to some of its proteins, called gliadins. This intolerance leads to abnormal immune response, which is followed by a chronic inflammation of the small intestinal mucosa with progressive disappearance of intestinal villi.

3. Epidemiology

The disease has a variable incidence, which in Europe is estimated between 0.3 and 1.2%, similar percentages are reported in North America and Australia. Recently, a high prevalence has been reported in people of Northern Africa (5–6% in populations of Western Sahara). In Italy the most recent statistics estimate a prevalence of 1/100, and each year about 5000 new cases are diagnosed. CD, once considered a disease of childhood, can affect individuals of all ages, with a preference for females (male/female ratio 1:2).

4. Clinical and laboratory aspects

4.1. Clinical aspects of CD

The variety of clinical manifestations which coeliac disease may present complicates its recognition. A correct diagnosis can not rely on a single test, but requires a precise reconstruction of a puzzle, whose pieces are represented by the clinical, serological, genetic and histological aspects. The evaluation of all these factors, apart from genetics, must take place while the patient is still on a diet containing gluten, since a gluten-free diet changes the clinical, serological and histological pattern, making it impossible to recognize the characteristic aspects of disease.

The significant improvement in our knowledge of intolerance to gluten has made it possible to identify the so-called

risk groups in which, on the basis of intestinal and extraintestinal symptoms, and of the presence of any associated diseases and familiarity, the possibility of coeliac disease must be investigated [4,5].

These risk groups are made up of:

1. *Subjects in whom coeliac disease is strongly suspected* (cases with severe malabsorption and with highly predictive associated diseases):
 - malabsorption syndrome with repeated diarrhea-like bowel movements, abdominal pain and marked weight loss;
 - dermatitis herpetiformis, also called coeliac disease of the skin, since in practically all cases there is more or less severe gluten-dependent intestinal damage.
2. *Subjects in whom coeliac disease is moderately suspected* (cases with atypical or extraintestinal symptoms and associated diseases):
 - atypical gastro-intestinal symptoms (dyspepsia, constipation, vomiting and intestinal subocclusion);
 - extraintestinal symptoms (anemia – most often due to a lack of iron but also to a lack of folic acid and vitamin B12), hyposomia, oral ulcers, hypertransaminasemia, osteopenia or osteoporosis, tooth enamel abnormalities, hemorrhagic syndrome due to vitamin K malabsorption, changes in the female reproductive system (late menarche, early menopause, recurrent miscarriage, premature labour);
 - associated diseases (diabetes mellitus type 1, Hashimoto thyroiditis, Graves' disease, selective IgA deficiency, alopecia areata, piebald skin, psoriasis, Addison's disease, Systemic Lupus Erythematosus, polymyositis, rheumatoid arthritis, cerebellar ataxia, epilepsy with or without cerebral calcifications, peripheral neuropathy, autoimmune hepatitis, primary biliary cirrhosis, idiopathic dilated cardiomyopathy, Berger's disease, Down's syndrome, Turner's syndrome, Williams' syndrome).
3. *1st degree relatives of coeliac patients* (high familiarity of coeliac disease that is present in 4–17% of 1st degree relatives of coeliac patients, but may also be found in high proportions in 2nd degree relatives).

A major role in the diagnostic process of coeliac disease is played by serology, which allows identification within the at-risk groups of the subjects who should undergo intestinal biopsy. While making it clear that no positive antibody test allows a diagnosis of coeliac disease without the necessary confirmation provided by an intestinal biopsy, some of the antibody markers show such a high diagnostic accuracy (with levels of sensitivity and specificity >95%) that they are highly predictive of coeliac disease.

4.2. Antibody markers

- IgA class antitransglutaminase antibodies (tTGA) are the tests with the highest sensitivity for coeliac disease (98%) with specificity estimated at around 90%. High titres of IgA class tTGA (>5 times the cut-off) are almost always

the expression of coeliac disease, while false positives (about 10%) almost always present medium-low titres (<2 times the cut-off)

- IgA class antiendomysial antibodies (EMA), while having a lower sensitivity compared to IgA class tTGA (90% vs. 98%), show an almost absolute specificity for coeliac disease. Antibody titres from >1:40 correlate with a greater severity of intestinal lesions, and low titre positives (1:5) are often an expression of infiltrative lesions of the intestinal mucosa (type 1), suggestive of coeliac disease.
- IgA class anti gliadin antibodies (AGA) are now an obsolete test with levels of sensitivity and specificity significantly lower than tTGA and EMA, and the search for their presence is useful only in early childhood (children aged <2 years); because they are the first antibodies to appear, they show a higher sensitivity than other tests in this age group. Positivity for IgA AGA associated with negativity for EMA and tTGA is almost never an expression of coeliac disease in adults and in children aged >2 years.
- With regard to the IgG class of antibodies, their use should be restricted to patients with selective IgA deficiency, because only in this subgroup of patients is the response indicative of coeliac disease. The recommended test is the detection of tTGA (+ AGA in children aged <2 years). The negativity of IgA tTGA with very low values (<0.1 AU) always suggests the presence of a selective IgA deficiency and indicates a search for IgG class tTGA.

4.3. Indications for biopsy in suspected coeliac disease

Intestinal biopsies taken from the first and second duodenal portion remain an essential means of confirming the diagnosis of coeliac disease. To retain its diagnostic validity, it is fundamental for the patient to be on a normal diet containing gluten at the time of the biopsy (often due to incorrect information, patients may have already been on a gluten-free diet for some time when they undergo the biopsy).

