An Update on Celiac Disease Histopathology and the Road Ahead

Fei Bao, MD; Peter H. R. Green, MD; Govind Bhagat, MD

• ***Context.*—Celiac disease (CD) is a common immune-mediated disorder that occurs in genetically predisposed individuals (carriers of HLA-DQ2 and DQ8 haplotypes) on consumption of wheat (gluten). It is characterized by inflammation of the small-intestinal mucosa and myriad gastrointestinal and systemic manifestations. Celiac disease is common in the general population (prevalence, 0.5%–1%). Currently, small-bowel biopsy is considered the gold standard for diagnosing CD. However, the role of serologic testing in the diagnosis of CD has evolved, from being a supportive test to supplanting intestinal biopsies in certain patient populations.**

***Objective.*—To summarize key aspects of histopathologic assessment, discuss the benefit of standardized pathology reports, impact of the site and number of small-bowel biopsy samples on diagnosis, and recommendations regarding serologic testing.**

***Data Sources.*—Literature review of publications on CD and experience with histopathologic review of biopsies at the Department of Pathology and Cell Biology, Columbia University Medical Center, New York-Presbyterian Hospital, New York.**

***Conclusions.*—Intraepithelial lymphocytosis in the context of villous atrophy is considered a characteristic histologic finding of CD; however, it is a rather nonspecific finding. A growing list of publications has also indicated that the detection of intraepithelial lymphocytosis in the absence of villous atrophy has rather low specificity for CD. Therefore, communication between pathologists and gastroenterologists is paramount, as is knowledge regarding the pertinent clinical and laboratory data, in distinguishing between CD and other disorders with similar histopathologic and clinical manifestations.**

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Celiac disease, also known as gluten-sensitive enteropathy, celiac sprue, or nontropical sprue (see reference for terminology) is a common immune-mediated disorder characterized by chronic inflammation of the small intestine, and the presence of systemic manifestations, which occurs in genetically predisposed individuals on consumption of certain grains, including wheat.[1] Celiac disease is believed to have been recognized as long ago as the 1st and 2nd century AD, when the characteristic stool findings were first described, with reference to ''…a celiac disease of chronic nature…,'' by the physician ''Aretaeus the Cappadocian.''[2] In 1888, Samuel Gee gave the first clear description, recognizing this disease in children. He termed the condition *the celiac affection* and suggested that dietary treatment might be of benefit.[3] In the mid-20th century, the link between CD and wheat was discovered and it was shown that the toxic component was gluten.[1] Histologic abnormalities of the small intestine, now considered characteristic for CD, were described by Paulley[4] in samples taken at surgery in 1954. In the 1990s, Marsh[5] classified the histologic patterns of small-intestinal mucosal injury, which were modified by Oberhuber et al[6] in 1999. The modified Marsh-Oberhuber classification is currently used by many pathologists.

The etiology of CD is multifactorial, with both genetic and environmental factors involved in disease development. Susceptibility to CD is primarily associated with the human leukocyte antigen HLA-DQ2 allele. The heterodimer DQA1*0501 and DQB1*0201 is detected in up to 95% of persons with celiac disease, with the remaining 5% expressing HLA-DQ8 (DQA1*0301, DQB1*0302). The frequency of these alleles in the general populations in Western countries is 20% to 30%.[7–9] Therefore, an individual not carrying DQ2 or DQ8 alleles is extremely unlikely to develop CD. Among autoimmune disorders, CD is one of the remarkably few where the offending antigen is known, that is, gluten (component of wheat, barley, and rye). Gluten is mostly made up of 2 groups of proteins: ethanol-soluble gliadins and ethanol-insoluble glutenins. Gliadin contains large amounts of the amino acids proline and glutamine. It is known that α-gliadin among other peptides is toxic to celiac patients. The pathogenesis of CD involves a $CD4^+$ T-cell mediated immune response to gliadin peptides, activation of a $CD8^+$ T-cell intraepithelial innate immune response, and production of antibodies against tissue transglutaminase, as well as anti-gliadin, anti-reticulin, and anti-endomysial antibodies.[10–13] In 1997, identification of tissue transglutaminase type II as the major autoantigen of CD (and also

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the epitope recognized by anti-endomysial antibodies) shed new light into the pathogenesis of this disease.[14] Tissue transglutaminase is an 85-kDa enzyme that is expressed in multiple tissues.[14] Gliadin can serve as a substrate of transglutaminase, which is activated upon injury or inflammation of the small bowel, and in the process becomes cross-linked to transglutaminase, thereby creating a neoantigen, which induces an immune response to the self-protein (transglutaminase). Moreover, tissue transglutaminase can selectively deamidate gliadin peptides, leading to enhanced T-cell stimulatory activity.[15] The ensuing inflammatory cascade produces inflammatory cytokines, proteinases, and other tissue-damaging mediators that induce mucosal tissue damage, leading to the characteristic histopathologic alterations recognized by pathologists.[16]

