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Review

Celiac disease: histology-differential diagnosis-complications. A practical approach

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Summary

Celiac disease is a multi-factorial chronic inflammatory intestinal disease, characterized by malabsorption resulting from mucosal injury after ingestion of wheat gluten or related rye and barley proteins. Inappropriate T-cell-mediated immune response against ingested gluten in genetically predisposed people, leads to characteristic histological lesions, as villous atrophy and intraepithelial lymphocytosis. Nevertheless, celiac disease is a comprehensive diagnosis with clinical, serological and genetic characteristics integrated with histological features. Biopsy of duodenal mucosa remains the gold standard in the diagnosis of celiac disease with the recognition of the spectrum of histological changes and classification of mucosa damage based on updated Corazza-Villanacci system. Appropriate differential diagnosis evaluation and clinical context also for the diagnosis of complications is, moreover, needed for correct histological features interpretation and clinical management.

Key words: celiac disease, sprue, small bowel, gluten.

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Introduction

Celiac disease (CD) is an immune-mediated inflammatory disorder of the small intestine occurring in genetically predisposed individuals when exposed to gluten [1]. CD can occur at any age, from early childhood to elderly, with two peaks of onset, one shortly after weaning with gluten in the first 2 years of life and the other during the second or third decade of life with a preference for females (male/female ratio 1:2). The disease has a variable incidence, with a worldwide prevalence of about 1:100; in Europe is estimated between 0.3 and 1.2% [2,3]. A correct diagnosis of CD 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. Nonetheless, CD still represents an under-recognized condition, due to heterogeneous symptoms and/or poor disease awareness, and the occurrence of diagnostic delay ranging from 4 to 13 years has been reported by some authors [4-9].

Clinical and laboratory aspects

The diagnosis of CD can be very challenging, since symptoms can significantly vary from patient to patient and this variability has been compared, not surprisingly, to a chameleon [10]. In 2011, the Oslo Classification ranked the clinical presentation of CD in classical, non classical, subclinical and refractory [11]. The gold standard for CD diagnosis is represented by the combination of both mucosal changes and positivity of serological tests [12,13].

Serologic markers

A major role in the diagnostic process of CD is played by serology, which allows identification of the subjects who should undergo intestinal biopsy; the following are the most important tests and their relative significance:

- **IgA class antitransglutaminase antibodies (tTGA)** are the tests with the highest sensitivity for CD (98%) with specificity estimated at around 90%. High titles of IgA class tTGA (> 5 times the cut-off) are almost always the expression of CD;
- **IgA class antiendomysial antibodies (EMA)**, this test has a lower sensitivity compared to IgA class tTGA (90% vs. 98%), but shows an almost absolute specificity for CD;
- **IgA class antigliadin 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); since they are the first antibodies to appear, they show a higher sensitivity than other tests in this age group. 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 the response is indicative for CD.

Genetic testing

CD is closely associated with histocompatibility antigens (HLA) DQ2 and DQ8. Practically all CD patients 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.
The genetic test should be performed in cases where there is a discrepancy between serology and histology and in 1st degree relatives to assess the genetic predisposition to CD.

The main clinical significance of genetic testing is to exclude a diagnosis of CD in the absence of HLA-DQ2 (and its fractions) and -DQ8 in cases of diagnostic doubt and predisposition to CD in family members of coeliac patients in the absence of HLADQ2 (and fractions) and -DQ8 [14,15].

Approach to duodenal biopsy

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. The following are some points which require a close working relationship between the endoscopist, the endoscopy-room nurse, the pathology laboratory technician and the pathologist [16].

Site of the biopsy

Biopsy by endoscopy should be performed in the bulb (in particular this site in children) and second duodenal portion; the recommendation in this field are at least 4 biopsies, 2 for each of sites mentioned above [17-19].

Orientation of the biopsy sample

Biopsy orientation is a crucial event for a proper histological assessment. We suggest the use of acetate cellulose filters previously cut and in this setting it is fundamental a strict relationship between endoscopist-nurse-technicians and pathologist. The endoscopist can place each mucosal sample in a straight line and with proximal-to-distal orientation onto the cellulose acetate filter with a “clarinet beak-shaped cut” (Bio-Optica). Such a filter allows a perfect adherence of biopsies and, being chemically inert and not reactive with fixative or processing chemicals, it does not alter the quality of histological sections at all. As the embedding phase starts, the filters are 90 degrees-rotated by technician, in order to ensure the best trans-sectional cut. The above described procedure is time- and money-sparing, allowing a significant improvement of diagnostic accuracy.