4.4. Who should undergo an intestinal biopsy?

- Subjects with positive serology characterized by the presence of IgA class antitransglutaminase and antiendomysial antibodies, and children younger than 2 years with isolated IgA AGA positivity. In some cases, the detection of very low antibody titres, particularly for IgA tTGA (test with 10% false positives), in the absence of EMA, suggests monitoring the patient for some time and re-testing before proceeding with an endoscopy investigation.
- Subjects with deficiency of IgA positive for IgG tTGA (and even children aged <2 years with positivity for AGA IgG with or without IgG tTGA) should also undergo intestinal biopsy.
- Subjects in whom coeliac disease is strongly suspected, in whom a severe malabsorption syndrome is present, irrespective of antibody test results (in practice it should be performed even if all the antibodies are negative), precisely because in highly symptomatic subjects it is possible to

find that they are serologically negative for coeliac disease.

- According to NIH guidelines [7], a duodenal biopsy is not essential in patients with dermatitis herpetiformis if the diagnosis is supported by immunofluorescence detection of granular deposits of IgA in the dermis. In these cases, in fact, the gluten-dependent intestinal damage is always present in a more or less severe form and gluten-free diet will lead to resolution of the skin lesions.

When the results of the intestinal biopsy and serological tests are consistent, the clinician is able to make the diagnosis of coeliac disease. The diagnosis is confirmed with the resolution of the clinical symptoms and negative serology tests after a reasonable period of strict gluten-free diet (usually 12 months). Therefore, provided that the clinical situation has improved and serological tests have become negative as a result of following this diet, an intestinal biopsy after gluten withdrawal is no longer considered essential for the definitive diagnosis of coeliac disease, not only in children [6], but also in adults [7].

4.5. Genetic testing

Coeliac disease is closely associated with histocompatibility antigens (HLA) DQ2 and DQ8. Practically all patients with coeliac disease are positive for one or both of these HLAs or for a fraction of the heterodimer, but genetic testing is never diagnostically significant since at least 30% of the general population present the same HLAs as coeliac patients.

1. When the genetic test should be performed:

- In cases where there is a discrepancy between serology and histology.
- In 1st degree relatives to assess the genetic predisposition to coeliac disease.

2. Significance of genetic testing:

- The main clinical significance of genetic testing is to exclude a diagnosis of coeliac disease in the absence of HLA-DQ2 (and its fractions) and -DQ8 in cases of diagnostic doubt.
- Exclusion of predisposition to coeliac disease in family members of coeliac patients in the absence of HLA-DQ2 (and fractions) and -DQ8.

4.6. Clinical notes

A close collaboration between pathologist and clinician is essential in order to address the concerns relating to the diagnosis of coeliac disease especially in cases that are difficult to evaluate.

The information that the clinician should provide the pathologist may be summarized as follows:

- Details of the patient's diet (normal or gluten-free, in the second case specifying how long the patient has been on a gluten-free diet).
- Level of clinical suspicion: high or moderate based on the symptoms.
- Whether the patient has a family history of coeliac disease (defining the degree of relationship).

- Serology with absolute (EMA), high (tTGA) or low (AGA) predictability. Always specify the antibody class, the presence of selective IgA deficiency, and the antibody titre if available (with its cut-off).
- Genetic test results (if performed in accordance with the indications), especially with regard to DQA1 05, DQB1 02 and DQB1 0302.

5. Approach to duodenal biopsy: the method

The biopsies that the pathologist receives nowadays are all performed by endoscopic examination, which, in addition to the duodenum, makes it possible to explore other districts of the gastro-intestinal tract. Biopsies performed through the use of the Crosby-Watson capsule by perioral route are now considered outdated and are no longer performed.

Here are some points which require a close working relationship between the endoscopist, the endoscopy-room nurse, the pathology laboratory technician and the pathologist.

5.1. Site of the biopsy

Biopsy by endoscopy is always performed in the second and third duodenal portion, as the bulb and the proximal duodenum can be a source of incorrect assessments; we recommend at least 4 biopsies, 2 for each of the areas mentioned above. Performing a biopsy only in the duodenal bulb may be a source of error, or may at least greatly reduce the sensitivity of the examination, and hence is strongly discouraged [8].

5.2. Orientation of the biopsy sample

This is essential for proper histological assessment.

Positioning of biopsies on cellulose acetate filters is advisable, with benefits for:

1. the laboratory staff, since with a simple 90° rotation it is possible to embed the combined biopsy-filter;
2. for subsequent histological evaluation by the pathologist.

The method based on experience acquired at St Mark's Hospital in London ensures histological samples in which it is possible to analyze the mucosa and, if necessary, the submucosa of the removed tissue, thus respecting the normal anatomical relationship between the different layers of the intestinal wall. In particular, after initial experience with filters that needed to be cut with obvious waste of time and human resources, a comprehensive kit has been developed, on which three easily-detachable cut filters with a bevelled end shaped like a clarinet mouthpiece are already fixed (Fig. 1).

After the fixing stage, the filter-biopsy combination is processed and then embedded. During this last phase the technician rotates the filter-biopsy combination 90 degrees in order to place the samples in their natural position.

After cutting, the biopsies are placed on a slide and, if necessary, the position of the bevelled end is marked on the label, to indicate the first biopsy.

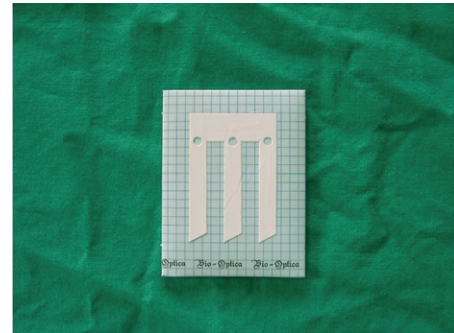


Fig. 1. Cellulose acetate filter with three easily-detachable cut filters.