CLINICAL SPECTRUM AND TERMINOLOGY

Celiac disease occurs both in adults and children with a female predominance (female to male ratio, 2–3:1).[17] Recent advances in the understanding of the disease, and the availability of, more sensitive serologic tests, as well as an appreciation of the multisystemic nature of CD, have led to the recognition that CD is more common than previously thought. It is estimated to affect between 0.5% to 1% of individuals in both Europe and the United States.[18–20] Recent studies have also suggested an increasing prevalence, approaching 2% in American and Finnish cohorts.[21–23] An increased incidence of CD has been documented among asymptomatic relatives of patients with CD. The prevalence ranges from 10% to 12% in first-degree relatives[24–26] and a high rate of concordance (70%) is observed in monozygotic twins.[27] The classical presentation of CD is diarrhea, abdominal pain, bloating, or discomfort. Less common presentations include constipation, weight loss, neurologic symptoms, dermatitis herpetiformis, delayed puberty, osteoporosis, infertility, vitamin and protein deficiencies, and elevated liver enzyme levels.[28] A substantial proportion of patients have had a previous diagnosis of irritable bowel syndrome[29] and it is now known that the presence of obesity does not exclude the possibility of CD.[30] Celiac disease with atypical symptoms is characterized by few or no gastrointestinal symptoms and the presence of extraintestinal manifestations. Recognition of atypical features of CD is partly responsible for the increased prevalence and now may be the most common presentation encountered at diagnosis.

Silent CD is associated with individuals who are asymptomatic, but have a positive serologic test result and show histopathologic changes typical for CD. These patients are usually detected via screening of high-risk individuals including first-degree relatives of celiac patients, or identified by endoscopy and biopsy conducted for other reasons.[5,31] Latent CD is defined by a positive serologic result but lack of villous atrophy on biopsy; individuals are asymptomatic, but later may develop symptoms and/or histopathologic changes.[5,31,32] Mild histologic alterations, such as increased numbers of intraepithelial lymphocytes (IELs) in the small-intestinal epithelium, may be seen in these patients. A whole host of confusing terms and neologisms have been propagated in the literature over the years, with some of the terms being used interchangeably while describing histologic and clinical aspects/presentations of CD. Hence, it is important to point out that the abovementioned terminology should be restricted to clinical presentations and use of words like *histologically silent celiac disease* or *subclinical celiac disease* should be avoided.

Celiac disease is associated with a host of autoimmune diseases such as type 1 diabetes mellitus, autoimmune thyroid disease, Addison disease, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and immunoglobulin (Ig) A deficiency.[33–38] There is also an increased prevalence of CD in patients with genetic disorders such as Down syndrome and Turner syndrome.[39,40]

The complications of CD typically occur after many years of disease in adults, partially due to continual ingestion of gluten, either intentionally or unintentionally. Serious complications include refractory celiac disease, which encompasses more indolent variants or precursors of enteropathy-associated T-cell lymphoma, and small-intestinal adenocarcinoma.[41,42] The overall risk of cancer is almost twice in patients with CD compared to the general population.[43] An increased risk of a variety of malignancies has been reported, including both T-cell and B-cell non-Hodgkin lymphoma subtypes that may either occur in the intestines or at extraintestinal locations, esophageal carcinoma, and carcinomas of the pancreatobiliary tract and small or large bowel.[44,45] Interestingly, it has been shown that the risk of mortality remains elevated up to 5 years post diagnosis and commencement of a gluten-free diet (GFD).[43–47] Adherence to a GFD is thought to reduce the risk of lymphoma. The risk of death from malignancy is reportedly higher in patients on a regular diet compared to those on a GFD,[48] and the overall risk of dying is higher in patients who do not respond to a GFD compared to those who do.[49]

DIAGNOSIS

In recent years, many studies[50,51] have advocated changes in the diagnostic algorithms for CD. The diagnostic approach to CD is changing, owing to better understanding of the clinical manifestations of CD and the availability of more sensitive and specific serologic tests, as well as testing for the HLA susceptibility alleles.[50–53] However, small-bowel mucosal biopsy is currently considered the gold standard for diagnosing CD. All serologic tests and small-bowel biopsies need to be performed while the patient is on a gluten-containing diet. According to the US National Institutes of Health consensus statement,[50] serologic testing is recommended as the first step in pursuing a diagnosis of CD. Duodenal biopsy is recommended in individuals with a positive celiac antibody test result, or when serologic results are nondiagnostic, or in individuals with suggestive clinical symptoms. With positive serologic results and a biopsy that shows the characteristic findings of intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy, a presumptive diagnosis of CD can be made. Definitive diagnosis is confirmed when symptoms resolve on commencing a GFD. A repeated biopsy to demonstrate normalized histology after a GFD is no longer required for a definitive diagnosis of CD. There is currently no consensus on the management of patients whose small-bowel biopsies reveal Marsh I lesions (intraepithelial lymphocytosis and preserved mucosal architecture) or when serologic test results for CD are negative despite severe mucosal

architectural abnormalities, so called seronegative CD (also see below).