Stains

The “old mistress” haematoxylin-eosin is sufficient to assess all the necessary morphological elements

(one or two sections can be used, if needed, for immunohistochemical assessment, generally for CD3 immunostain which is useful for a correct count of T lymphocytes) [20].

Histopathological aspects of normal and pathological duodenal mucosa

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).

Intra-epithelial lymphocytic infiltrate: the number of intraepithelial lymphocytes (T lymphocytes; IEL) is subject to individual variability. The majority of normal subjects have less than 25 lymphocytes per 100 epithelial cells; based on the experiences of Hayat and Veress [21,22] a count of IEL over 25/100 epithelial cells is considered pathological. The intraepithelial lymphocyte count is very important and should always be done, especially in the initial lesions, using anti-CD3 antibodies.

Glandular crypts: the crypts are comprehensive of epithelial cells, endocrine cells, goblet cells and Paneth cells; mitosis are in general 1 for any crypt.

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; eosinophils must never be more than 5 per field at 40× [20].

Pathological intestinal mucosa

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

- **Increased intraepithelial T-lymphocytes:** a value of 25 T-lymphocytes/100 enterocytes is considered a pathological condition also called "lymphocytosis".
- **Crypt hyperplasia**: extension of the regenerative epithelial crypts associated with 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 is specific for CD; the diagnosis of CD is based on the identification of histological alterations 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 [23].

Type 1 or infiltrative lesion

1 Villi within normal morphological limits (normal villous/crypt ratio 3:1);
2 increased number of IEL (greater than 25/100 epithelial cells).

Type 2 or hyperplastic lesion

1 Villi architecturally within normal morphological limits (like type 1);
2 increased number of IEL (greater than 25/100 epithelial cells) (like type 1);
3 hyperplasia of the glandular elements (regenerative aspects highlighted by the reduced mucinous activity and increased number of mitoses).

Type 3 or destructive lesion

1 Varying degrees of villous atrophy associated with hyperplasia of glandular crypts;
2 surface enterocytes with reduced height, irregular brush border and sometimes cytoplasmic vacuoles;
3 increased number of IEL (like type 1 and 2 lesions).

A combination of the three factors described above with adeguate clinical informations (i.e. anamnesis and serological/genetic data) is consistent with a CD diagnosis. This classification is universally recognized for the diagnosis of CD, and extensively validated; the only point worthy of observation and critical analysis is that mild, moderate or severe atrophy (total villous flattening) are all grouped together in a single category: the type 3 lesion.

A modification to this classification has been proposed by Oberhuber et al. [24] who divided the Marsh type 3 lesion into three subgroups:

3a mild villous atrophy and pathological increase of IEL;
3b moderate villous atrophy and pathological increase of IEL;
3c total villous atrophy and pathological increase of IEL.

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 [25,26]; in particular, the lesions that characterize CD have been divided into two categories: **non-atrophic (grade A) and atrophic (grade B).**

Grade A lesions are characterized by normal villi but with a pathological increase in IEL.

Grade B lesions are further subdivided into:

Table I. Comparison of Marsh - Corazza-Villanacci - Villanacci classification schemes.

Marsh mod. Oberhuber		Corazza-Villanacci	Villanacci
Lesions	**Diagnostic Criteria**	**Lesions**	**Lesions**
Type I lesion *infiltrative*	No architectural changes **(villous/cript ratio preserved)** Increased IELs count **(> 25/100 epithelial cells)**	**Grade A lesion not atrophic** No architectural changes **(villous/cript ratio preserved)** Increased IELs count **(> 25/100 epithelial cells)**	**A Non atrophic type** No architectural changes **(villous/cript ratio preserved)** Increased IELs count **(> 25/100 epithelial cells)**
Type II lesion *hyperplastic*	No architectural changes **(villous/cript ratio preserved)** Crypt hyperplasia **(mitoses > 1/crypt)** Increased IELs count **(> 25/100 epithelial cells)**		
Type III A lesion *destructive*	Villous atrophy **(mild degree)** Crypt hyperplasia **(mitoses > 1/crypt)** Increased IELs count **(> 25/100 epithelial cells)**	**Grade B1 lesion *partial atrophy*** Villous atrophy **(mild-moderate degree)** Crypt hyperplasia **(mitoses > 1/crypt)** Increased IELs count **(> 25/100 epithelial cells)**	**B Atrophic type** Villous atrophy **(mild-moderate-severe degree)** Crypt hyperplasia **(mitoses > 1/crypt)** Increased IELs count **(> 25/100 epithelial cells)**
Type III B lesion *destructive*	Villous atrophy **(moderate degree)** Crypt hyperplasia **(mitoses > 1/crypt)** Increased IELs count **(> 25/100 epithelial cells)**		
Type III C lesion *destructive*	Villous atrophy **(severe degree)** Crypt hyperplasia **(mitoses > 1/crypt)** Increased IELs count **(> 25/100 epithelial cells)**	**Grade B2 lesion *total atrophy*** Villous atrophy **(severe degree)** Crypt hyperplasia **(mitoses > 1/crypt)** Increased IELs count **(> 25/100 epithelial cells**	