When properly carried out, this method is of great benefit to the pathologist, but also to the technician who during the embedding phase does not have to search for the individual biopsies, which are sometimes fragmented and have no guiding landmark.

The use of cellulose acetate filters allows perfect adhesion of the biopsies, avoiding their dispersion in the fixation medium. These filters also do not react chemically with the fixatives and reagents used during the processing of the sample; during the cutting phase they do not offer resistance to the blade and, unlike tissue paper, they do not fray.

This method, which can be applied on all segments of the gastro-intestinal tract, has led to considerable diagnostic and economic benefits by reducing the time, the number of embeddings, and consequently the number of sections to be cut and colored.

For its obvious advantages, the use of the kit is strongly recommended.

5.3. Stains

It is sufficient to stain with Haematoxilin & Eosin, possibly associated with an Alcian Blue-PAS, to assess all the necessary morphological elements (one or two sections can be used for immunohistochemical assessment if necessary).

6. Histopathological aspects of normal and pathological duodenal mucosa

6.1. Normal intestinal mucosa

Villi: Digitiform appearance with the ratio between the height of the villi and of the crypts always in favor of the villus (3 : 1 or more).

Enterocytes: Normal height with 29–34 μm clear brush-border.

Intra-epithelial lymphocytic infiltrate: The number of intra-epithelial lymphocytes (T lymphocytes) is subject to individual variability. The majority of normal subjects have less than 20 lymphocytes per 100 epithelial cells; based on the experiences of Hayat [9] and Veress [10], a count of IEL between 25 and 29/100 epithelial cells is considered borderline and pathological over 30/100 epithelial cells.

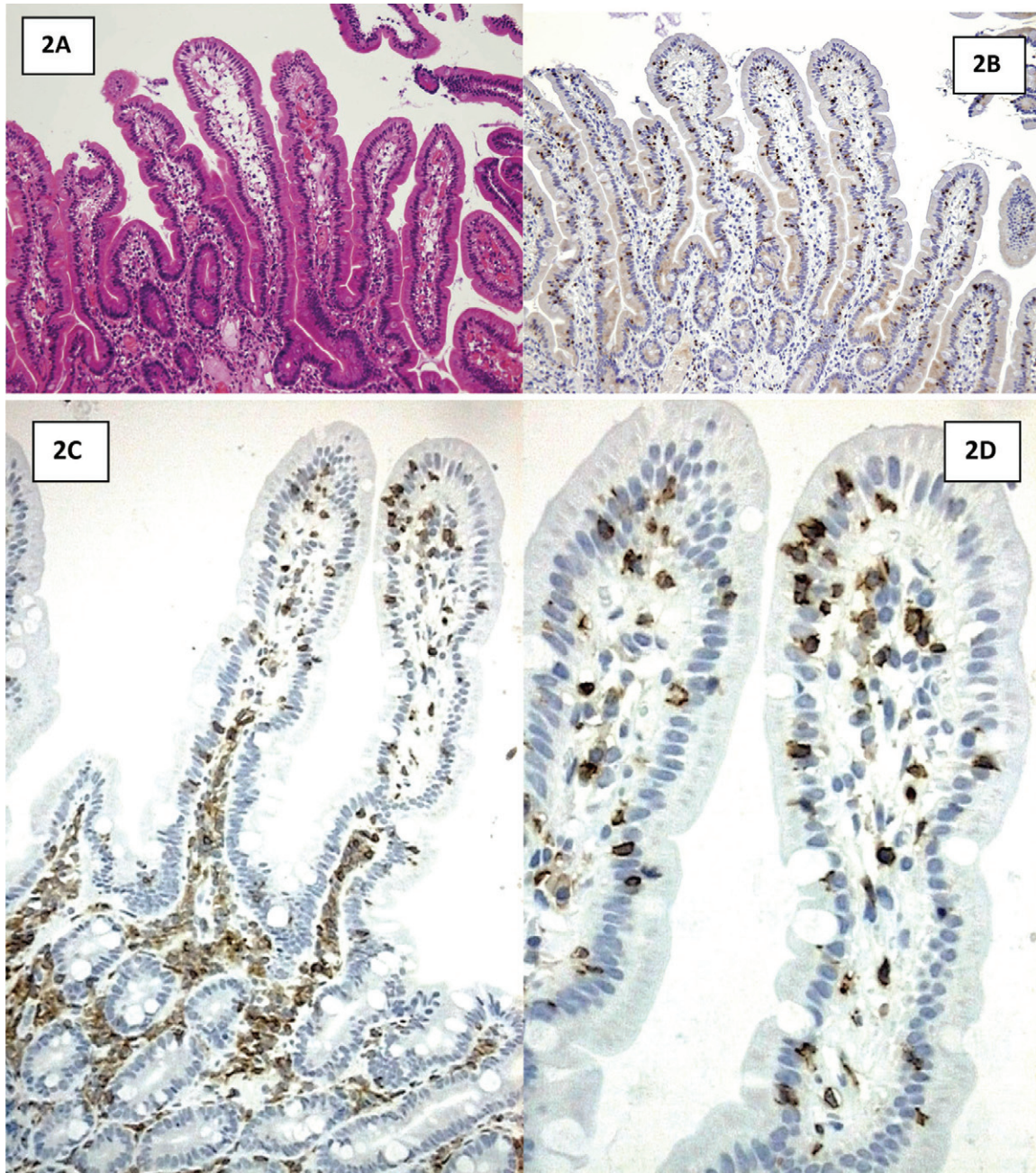


Fig. 2. Normal duodenal mucosa: villus/crypt ratio 3:1. Intraepithelial lymphocytes within the normal range.