Serologic Testing

Serologic testing plays an important role in the diagnosis of CD. The most sensitive antibody tests detect IgA-class antibodies against tissue transglutaminase (tTGA) and endomysium (EMA). The anti-gliadin antibodies are no longer considered sensitive or specific enough to be used for routine clinical detection of CD, except in children younger than 18 months of age, because anti-gliadin IgA antibodies are considered to be the first autoantibodies to appear after intestinal exposure to a gluten-containing diet.[54] Numerous assays have been developed to detect IgA and IgG antibodies directed against tTGA and EMA, including enzyme-linked immunosorbent assays (ELISAs) to quantify tTGA and indirect immunofluorescent assays to detect EMA. The IgA recombinant anti-human tTGA ELISA assay is superior to the anti–guinea pig tTGA assay and is more cost-effective than the endomysial antibody test.[55,56] Serologic IgA tTGA antibody testing is considered to be the most sensitive method for detecting CD, with sensitivity approaching 97% in clinical practice,[54] while IgA EMA antibodies are highly specific markers for CD (approaching 100%).[56–58] The presence and titers of both tTGA and EMA antibodies have been shown to correlate with the degree of mucosal damage. Studies have shown that EMA seropositivity correlates with more severe villous atrophy, but not with the presence of gastrointestinal symptoms or the mode of presentation of CD.[59] One study[60] has also demonstrated that IgA tTGA levels of 100 U or greater occur almost exclusively in individuals, both adults and children, manifesting severe degrees of villous atrophy (Marsh 3 lesions). Celiac patients with lesser degrees of villous atrophy are less likely to have positive serologic findings. Seronegative CD does occur, accounting for up to 15% of all celiac patients.[59] Recently, a new test for detecting antibodies against deamidated gliadin peptides (DGP) was introduced, which displays promising results and a high specificity (99%), approaching that obtained with IgA EMA tests.[57,61] One study[62] demonstrated that removing gliadin from the diet leads to a more dramatic drop in antibody titers against DGP, indicating that DGP IgA testing might have greater sensitivity for the detection of adequate adherence to a GFD. However, its relevance in the diagnosis of different disease phases and patient populations and its reproducibility in the clinical setting remains to be investigated. A summary of the sensitivities and specificities of tests incorporating IgA antibodies directed against tTGA, EMA, and DGP is listed in Table 1.

Table 1. Sensitivities and Specificities of Immunoglobulin A (IgA)–Class Antibodies Against Endomysial Antigen (EMA), Tissue Transglutaminase (tTGA), and Deamidated Gliadin Peptides (DGPs)

IgA-Class Antibodies	Sensitivity, %	Specificity, %
EMA	89–92[55,57,58]	98–100[55–58]
tTGA	93–97[54,55,61]	95–97[54,56,58]
DGPs	84–88[57,61]	94–99[57,61]

Seronegative Celiac Disease

Seronegative CD poses a clinical challenge and requires integration of clinical, genetic, and histopathologic criteria as the individuals lack serum autoantibodies.[63] These individuals are more likely to have either normal villous architecture with increased IEL counts or mild degrees of villous atrophy, and they likely lack a humoral response to the autoantigens—tissue transglutaminase or gliadin.[64] The sensitivity of serum anti-EMA or tTGA antibody–based tests is low, reportedly 31% to 70% for CD with mild villous atrophy.[65,66] Newer tests for antibodies against DGP can detect 20% to 30% of persons with IgA tTGA seronegative disease.[67] This suggests that more sensitive tests for detection of serum autoantibodies are needed in certain cases, especially those manifesting limited mucosal inflammation. Detection of mucosal IgA tissue transglutaminase immune deposits might be one of the most sensitive approaches for identifying CD with minimal or no villous atrophy. Presence of these deposits in the lamina propria indicates activation of the local mucosal antibody response in the absence of a systemic humoral immune response. The sensitivity and specificity of this method is reported to be 93%.[68] Evaluation of the number and pattern of IELs at the villous tips and detecting increased intraepithelial γδ T cells in biopsy samples also help in diagnosing cases of CD with mild inflammation, lacking serologic abnormalities.[69]