Grade B1 in which the villus/crypt ratio is less than 3:1 and pathological increase of IEL is present;
Grade B2 in which the villi are no longer identifiable and pathological increase of IEL is present.
Recently a simplified classification with only two entities was proposed [27] (Tab. I).

Immunohistochemistry

One of the key points in the CD diagnosis is the number of IEL, which are CD3 and CD8 positive T lymphocytes; in pathological conditions, their number should be more than 25 lymphocytes per 100 epithelial cells. 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 antibodies which often allows for a more accurate evaluation of T lymphocytes (Fig. 1) [28-31].

Non celiac gluten sensitivity (NCGS)

Non-celiac gluten sensitivity (NCGS) is "**a clinical entity induced by the ingestion of gluten leading to intestinal and/or extraintestinal symptoms that improve once the gluten-containing foodstuff is removed from the diet, and CD and wheat allergy have been excluded**" [32]. The histologic characteristics of NCGS are still under investigation, ranging from normal histology to a slight increase in the number of T lymphocytes in the superficial epithelium of villi. Some authors described a normal number of T lymphocytes but a peculiar disposition of this cells in small "cluster" of 3-4 elements in the superficial epithelium, as well as the linear disposition in the deeper part of the mucosa together with an increased number of eosinophils (> 5/HPF) in lamina propria. Further studies are needed to assess these findings as specific for NCGS [33] (Fig. 2).

Differential diagnosis

CD shares its duodenal histopathologic features with a large variety of intestinal disorders. Thus, a correct diagnosis may be reached only by an integrated evaluation of clinical manifestations, laboratory and HLA tests and endoscopic findings, as well as histopathologic findings. Main conditions with histologic changes that can overlap with CD and some tips for differential diagnosis are summarized in Table II.
Common entities characterized by intraepithelial lymphocytosis without villous atrophy include several