The intra-epithelial lymphocyte count is very important and should always be done, especially in the initial lesions, following the indications given below:

- always count the T lymphocytes with the help of immunohistochemical investigations using anti-CD3 antibodies;
- evaluate the biopsies perfectly oriented with a precise alignment of the surface-coating epithelial cells;
- do the count both in the apical portions and along the edges of the villi; it is important to have accurate and reproducible fields. Counts done only on the apical portions have proved unreliable (Fig. 2A–D).

Glandular crypts: The crypts basically have the task of performing a regenerative function, which means it is possible to find evidence of mitosis; the normal range is usually one

mitosis per crypt. Alongside the epithelial cells are endocrine cells, goblet cells and Paneth cells, but these have no value as regards the diagnosis of coeliac disease.

Lamina propria: Plasma cells, eosinophils, histiocytes, mast cells and lymphocytes are normally found in the lamina propria. Neutrophils are generally absent, except in cases of active duodenitis with possible gastric metaplasia closely related to *Helicobacter pylori* infection.

The cellular component mainly consists of plasma cells and lymphocytes, the latter sometimes in the form of lymphoid aggregates and eosinophilic granulocytes whose value must never be greater than 60 for 10 fields of vision examined at 40 \times .

7. Pathological intestinal mucosa

7.1. Basic lesions

The histological diagnosis of CD consists of an integrated assessment of the following elementary lesions:

- *Increased intraepithelial T lymphocytes*: a value between 25 and 29 IEL/100 enterocytes is considered border-line value; >30 IEL/100 enterocytes represents a pathological “lymphocytosis”.
- *Decreased enterocyte height*, flattening of enterocytes, intracytoplasmic vacuolation as well as reduction or absence of brush-border are possible but not specific.
- *Crypt hyperplasia*: extension of the regenerative epithelial crypts associated with changes in the presence of more than 1 mitosis per crypt.
- *Villous atrophy*: decrease in villous height, alteration of normal crypt/villous ratio (3:1) until total disappearance of villi. This assessment requires proper orientation of the biopsies.

None of these elementary lesions of CD is exclusive; the diagnosis of CD is based on the identification of histological lesions accompanied by clinical and serological consistent data. On the basis of the presence of one or more of these elementary lesions the histopathology of CD is subdivided into different diagnostic categories according to the Marsh classification [2].

Marsh classification

Type 1 or infiltrative lesion

1. Villi architecturally within normal morphological limits (normal villa/crypt ratio 3:1);
2. Increased number of intraepithelial lymphocytes (greater than 25–30 per 100 epithelial cells) (Fig. 3A–D).

Type 2 or hyperplastic lesion

1. Villi architecturally within normal morphological limits (like type 1);
2. Increased number of intraepithelial lymphocytes (greater than 25–30 per 100 epithelial cells) (like type 1);
3. Hyperplasia of the glandular elements (regenerative aspect of the glandular elements highlighted by the reduced muciferous activity and increased number of mitoses).

Type 3 or destructive lesion

1. Varying degrees of villous atrophy associated with hyperplasia of glandular crypts;
2. Reduced surface enterocyte height, with irregular brush-border and sometimes cytoplasmic vacuoles;
3. Increased number of intraepithelial lymphocytes (like type 1 and 2 lesions).

The combination of the three factors described above is consistent with a diagnosis of coeliac disease or gluten-sensitive enteropathy, which should be considered as synonyms. These three patterns, albeit schematic, represent the histological lesions seen in coeliac disease and it is important