Site and Number of Small-Bowel Biopsies

Small-bowel biopsy remains the gold standard for diagnosing CD. It is also now well recognized that CD is a patchy disease.[70–72] However, there are no uniform, agreed-upon recommendations or guidelines for the number or site of biopsies required for diagnosis. A recent large-scale retrospective study conducted in the United States demonstrated that the probability of a new diagnosis of CD was doubled when 4 specimens or more were submitted for histopathologic assessment.[71] Inadequate sampling may lead to false-negative diagnosis, and poorly oriented biopsy specimens can cause both underinterpretation and overinterpretation of the histologic abnormalities associated with CD. Historically, biopsies were taken from the jejunum to diagnose CD.[73,74] Studies since the mid-1990s have shown that biopsies from the second part of the duodenum are sufficient for diagnosis without loss of sensitivity or specificity.[75,76] Biopsies from the duodenal bulb should be interpreted with caution because this area is exposed to gastric acid and is prone to peptic injury; Brunner glands can also be prominent in the bulb and they may cause artifactual distortion of villi. However, the duodenal bulb is also the most sensitive site to detect mucosal injury induced by gluten, as the severity of mucosal damage is thought to follow a proximal to distal gradient. Many investigators have demonstrated that CD-related histologic lesions are present at the bulb, and importantly, isolated mucosal abnormalities at this site can be seen in up to 10% of CD cases, in both adults and children.[77–79] Hence, in practice, it seems reasonable to suggest that at least 4 to 6 endoscopic biopsy specimens be taken from the duodenum, with 2 samples from the bulb region.

HISTOPATHOLOGIC EVALUATION OF CELIAC DISEASE

Histopathologic evaluation of small-bowel biopsies should be performed on well-oriented biopsy pieces that

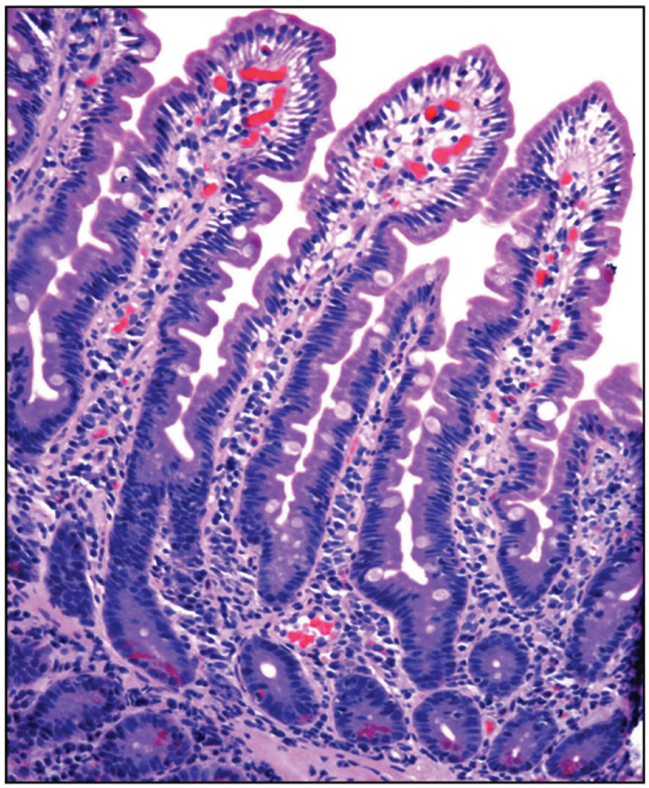

Figure 1. *Normal duodenal mucosa, with a normal villous to crypt ratio and scattered intraepithelial lymphocytes present with a normal ''decrescendo'' pattern (hematoxylin-eosin, original magnification ×100).*