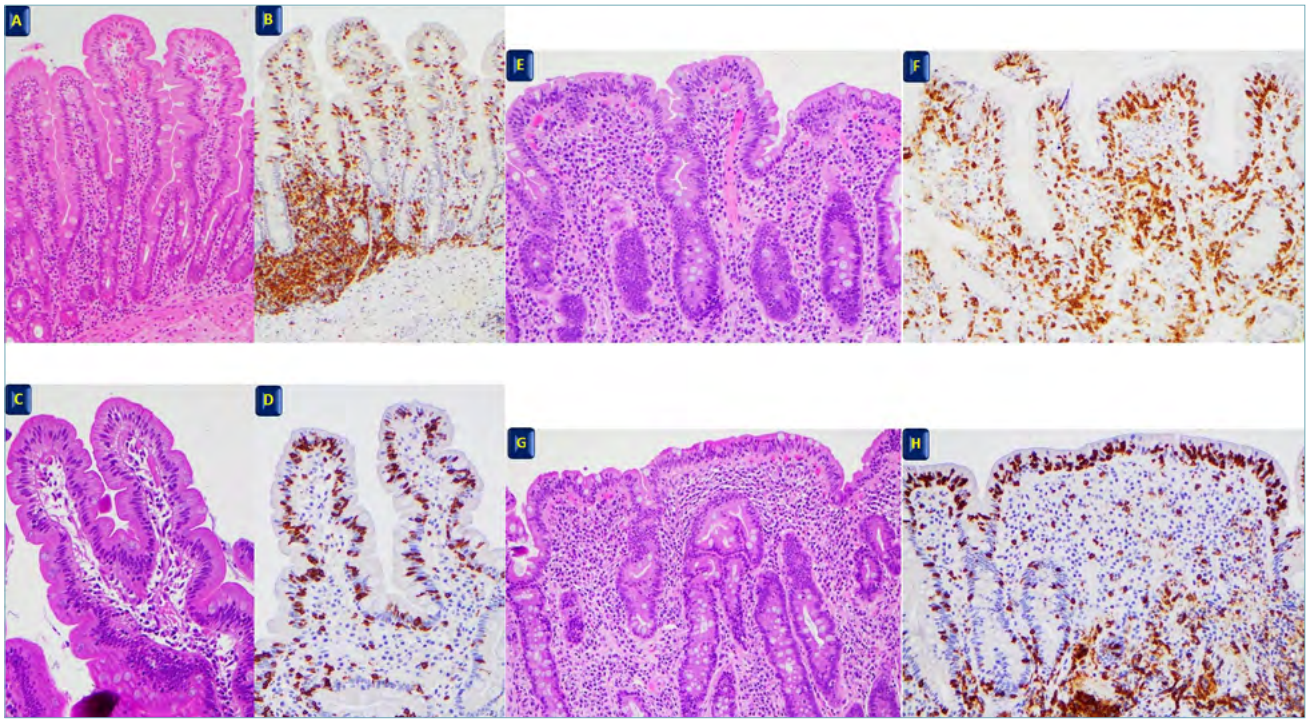

Figure 1. (A-B): normal duodenal mucosa; villous/crypt ratio over 3:1; number of T lymphocytes < 25 x 100 epithelial cells. (A) H&E x 10, (B) CD3 immunostain x 10. (C-D): **Type 1 - Grade A lesion**; normal villi but with pathological increase of T lymhocytes > 25 x 100 epithelail cells. (C) H&E x 20, (D) CD3 immunostain x 20. (E-F): mild to moderate villous atrophy **Type 3A-3B** - **Grade B1** with pathological increase of T lymphocytes. (E) H&E x 20, (F) CD3 immunostain x 20. (G-H): severe villous atrophy **Type 3C - Grade B2** with pathological increase of T lymphocytes**.** (G) H&E x 20, (H) CD3 immunostain x 20.

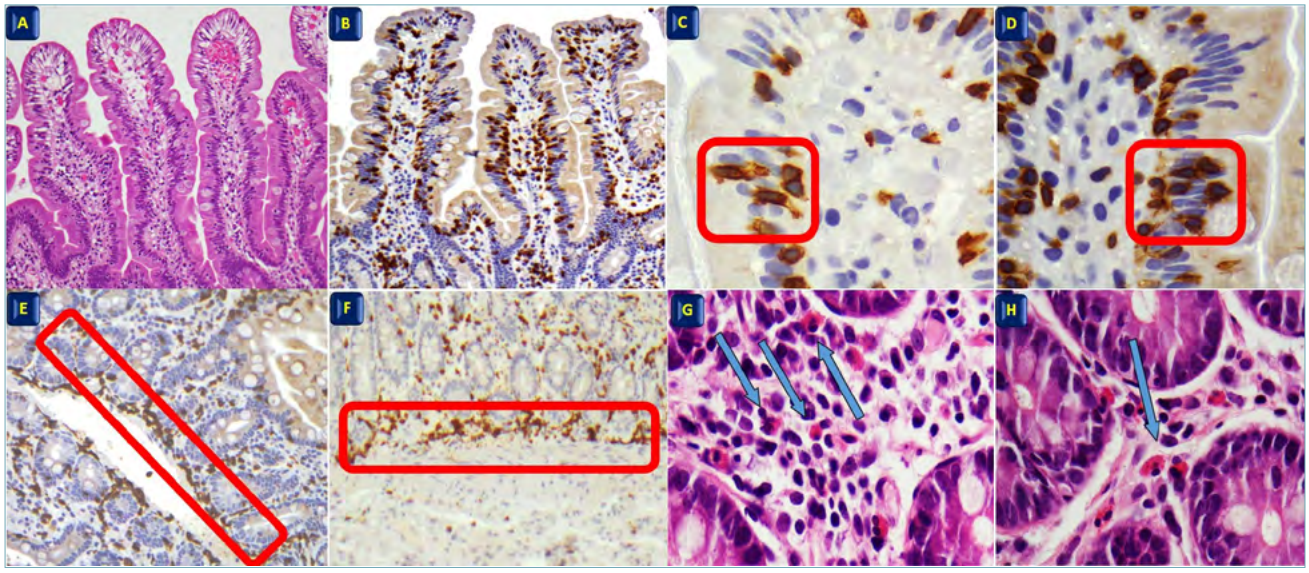

Figure 2. (A-B): normal villi; T lymphocytes < 25 x 100 epithelial cells. (A) H&E x 10, (B) CD3 immunostain x 10. (C-D): cluster of T Lymphocytes in the superficial epithelium. (C-D) CD3 immunostain x 60 red rectangle. (E-F): linear disposition of T lymphocytes in the deeper part of the mucosa. CD3 immunostain x 4 red rectangle. (G-H): eosinophils in lamina propria.