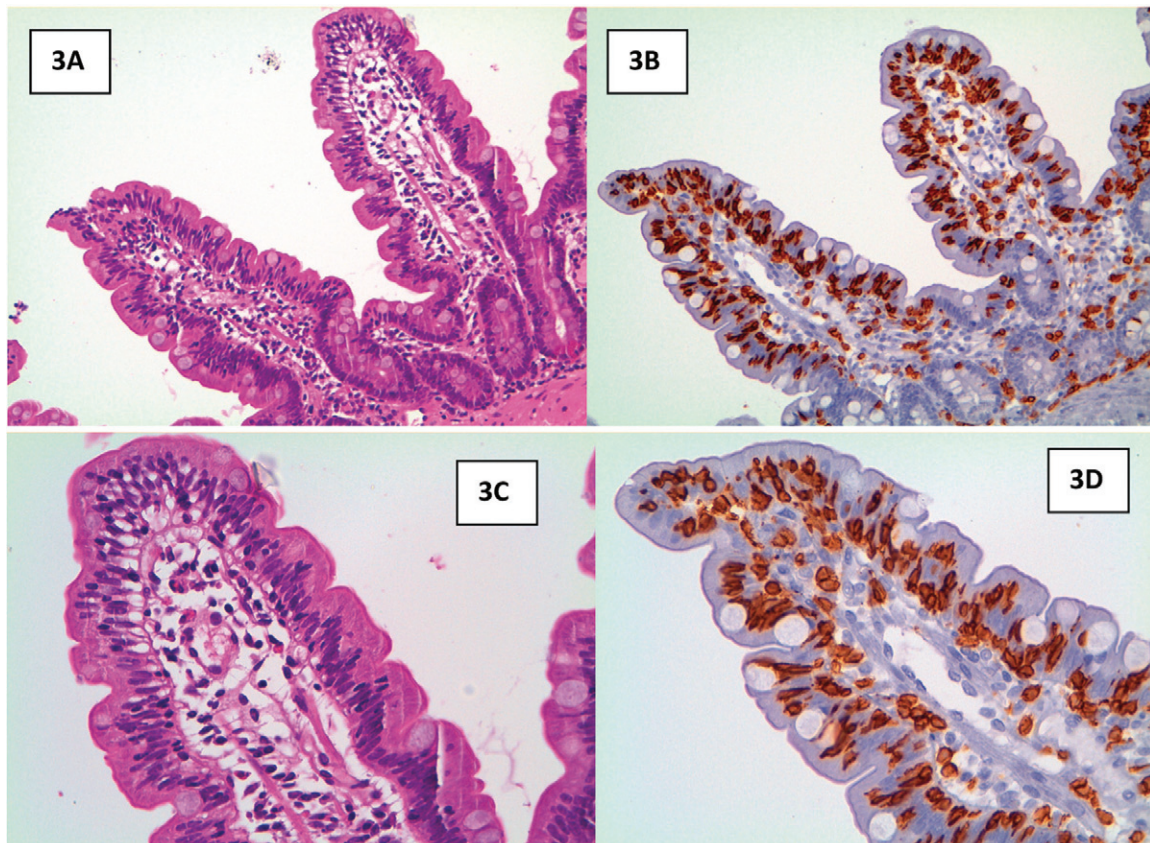


Fig. 3. Type 1/2 infiltrative lesion according to Marsh-Oberhuber. Grade A new classification.

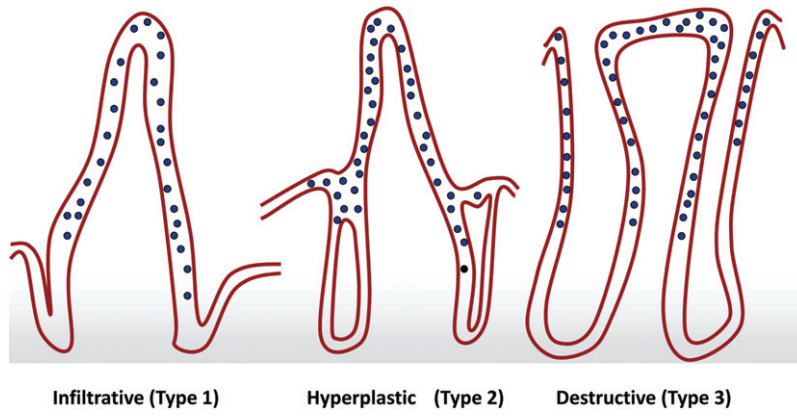


Fig. 4. From Marsh [2], amended.

to consider them as dynamic and progressive both in one direction and the other and not static, since they depend on how much gluten the patient has been exposed to and for how long.

Figure 4 summarizes the above description.

This classification is universally recognized for the diagnosis of coeliac disease, and extensively validated; the only point worthy of observation and critical analysis is that the cases with mild, moderate or severe atrophy (total villous flattening) are all grouped together in a single category: the type 3 lesion.

An amendment to this classification has been proposed by Oberhuber et al. [11], who divided the Marsh type lesion 3 into three subgroups.

- 3a** mild villous atrophy and pathological increase of intraepithelial lymphocytes.
- 3b** moderate villous atrophy and pathological increase of intraepithelial lymphocytes (Fig. 5A, B).
- 3c** total villous atrophy and pathological increase of intraepithelial lymphocytes (Fig. 6A, B).

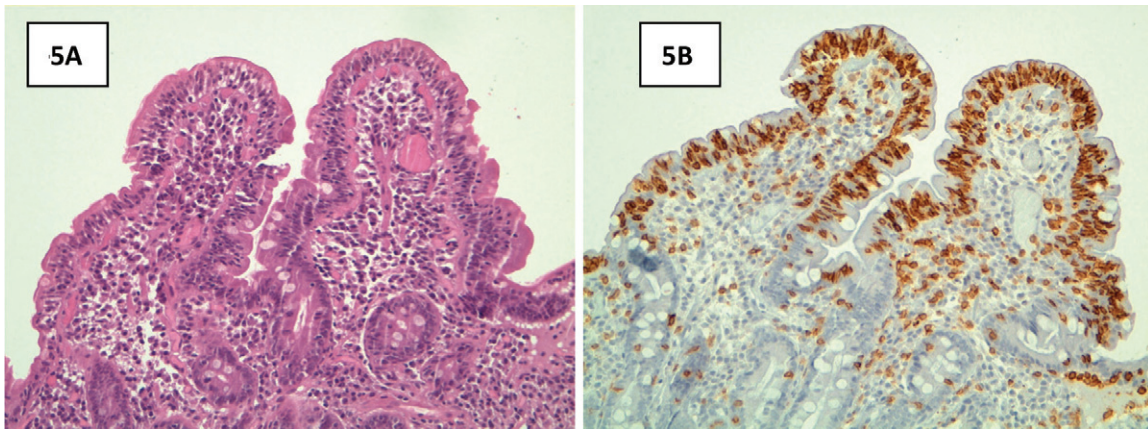


Fig. 5. Type 3a–3b lesion according to Marsh-Oberhuber. Grade B1 new classification.