contain at least 3 or 4 consecutive villous-crypt units visualized in their entirety and arranged parallel to each other. Scattered intraepithelial lymphocytes are present normally, which are more prominent along the lateral edge of villi, decreasing in number from the villous base toward the tip, the so-called decrescendo pattern,[80] shown in Figure 1. Loss of this pattern is considered more sensitive, though not specific, for CD.[81,82] The upper limit of normal IEL numbers was previously considered to be 40 lymphocytes per 100 epithelial cells, which was derived from older studies assessing jejunal biopsy samples and used as a diagnostic criterion in the Marsh-Oberhuber classification.[6] More recent studies[83] have shown that the upper limit of normal for duodenal IELs is closer to 25 IELs per 100 epithelial cells (mean + 2 SD). Counts between 25 and 29 IELs per 100 epithelial cells are considered a borderline increase, while a count of 30 or more represents a definite increase in IELs.[84] A simpler method, whereby villous tip IELs are counted, has been proposed. IELs are counted in 20 villous-tip enterocytes in 5 randomly selected villi. The upper limit of normal for IELs is 5 of 20 enterocytes, with counts of 6 or more representing an increase in IELs. It is a fast and simple alternative to the more cumbersome method of counting intraepithelial lymphocytes along 100 or 500 enterocytes and correlates well with the traditional methods.[82] Clustering of IELs at or close to the villous tips is also a helpful feature supporting a diagnosis of CD. In cases for which the IEL count is only mildly elevated or when tissue histology is compromised (eg, thick sections, staining artifacts), immunohistochemistry for CD3 is helpful for highlighting the distribution pattern of IELs. Although there are a variety of ways for counting IELs, absolute counts are time-consuming and impractical for routine practice. There is little need for counting IELs when there is diffuse marked intraepithelial lymphocytosis. However, counting IELs, with or without the aid of an immunohistochemical stain for CD3, is helpful in cases with patchy and/or mild increases in IEL numbers. Immunophenotypic studies have shown that the increased IEL numbers represent an expansion of both cytotoxic T cells (60%–70% of the cytotoxic T cells express CD8) bearing the αβ T-cell receptor, and γδ T-cell receptor–positive lymphocytes (predominantly $CD8^-$), with the former predominating. The γδ T cells comprise 1% to 10% of IELs in normal small-intestinal mucosa, but increase in patients with CD, where they represent up to 15% to 30% of all IELs.[85,86]

Microscopic examination of the small-bowel biopsies should be performed in a sequential algorithmic manner, ensuring inspection and evaluation not only of the mucosa and submucosa but also of the luminal aspect, to identify adherent or free-floating infectious organisms, foreign objects, and others. The surface epithelium, villous architecture, and lamina propria should be carefully examined in cases where CD is clinically suspected. Below is a check list for assessment of small-bowel biopsies when the differential diagnosis of CD is entertained.

- Luminal surface and epithelial-lumen interface: Check for infectious agents, such as *Giardia* organisms, *Cryptosporidium* organisms, or *Helicobacter pylori*, in cases of gastric epithelial metaplasia of the surface epithelium.
- Enterocytes: Presence versus loss of brush border, shape and height of enterocytes, intracytoplasmic vacuolation, presence of goblet cells or Paneth cells, denudation or damage of the surface enterocytes. Extension or hyperplasia of crypts with regenerative changes and increased mitotic activity at the base of the crypts.
- Intraepithelial lymphocytes.
  - Pattern of IELs: Diffuse or patchy increase.
  - Number of IELs: Mild, moderate, or severely increased.
  - In case of uncertainty: Count the number of IELs along the entire crypt-villus units (normal, <25 IELs per 100 enterocytes; borderline increased, 25–29 IELs per 100 enterocytes; or definitely increased, ≥30 IELs per 100 enterocytes). Alternatively, use the villous-tip method (normal, ≤5 IELs per 20 enterocytes; or definitely increased, ≥6 IELs per 20 enterocytes).
- Basement membrane: Presence of thickening of basement membrane, with or without subepithelial collagen deposition, and entrapment of capillary vessels, fibroblasts, or inflammatory cells.
- Villous and crypt architecture: Villous to crypt ratio; presence of partial, subtotal, or total villous atrophy accompanied by crypt hyperplasia.
- Lamina propria: Lamina propria normally contains a mixture of plasma cells, lymphocytes, and occasional eosinophils and macrophages, which are normally found in the lower portions or base of the lamina propria, while the villi appear relatively empty with few inflammatory cells. The presence of more than rare neutrophils in the lamina propria is not a normal finding. While assessing the lamina propria, the type and extent of inflammation should be documented in the report.