Table II. Major CD non-neoplastic mimickers and histopathologic features useful for differential diagnosis.

Mimicker	Increased IELs	Villous atrophy	Histopathologic tips for differential diagnosis
Infectious diseases			
Parasitic infestation	Rare (in children)	Rare (in children)	Identification of parasites (e.g. *Giardia)*; increased eosinophils in lamina propria
HP-positive gastritis and peptic duodenitis	Possible	Possible, mild (if present)	Foveolar metaplasia of the duodenum; increased plasma cells in lamina propria; neutrophilic infiltration in lamina propria and epithelium; changes more prominent in the bulbus; *HP* in gastric biopsies
Tropical sprue	Yes	Yes, usually low-grade	Extensive ileal involvement
Bacterial overgrowth	Yes	Possible	Mild lesions
Whipple disease	Rare	Yes	PAS-positive macrophages in lamina propria
Viral gastroenteritis or post-infectious changes	Yes	Possible, variable grade	Mucosal recovery after infection resolution
Drugs			
NSAIDs	Possible	Rare, patchy, mild	Erosions, neutrophilic infiltration in lamina propria
Antineoplastic and immune modulatory drugs (including immune checkpoint inhibitors)	Rare	Possible	Crypt architectural distortion; neutrophilic infiltration in lamina propria; foci of crypt apoptosis; involvement of other gastrointestinal tracts (gastritis, colitis)
ARBs use (Olmesartan and others)	Possible	Frequent, variable grade	Neutrophilic infiltration in lamina propria; deposition of subepithelial collagen, foci of crypt apoptosis
Other immune-inflammatory conditions			
Collagenous sprue	Yes	Frequent, variable grade	Deposition of subepithelial collagen
Immunodeficiencies (including CVID)	Yes	Possible, variable grade	Depletion of plasma cells in lamina propria, follicular lymphoid hyperplasia; concomitant giardiasis
Autoimmune enteropathy	Possible (celiac pattern)	Yes, variable grade	Neutrophilic infiltration in lamina propria; crypt apoptosis; reduction in goblet and Paneth cells; diffuse involvement of other gastrointestinal tracts (gastritis, enteritis, colitis)
Crohn's disease and ulcerative colitis-associated duodenitis	Rare	Rare, patchy (if present)	Erosions/ulcerations, neutrophilic inflammation; crypt distortion; microgranulomas; basal plasmacytosis; ileal and colonic involvement
Eosinophilic gastroenteritis and food protein-sensitive enteropathies (including gluten-sensitive enteropathy)	Possible	Possible, usually not severe	Increased eosinophils in lamina propria; involvement of other gastrointestinal tracts (enteritis and colitis)

Legend: ARB: angiotension receptor blocker; CVID: common variable immunodeficiencies; HP: Helicobacter pylori; IEL: intraepithelial lymphocyte; NSAID: non-steroidal anti-inflammatory drug; PAS: periodic acid Schiff.

medications, *Helicobacter pylori* gastritis, duodenal parasitic infestations, and autoimmune conditions. Potential confounders which typically cause villous atrophy comprise olmesartan and other angiotensin receptor blockers, various immunomodulatory drugs, common variable immunodeficiency, autoimmune enteropathy, Whipple disease and tropical sprue [1,34].

Several **medications**, including nonsteroidal anti-inflammatory drugs (NSAIDs), immunomodulatory and antineoplastic drugs, can mimic CD histologically; however, villous atrophy is seldom described in these cases. In addition, use of NSAIDs has been reported to usually cause mucosal erosions/ulcerations with inflammatory infiltrate composed with plasma cells and neutrophils [35], while crypt architectural distortion, neutrophilic infiltration, ischemic changes, villous blunting, epithelial cell apoptosis in crypts and neutrophilic cryptitis were occasionally described in individuals treated with checkpoint inhibitors or kinase inhibitors [36,37]. Olmesartan, an angiotensin II receptor blocker, was proved to cause partial to complete villous atrophy and increased IEL, thus mimicking CD histology [38].