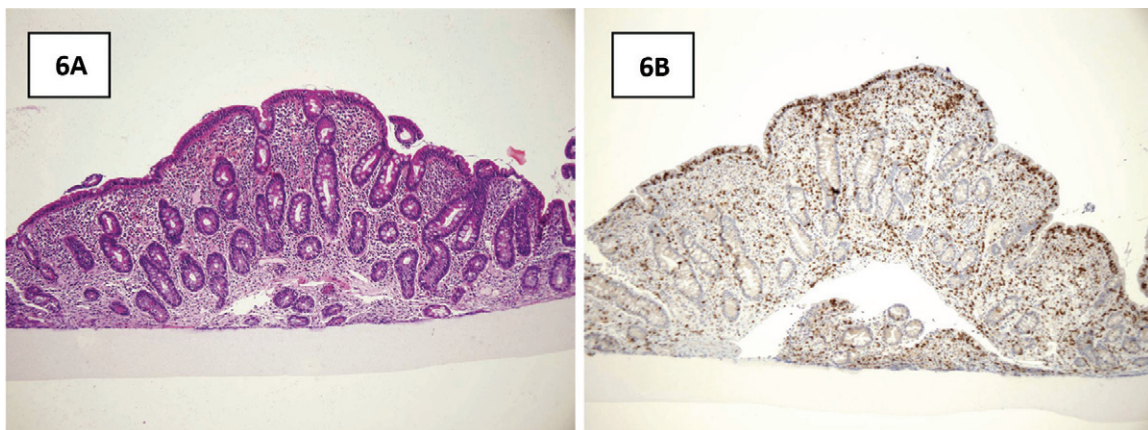


Fig. 6. Type 3c lesion according to Marsh-Oberhuber. Grade B2 new classification.

Without prejudice to all the other morphological criteria described above, this classification provides a better description of the spectrum of lesions that may occur both in coeliac disease patients on a normal diet and in those on a gluten-free diet.

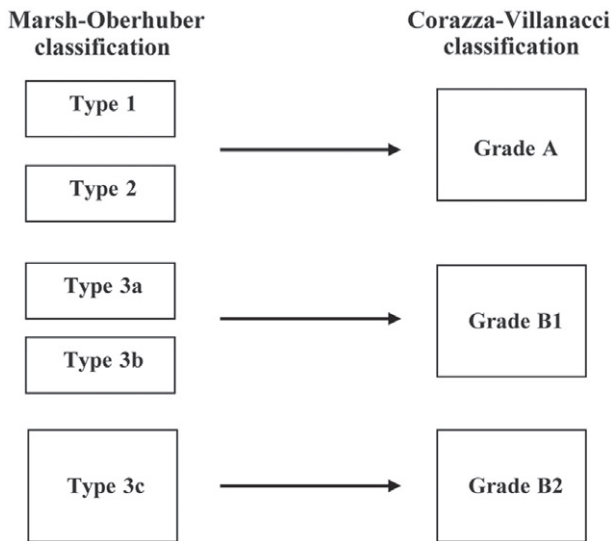
Along the same lines, and in an attempt to simplify and standardize the work of pathologists and facilitate the relationship between pathologists and clinicians, a new version of the histological classification has recently been proposed by Corazza and Villanacci [12,13]; in particular, the lesions that characterize coeliac disease have been divided into two categories: *Non-atrophic (grade A)* and *atrophic (grade B)*.

Grade A lesions are characterized by a pathological increase in intraepithelial lymphocytes, best recognized by the use of immunohistochemical techniques.

Grade B lesions are further subdivided into:

Grade B1 in which the villus/crypt ratio is less than 3:1, with villi still identifiable, and

Grade B2 in which the villi are no longer identifiable:



8. Diagnostic criteria

The diagnosis represents the culmination of what is described above and must comprise all the morphological requirements so as to allow a direct, clear and simple understanding of the morphological situation of the duodenal mucosa by the clinician.

A two-step proposal is presented below:

8.1. Assessment of the morphological pattern divided according to description and diagnosis

The description should report, in sequence, the same morphological elements listed above, namely: villous trophism, number of intraepithelial lymphocytes, features and glandular structures of the lamina propria, concluding with compatibility or non-compatibility with the pattern of a coeliac patient based on complete clinical and laboratory data.

The final diagnosis in the case of an atrophic lesion, culminating in a “consistent” with a CD with atrophic lesions (type 3a, 3b or 3c); in the case of non-atrophic lesions culminating in finding attributable to intraepithelial lymphocytosis, stressing that these injuries are “suggestive for” but not exclusive of CD and should therefore necessarily be placed in the right clinical setting and supported by a serological confirmation.

As a brief addition to the above we propose that the term sub-atrophy, in itself unclear and misleading, should no longer be used. Instead, it is better to specify whether the villi are normal or atrophic, and in the latter case, the degree of atrophy, from mild to moderate to severe. In the event of severe atrophy it is possible to use the term total or severe atrophy. Scores should not be attributed to the individual morphological elements as they are too subjective and of little or no use for the final diagnosis.

8.2. The histology report: checklist

Name _____ Sex M/F _____ Date of Birth ____/____/____
 1st Biopsy _____ No. _____ Control _____ No. _____
 No. of biopsies _____ Oriented _____ Non-Oriented _____
 Villi: normal atrophy _____ mild _____ moderate _____ severe _____
 Villus/crypt ratio: normal [3:1] _____ altered _____
 Intraepithelial lymphocytes: normal _____ increased _____
 (normal: less than 25–30 lymphocytes/100 epithelia;
 increased: more than 25–30 lymphocytes/100 epithelia)
 Evaluation with CD3 _____
 Glands: normal _____ hyperplastic _____
 Lamina propria _____

DIAGNOSIS

| Oberhuber-Marsh: | New grading system: |
|------------------|---------------------|
| Type 1 _____ | Grade A _____ |
| Type 2 _____ | |
| Type 3a _____ | Grade B1 _____ |
| Type 3b _____ | |
| Type 3c _____ | Grade B2 _____ |

Comments: _____

9. Immunohistochemistry

One of the key points in the diagnosis of coeliac disease is the number of intraepithelial lymphocytes, which are CD3 and CD8 positive T lymphocytes; in pathological conditions, their number should be more than 25 lymphocytes per 100 epithelial cells (border-line value 25–30).