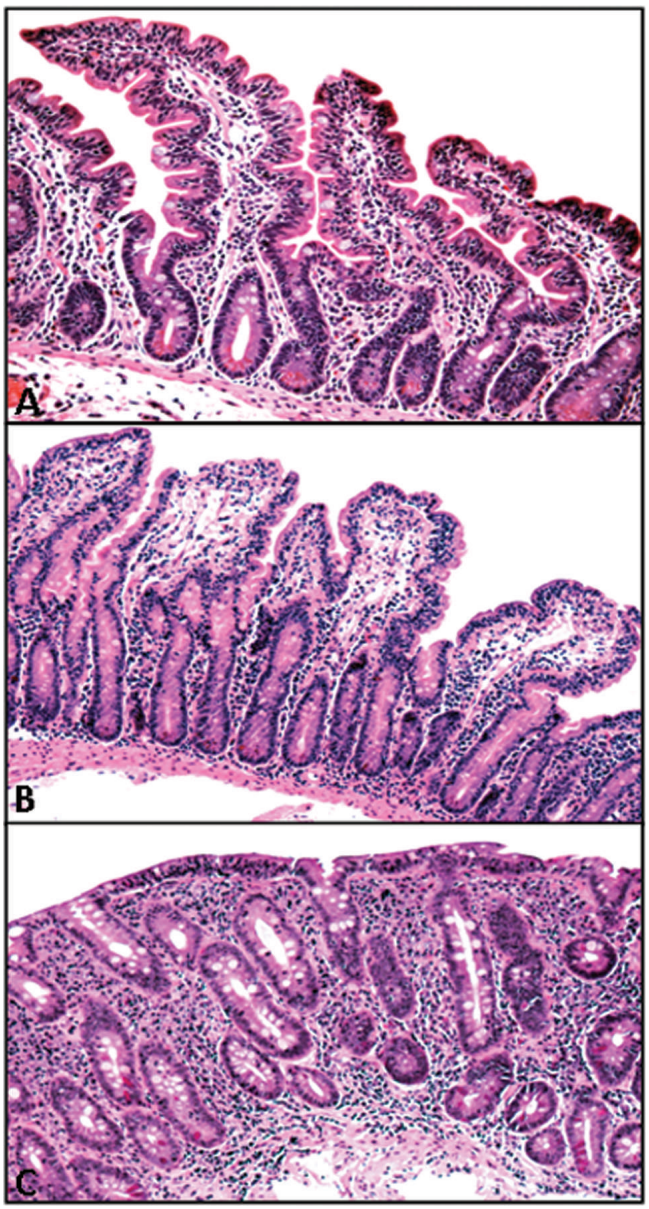

Figure 2. *Different grades of duodenal mucosal lesions of celiac disease. A, Infiltrative type (type 1) or nonatrophic lesion (grade A) with normal crypt and villous architecture and increased numbers of intraepithelial lymphocytes (IELs). B, Destructive type (type 3b) or atrophic lesion (grade B1) with moderate villous atrophy and diffuse increase in IELs. C, Flat lesion (type 3c or grade B2) with total villous atrophy and diffuse increase in IELs (hematoxylin-eosin, original magnifications ×100 [A through C]).*

Old and New Classifications of Celiac Disease

Marsh[5] introduced a grading scheme to classify the morphologic spectrum of gluten-sensitive enteropathy in 1992, based on his clinical research on a variety of small-intestinal disorders and observations of the intestinal response to gluten challenge, reported as a series of studies. Oberhuber et al[6] modified some of the parameters and published a modified scheme in 1999. The Marsh-Oberhuber classification describes 5 interrelated states of small-intestinal mucosal injury (types 0–4) as follows:

Table 2. Old and New Classifications for Histopathologic Evaluation of Celiac Disease–Associated Mucosal Changes[a]

Marsh-Oberhuber Classification	Corazza-Villanacci Classification
Type 1	Grade A
Type 2	
Type 3a	Grade B1
Type 3b	
Type 3c	Grade B2
Type 4	Deleted

[a] Comparison of a new histopathologic classification scheme proposed by Corazza and Villanacci with the Marsh-Oberhuber classification in evaluation of celiac disease–associated mucosal lesions.

- Type 0: Preinfiltrative, normal small-intestinal mucosa with less than 30 IELs per 100 enterocytes.
- Type 1: Infiltrative type, which is characterized by a normal villous and crypt architecture (normal villous to crypt ratio of >3:1) and an increased number of IELs (≥30 IELs per 100 enterocytes).
- Type 2: Infiltrative-hyperplastic type, which is characterized by a normal villous architecture and crypt hyperplasia with an increased number of IELs (≥30 IELs per 100 enterocytes). This stage is only very rarely encountered in patients with CD and has mainly been observed under experimental conditions, after commencement of a GFD or time-dose–related gluten challenge studies,[87] and in patients with dermatitis herpetiformis.[88]
- Type 3: Destructive (flat mucosa) type of CD lesion. It is divided into 3 different subgroups depending on the degree of villous atrophy.

1. Type 3a: Mild villous atrophy with villous to crypt ratio of <3:1 or 2:1, and an increased number of IELs (≥30 IELs per 100 enterocytes).
2. Type 3b: Marked villous atrophy with villous to crypt ratio of <1:1, and an increased number of IELs (≥30 IELs per 100 enterocytes).
3. Type 3c: Total villous atrophy with completely flat mucosa and an increased number of IELs (≥30 IELs per 100 enterocytes).