Helicobacter pylori (HP) infection may determinate an epithelial lymphocytosis generally with minimal villous changes. Foveolar gastric metaplasia and marked neutrophilic infiltration in epithelium and/or lamina propria may help in distinguishing HP-related **peptic duodenitis** from microscopic alterations of CD [39]. Although intestinal **parasitic organisms** may show every CD histopathologic hallmark, a high number of eosinophils in lamina propria should prompt the pathologist to search for the presence of parasites. Giardiasis, caused by *Giardia lamblia*, is one of the most common intestinal parasitic disease. Giardia can be easily identified in duodenal biopsy samples as a pear-shaped organism with two paired nuclei, located in lumen, adjacent to the epithelium. It usually does not determine significant histologic lesions, even if villous blunting, intraepithelial lymphocytosis and/or crypt hyperplasia are rarely observed in children [40]. Nevertheless, the presence of villous atrophy in association with signs of parasitic infections should hint the possibility of an underlying CD [41].

Food protein-sensitive enteropathies can also reproduce CD histologic abnormalities, but they tend to be transient or to respond to dietary allergen withdrawal. In duodenal biopsies from patients with **pernicious anemia**, partial villous blunting and mucosal chronic inflammatory infiltration may be detected, along with the more typical epithelial megaloblastic changes [42].

Collagenous sprue is a rare malabsorption condition which is often misdiagnosed as CD; however, the identification of a thick subepithelial collagen type I band with inflammatory cells and capillaries entrapped may lead to a correct diagnosis [43,44]. A significant, albeit variable, fraction of cases is associated with CD and may be treated with combinations of a gluten-free diet and immunosuppressive therapy.

Common variable immunodeficiency (CVID) enteropathy may mimic CD. Nevertheless, two distinguishing features, usually absent in CD individuals, may be found in duodenal samples of CVID patients: depletion in plasma cells (present in about two-third of cases) and follicular lymphoid hyperplasia [45]. Furthermore, pathologists should always search for a coexisting *Giardia lamblia* infection, as it was reported in 23% of cases by Malamut et al. [46]. In a minority of CVID patients, villous atrophy is gluten-sensitive [47].

Autoimmune enteropathy, a disease characterized by small intestinal mucosal atrophy and circulating autoantibodies towards enterocytes and/or goblet cells, may show an active enteritis pattern, characterized by expansion of the lamina propria by mixed inflammation with neutrophil infiltrates, or a CD-like pattern [48-50]. Foci of apoptotic epithelial cells and reduction in goblet and Paneth cells may rarely be observed. Importantly, biopsies from other gastrointestinal sites often show histologic abnormalities and may aid for diagnosis.

Lastly, it should be remembered that forms of **idiopathic villous atrophy** (villous atrophy or sprue of unknown aetiology) may cause diagnostic challenges. Some of these patients have spontaneous histological recovery and are associated with excellent survival, whereas others show persistent villous atrophy, with or without associated lymphoproliferative disorders [51].

Complications of CD

Refractory celiac disease (RCD). RCD a condition characterized by prolonged villous atrophy in duodenal biopsies of a CD patient, along with malabsorption symptoms, despite a strict adherence to gluten-free diet (GFD) over a minimum period of 12 months [52,53]. Other causes of persistent villous atrophy or slow-to-respond CD must be carefully excluded before making a diagnosis of RCD. Endoscopic abnormalities such as mucosal erosions, ulcerations (ulcerative duodeno-jejunitis) or strictures may be observed.

It is a rare CD complication with a variable incidence and prevalence. A systematic review by Rowinski and Christensen[54] showed a cumulative incidence of 1-4% over 10 years and a prevalence of 0.31%-0.38% in CD patients, while a study based on a cohort of celiac individuals in Austria reported an incidence over 25 years of 2.6% [55]. Globally, RCD incidence seems to be decreasing during the last 20 years, probably because of increased awareness, stricter adherence to GFD and greater availability of gluten-free products [55,56]. Mean age at RCD diagnosis has been reported to be abound 63 years. Generally, the median time between the diagnosis of CD and the diagnosis of RCD is 21 months, although rare cases of RCD primarily diagnosed at the time of first presentation of malabsorption symptoms have been described [57].