The definition of a precise cut-off between normal, abnormal and border-line pathological lesions is of particular importance given the increase in coeliac disease diagnosed in the early/subclinical stage.

The counts can be performed reasonably well on the normal and irreplaceable hematoxylin-eosin but we suggest, especially in the initial forms, that an immunohistochemical assessment should always be carried out with monoclonal

CD3 which often allows for a more accurate display of lymphocytes, following a series of procedures (see the section on intraepithelial lymphocyte infiltration). Evaluation with CD8 may also help, and is particularly useful in cases of elderly subjects where it is possible to find refractory forms which do not respond to diet, regarded by many as pre-lymphomatous and in which the expression of CD8 may be negative with respect to the “norm” [14].

As frozen material is available, immunohistochemical typing for the gamma-delta receptor of T lymphocytes can be carried out; in normal conditions this receptor is not expressed by more than 2–3% of T lymphocytes while in coeliac disease it may reach 20–30% – a particularly useful marker in initial lesions. This assessment is, however, based on the use of frozen material and is not therefore recommended in routine practice.

10. Differential diagnosis

The above summarizes the morphological lesions which may occur with coeliac disease and where the pathologist clearly has a key role, if only to exclude the possibility of clinically suspected malabsorption which may also be:

- Parasitic (*Giardia lamblia*, *Cryptosporidium*, *Microsporidium*)
- Infectious (Whipple’s disease)
- Viral (cytomegalovirus, herpes virus)
- Idiopathic (Crohn’s disease)
- Neoplastic.

The most important problem today in the diagnosis of coeliac disease is represented by early lesions, i.e. normal villi with a pathologic increase in intraepithelial T lymphocytes. This issue is appropriately dealt with in the excellent review by Brown et al. [15], summarized in Table 1.

Table 1 shows that in addition to coeliac disease, there are a number of pathological conditions that have the same morphological aspect as coeliac disease in its early stages, i.e. normal villous architecture but with a pathological increase of

Table 1

Causes of proximal small intestinal intraepithelial lymphocytosis with normal villus architecture*

- Gluten sensitivity[†]
- Non-gluten food hypersensitivity (e.g., cereals, cow’s milk, soy products, fish, rice, and chicken)
- Infections (e.g., viral enteritis, *Giardia*, *Cryptosporidia*, *Helicobacter pylori*)[†]
- Bacterial overgrowth
- Drugs (e.g., NSAIDs)[†]
- Immune dysregulation (e.g., Hashimoto thyroiditis, rheumatoid arthritis, SLE, autoimmune enteropathy)[†]
- Immune deficiency (e.g., IgA deficiency, CVID)
- Inflammatory bowel disease
- Lymphocytic and collagenous colitis

*NSAIDs, non-steroidal anti-inflammatory drugs; SLE, systemic lupus erythematosus; IgA, immunoglobulin A; CVID, common variable immunodeficiency.

[†]Most common associations.

IELs (>25–30/100 epithelial cells) (lesion type 1 according to Marsh, Grade A according to the new proposed classification). These conditions include hypersensitivity to other foods (milk, cereals, soybeans, fish, etc.), infections (*Helicobacter pylori*, *Giardia*, etc.), the use of drugs, immunodeficiencies and immunodysregulation (Hashimoto thyroiditis, systemic lupus erythematosus, rheumatoid arthritis, etc.) and, not least, chronic idiopathic inflammatory bowel colitis or colitis with a different etiology, such as lymphocytic and collagenous colitis.

The question that we must therefore ask is: How can we discriminate between different pathological conditions where the morphology is essentially superimposable? A proper clinical evaluation based on histological and laboratory data is crucial. We must not forget that a diagnosis of coeliac disease is a “marker” which remains throughout life with obvious therapeutic and behavioral relapses. The table below helps understand how important the need for collaboration between the pathologist and endoscopist is in the detection of other conditions, such as infection with *Giardia lamblia* or other parasites, the possibility of presentations of immunodeficiencies morphologically superimposable on coeliac disease and not least the localization of Crohn’s disease or particular forms of enteritis within the sphere of untreatable diarrhea, such as autoimmune enteritis, tufting enteropathy, a disease caused by atrophy of the microvilli, and cases of graft-versus-host disease, all conditions in which the morphological element is fundamental.

Three conditions, however, deserve special mention:

- Forms of so-called “autoimmune enteritis” possible in children with immunological deficiency (common variable immunodeficiency, X linked agammaglobulinemia) in which the intestinal biopsy may be fully comparable to the pattern of coeliac disease [16].
- Damage by drugs: there is increasing evidence in the literature showing that the use of drugs, especially non-steroidal anti-inflammatory drugs (NSAID), are capable of causing morphological alterations identical to those of coeliac disease, and it is therefore important to keep this possibility in mind in cases of elderly patients, especially when the serological markers are all negative [15].
- The possibility that concurrent infection with *Helicobacter pylori* in the stomach can produce a morphological pattern very similar to that of initial lesions of coeliac disease as recently reported [17].