- Type 4: Atrophic type (also referred to as the *hypoplastic lesion*) is a very rare pattern, characterized by flat mucosa with only a few crypts visualized and near-normal IEL counts. It is usually found in patients with refractory sprue, ulcerative jejunoileitis, and enteropathy-associated T cell lymphoma.[89]

The Marsh-Oberhuber classification is currently used by many pathologists to evaluate the duodenal mucosal lesions in patients with CD. However, types 1 and 2 mucosal alterations are often not recognized, and lesions representing type 3a and 3b in the Marsh scheme are fraught with a high degree of interobserver variability, even among expert gastrointestinal pathologists. A new histologic classification has recently been proposed by Corazza and Villanacci,[90] which has divided the mucosal lesions of CD into 2 categories: nonatrophic lesion (grade A; demonstrated in Figure 2, A) and atrophic lesion (grade B). Grade B lesions are further subdivided into B1 (demonstrated in Figure 2, B) and B2 (demonstrated in Figure 2, C) by the presence or absence of villi.

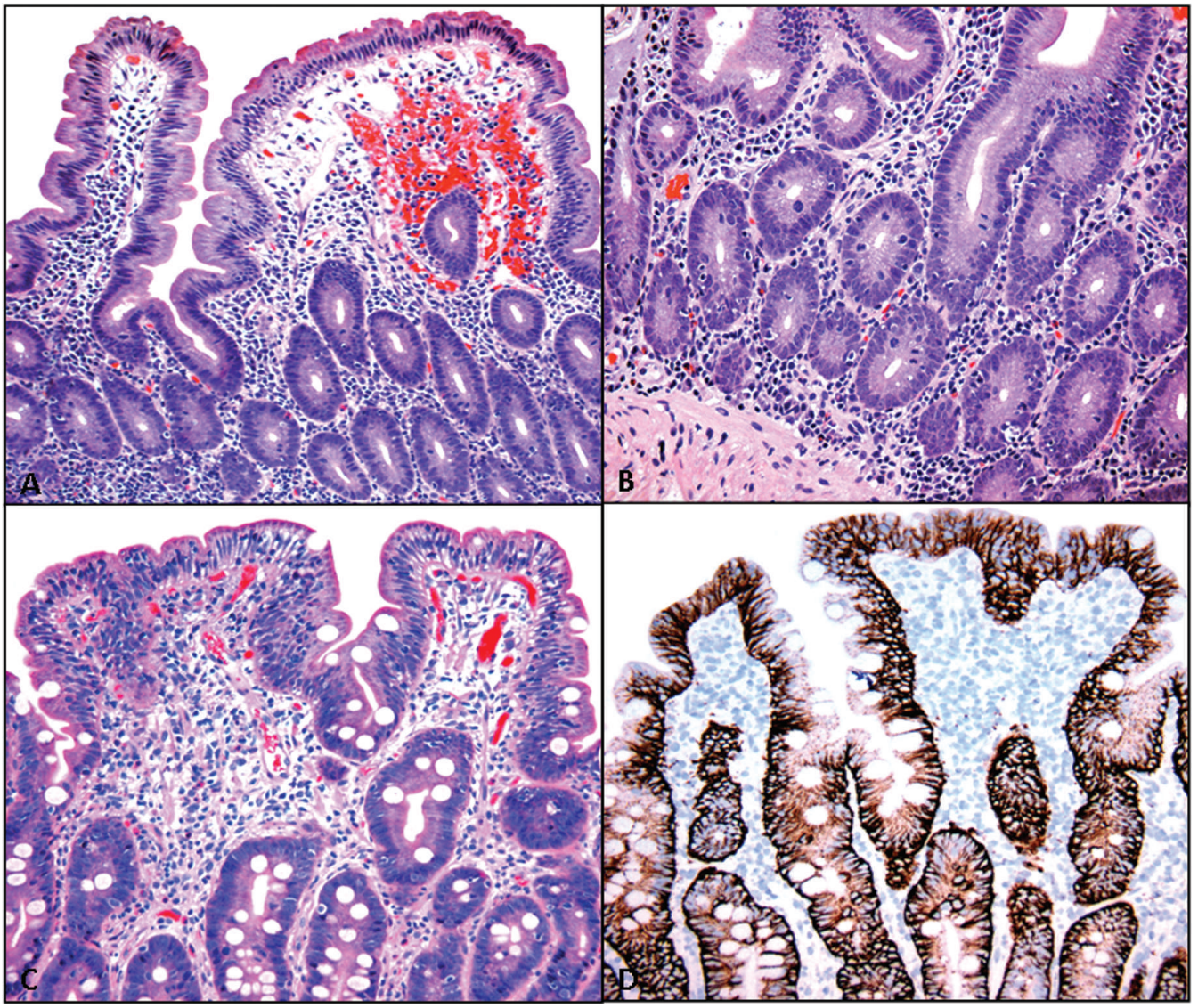

Figure 3. *Histopathologic findings in patients with autoimmune enteropathy (AE) and common variable immunodeficiency (CVID). A, Duodenal biopsy specimen from a patient with AE illustrates moderate villous atrophy and absence of goblet cells and Paneth cells, without surface lymphocytosis. B, Duodenal biopsy specimen from a patient with AE shows absence of goblet cells and Paneth cells, with mild lymphocytosis, crypt apoptosis, and mitosis in the deep crypt epithelium. C, Duodenal biopsy specimen from a patient with CVID demonstrates moderate villous atrophy and surface intraepithelial lymphocytosis, with absence of plasma cells. D, An immunohistochemical stain for CD138 confirms the absence of plasma cells in the lamina propria (hematoxylin-eosin, original magnifications ×100 [A, B] and ×200 [C]; original magnification ×200 [D]). Photomicrographs A and B courtesy of Roger Moreira, MD, Columbia University Medical Center, New York, New York.*

- Grade A: Nonatrophic, with normal crypt and villous architecture and increased IEL numbers (>25 IELs per 100 enterocytes).
- Grade B1: Atrophic, with villous to crypt ratio <3:1, but the villi are still detectable and IEL numbers are increased (>25 IELs per 100 enterocytes).
- Grade B2: Atrophic and flat, where the villi are no longer detectable and increased IEL numbers are noted (>25 IELs per 100 enterocytes).

The new classification is simpler and has demonstrated better interobserver agreement than the more cumbersome Marsh-Oberhuber classification.[91] Its use may contribute to more uniform diagnostic reporting in cases of CD and enhance communication between pathologists and clinicians. Table 2 compares the old and new classifications for histopathologic evaluation of CD-associated mucosal changes.

DIFFERENTIAL DIAGNOSIS