Two types of RCD have been recognized on the bases of their clinical, histologic and molecular features. Type I RCD is characterized by an usual immunophenotype of IEL (i.e. retained expression of surface CD3, CD8 and CD103) and absence of a monoclonal T cell receptor (TCR) gene rearrangement, whereas type II RCD is marked by an aberrant intraepithelial lymphocyte immunophenotype (i.e. > 50% of intraepithelial T cells lacking CD8 by immunohistochemistry on formalin-fixed paraffin-embedded sections and/or >20-25% CD45+ T cells lacking surface CD3 on flow cytometry), and a monoclonal TCR gene rearrangement [54]. TCR gene rearrangement clonal analysis by multiple polymerase chain reaction may be efficiently performed also on formalin-fixed paraffin-embedded

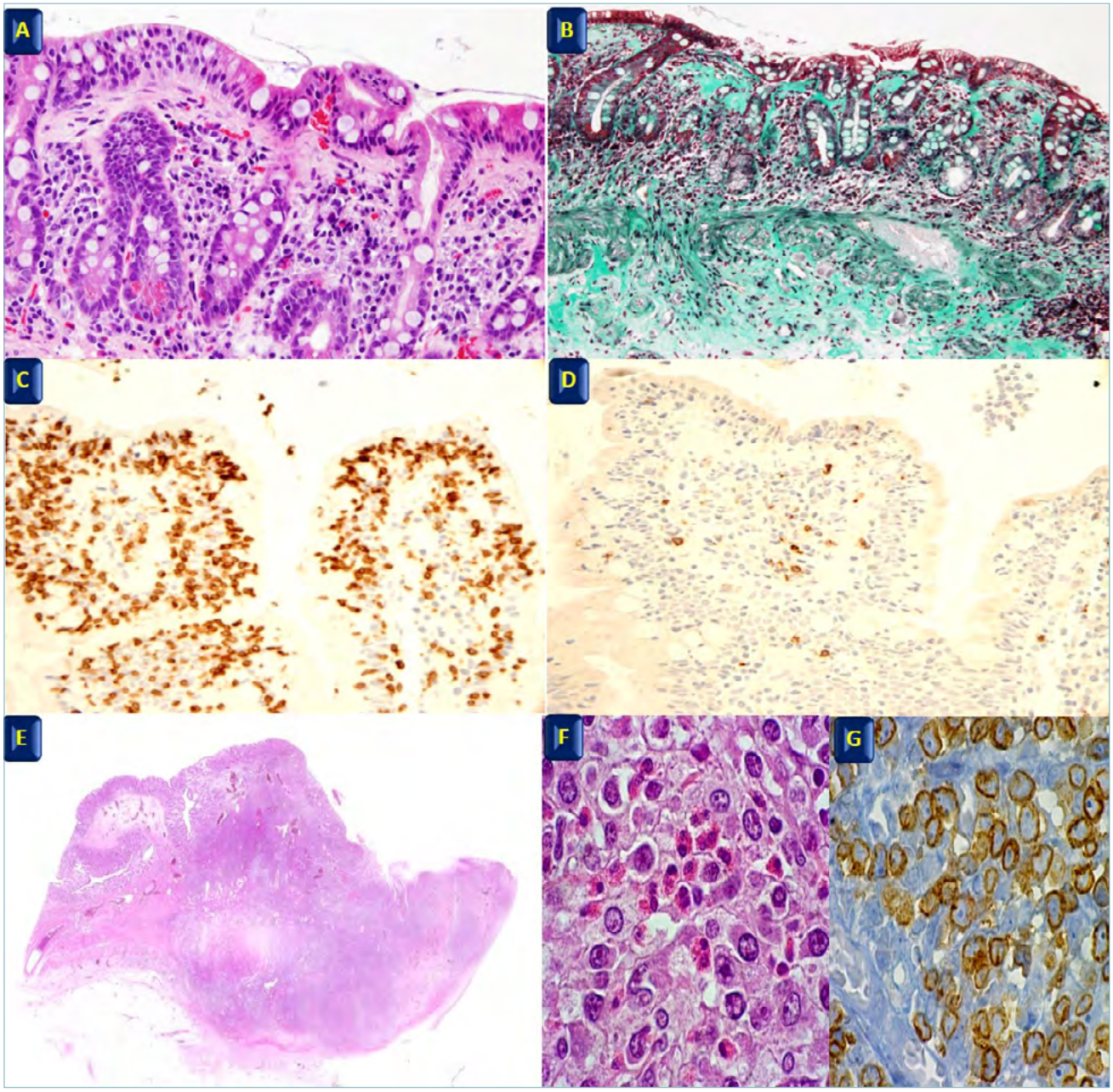

Figure 3. (A-B): **collagenous sprue**; pathological increase in the thickness of the connective tissue band under the superficial epithelium > 10 mμ; (A) H&E x 20; (B) Trichrome stain x 20. (C-D): **refractory celiac disease**; pathological increase of T lymphocytes CD3 positive (C) negativity for CD8 (D). C-D x 20. (E-F): **enteropathy type T cell lymphoma**; (E) x 4**,** (F) H&E x 40; (G) CD3 immunostain x 40.