11. Complications that can be confirmed histologically

Unlike what occurs in children, there is considerable evidence that coeliac disease in adults, especially if diagnosed late and even more so if not dealt with by a timely and rigorous gluten-free diet, is burdened by a higher mortality rate than in the general population.

The removal of gluten from the diet therefore determines not only an improvement of the histological and clinical aspects, but also prevents the complications which must

always be suspected if an adult patient continues to be unwell, despite the diet.

These complications are due to:

- *Collagenous sprue*: The patient does not respond to diet and histology shows fibrous tissue in the intestinal wall at the level of the superficial subepithelial layer. This morphological pattern is very similar to the condition of collagenous colitis described in the colon, where the thickness of the connective band best highlighted with Masson's trichrome is more than 15 millimicrons, although this is a very rare event is described in the literature.
- *Refractory sprue*: This condition reproduces the same clinical picture as collagenous sprue but can be identified by immunohistochemical staining, demonstrating that T lymphocytes, which in normal conditions express CD3 and CD8, in this case present only the expression of CD3 and not of CD8 [14].
- *Ulcerative jejunoileitis*: Presence of extensive ulceration of the intestinal mucosa, often related to refractory sprue.
- *Lymphoma*: This is the most serious complication and should always be suspected when histology shows a prevalence of atypical monomorphous lymphocytic elements. In these cases it is useful to carry out immunophenotyping of the lymphoid population, which is almost always type T [18–20].

12. Summary

12.1. What are the “certainties” in the diagnosis of coeliac disease?

An obvious prerequisite for certainty in the diagnosis of coeliac disease (CD) from the anatomic-pathological point of view is the observation and respect of a number of key points:

1. Close collaboration between clinicians, laboratory technicians and endoscopists.
 2. An adequate number of biopsies (at least 4, 2 in the distal and 2 in the proximal duodenum).
 3. Correct orientation of the biopsy (the use of pre-cut cellulose acetate filters).
 4. Sufficient clinical information.
 5. Excellent quality of the biopsy samples.
- With these details it is clear that “certainty” in the diagnosis of CD is only possible if the villous atrophy is associated with a pathological increase in the number of intraepithelial lymphocytes (value exceeding 25–30/100 epithelial cells). In this situation, by applying the three classifications now known and validated (Marsh, Marsh-Oberhuber and Corazza-Villanacci) there is no problem in the diagnosis and comparison with clinical and laboratory data.
 - The degree of atrophy should be certain and not merely pseudo-atrophy due to incorrect orientation and cutting of the villi. Assessment of the number of intraepithelial

lymphocytes is useful in these cases and “must” always be pathological (>25–30/100 epithelial cells), best evaluated both with H&E staining and with immunohistochemistry staining for CD3.

- Attention should be paid to biopsies taken from the duodenal bulb, where the presence of Brunner glands can lead to a false diagnosis of atrophy; biopsies of the bulb should always be compared with those taken from the distal portions, especially in the early stages of the disease, which has a progression of the pathological process in a cranio-caudal direction.
- If there are varying degrees of atrophy, these should all be described, not just the most severe degree. An assessment of compatibility should only be included in the description of the case, while the term “coeliac disease” should be avoided in the final diagnosis, which should be limited to a description, giving the clinician a precise “snapshot” of the state of the duodenal mucosa. The final diagnosis of CD should be made solely and exclusively by the pediatric or adult clinical gastroenterologist.

12.2. What are the “doubts” in the diagnosis of coeliac disease?

The points that cause doubt and require caution on the part of the pathologist in the diagnosis of CD are clearly represented by the cases in which there are initial lesions (Marsh 1–2, and Grade A in accordance with the new proposed classification); in these cases it is necessary to:

1. Carefully assess the orientation of the biopsies.
 2. Consider whether the villus/crypt ratio of at least 3:1 is respected.
 3. Carefully count the number of lymphocytes in the surface coating epithelium.
 4. Always carry out additional immunohistochemical evaluation with CD3.
 5. Compare the clinical and laboratory data.
- The two key elements that must be assessed are the absence of atrophy and the increase in the number of intra-epithelial lymphocytes; it is therefore crucial to always associate immunohistochemical evaluation with CD3. The presence or absence of hyperplasia of the glandular elements is totally irrelevant for practical and therapeutic purposes.
 - Do not forget that the “slide” is proof of the assessment by the pathologist and as such can be compared and re-assessed by other colleagues and specialists; it must also be strongly emphasized that the histological assessment must be conducted solely by the pathologist and not by other “specialists”.
 - As with “certain” cases, it is even more important in doubtful cases to merely express an opinion of possible compatibility with CD in the description, describing only the histological aspects in the final diagnosis.
 - Exclude, if possible, a concurrent infection due to *Helicobacter pylori* (it is advisable to always take biopsies of

the antral and oxyntic gastric mucosa), immunodeficiencies, parasitic infections, allergies to other dietary factors and use of drugs.

- In doubtful situations in which the final pattern is unclear, it is useful to bear in mind the excellent review by Brown et al.: the pathologist must be sure that he is faced with a pathological condition unequivocally demonstrated by the increase in the number of intraepithelial T lymphocytes confirmed by the evaluation with CD3. The final diagnosis will be based on comparison of the clinical and laboratory data.
- In pediatric patients in the first year of life, the possibility of intolerance to cow's milk proteins should not be forgotten; in such cases the eosinophilic granulocyte count may be useful (pathologic value above 60 for 10 fields of vision at 40×).

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Conflict of interest

The authors have no conflict of interest to report.

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