tissues. As samples from duodenal mucosa from normal, CD or RCD type I patients may occasionally show TCR-β or TCR-γ clonality, the diagnosis and typing of RCD should be only made by a gastroenterologist after an integrated evaluation of clinical information, histology, intraepithelial lymphocyte immunophenotype (by immunohistochemistry or flow cytometry) and clonal analysis [58,59]. Flow cytometry seems to be better than CD8 immunohistochemistry in differential diagnosis between type I and II RCD. However, a recent study found that immunohistochemical expression of a NK biomarker, NKp46, on

T-cell surface, may help in distinguishing RCD type II (NKp46-positive) from RCD type I, usually showing no or few NKp46-positive T-cells [60]. Histology of RCD type I may be indistinguishable from untreated responsive CD; however, a collagenous sprue-type pattern and basal plasmocytosis are rarely noted in RCD type I. Making a correct diagnosis is of pivotal importance because type I and type II RCD have very different prognosis, therapy response and rate of development of lymphoproliferative malignancies. RCD type I has 5-year survival rates up to 95%, response rate to corticosteroids of 90% and rates of developing enteropathy-associated T-cell lymphoma (EATL) 5 year after RCD diagnosis lower than 14%; on the other hand, RCD type II has a 5-year survival of 58%, lower response rate to corticosteroids and higher rates of developing EATL [54,61].

Lymphoproliferative malignancies. CD individuals, especially those with longstanding disease, have a relative risk of developing extra-nodal non-Hodgkin lymphoma around 3-4 times higher than general population [60].

EATL is an aggressive malignancy complicating CD, commonly involving jejunum and ileum and characterized by markedly atypical malignant cells, densely infiltrating both the epithelium, which typically shows severe villous atrophy, and the lamina propria, extending below *muscularis mucosae*. Neoplastic cells are positive for CD3 and CD103, negative for CD5 and CD4, express CD8 variably, contain cytotoxic granule-associated proteins and harbor a clonal rearrangement of TCRγ and/or TCRβ genes [61].

EATL should be distinguished from monomorphic epitheliotrophic intestinal T-cell lymphoma (MEITL), composed of monomorphic, not significantly atypical, small- to medium-sized T cells, immunoreactive for CD3, CD8, CD56, CD103, and TIA1 and negative for CD5, CD4, CD30. Neoplastic T cells infiltrate both the lamina propria and the epithelium, causing partial or severe villous atrophy. Although the latest WHO classification of lymphoid neoplasms denied any association of MEITL with CD, it was recently described in two CD patients [62]. Both EATL and MEITL have an ominous prognosis, with a reported 5-year survival rate lower than 20% [63] (Fig. 3).

Small bowel carcinoma (SBC). Patients with CD have an increased risk of developing SBC. CD-associated SBC was shown to arise after a median CD duration of 17 months in patients with a median age of 53 years and to predominantly involve the jejunum. CD-associated SBCs harbor mismatch repair deficiency more frequently in comparison with Crohn's disease-associated or sporadic SBCs [64,65]. In addition, they often showed a high number of tumor infiltrating lymphocytes and a subset of them has a medullary-type histology [66]. Importantly, they usually display a relatively indolent behavior. SBC in CD patients is rarely associated with adjacent preinvasive neoplastic/dysplastic lesions, which, like their invasive components, usually express nuclear β-catenin, while retaining mismatch repair protein expression**.** Recently, Giuffrida and colleagues found that CD-associated SBCs are often infiltrated by PD-1-positive T-cells and show expression of PD-L1 in neoplastic/immune cells (combined positive score ≥ 1) in more than one third of cases [67].

Liver complications. Some CD patients may have altered liver function tests and/or develop a wide spectrum of liver diseases, encompassing cryptogenic hepatitis, steatohepatitis, cirrhosis, as well as liver autoimmune disorders [68].

Conclusion

CD is a very common disorder affecting most people in the silent form. Many of these patients are identified through screening of at-risk groups or after malabsorption symptoms onset, rarely for disease-associated complications. CD diagnosis and its differential diagnosis is made from integrations between typical histological findings and clinical, serological and immunological features. Corazza-Villanacci System is a helpful method to assess mucosal damage and and the response to gluten-free diet in patient follow-up.

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