Differential diagnosis of CD includes a variety of disorders that manifest villous atrophy and/or increased numbers of IELs. Intraepithelial lymphocytosis is a characteristic histologic feature of CD; however, it is a rather nonspecific finding. In some series, up to 2.5% of duodenal biopsy specimens show increased IEL counts (>25 IELs per 100 enterocytes) in the absence of villous architectural changes.[92] Common causes for increased IEL counts with normal villous architecture are listed in Table 2. *Helicobacter pylori*–associated gastroduodenitis, medications (primarily nonsteroidal anti-inflammatory drugs), infections, and immune dysregulation are the

most common disorders associated with this pattern. The prevalence of CD as the etiology for cases that display increased IEL counts and architecturally normal duodenal biopsy specimens is only about 10%.[93] The titers of autoantibodies for CD, including tTGA and EMA, have been shown to correlate with a greater degree of villous atrophy; hence, celiac patients with normal villous architecture are less likely to have positive celiac serologic test results. A major challenge facing both clinicians and pathologists is to diagnose CD when Marsh type 1 or grade A lesions (according to the Corazza-Villanacci classification) are present. The specificity of histopathologic findings in the small-bowel biopsy is much greater when villous atrophy (partial, subtotal, or total) is present. However, other entities such as tropical sprue, autoimmune enteropathy (AE; illustrated in Figure 3, A and B), common variable immunodeficiency (CVID; illustrated in Figure 3, C and D), collagenous sprue (illustrated in Figure 4, A and B), and drug-induced mucosal injury (such as colchicine toxicity, illustrated in Figure 4, C), among other disorders listed in Table 3, can all cause villous atrophy, with or without a concomitant increase in IEL numbers. Because of the complex nature and myriad presentations of CD, the profound changes in lifestyle, as well as the social impact when labeled or diagnosed with CD, communication between pathologists and clinicians is of paramount importance to correctly interpret clinical, histologic, and laboratory data. This better enables inclusion or exclusion of CD as a cause for the patient's symptoms and engenders a clinically relevant discussion regarding important histopathologic findings and possible differential diagnoses. The final diagnosis of CD should be made by a gastroenterologist or patient's pediatrician upon factoring all the data including histopathologic findings.

Autoimmune Enteropathy

Autoimmune enteropathy comprises a rare group of immune-mediated disorders involving the intestines, which occur primarily in young children and infants, but can also affect adults in some instances. In young males, an X-linked severe form of the disorder is associated with immune dysregulation and polyendocrinopathy due to a germ line mutation in the *FOXP3* gene located on the X chromosome.[94] The disease commonly affects the proximal small bowel, with involvement of the stomach and colon described in some cases. Histopathologic changes in AE show some similarities with CD. The small-bowel biopsy specimens show variable, sometimes total, villous atrophy; crypt hyperplasia with dense lymphoplasmacytic infiltrate in the lamina propria; and neutrophils with crypt abscesses in severe cases.[95–97] While IELs may be seen in the intestinal epithelium, they are more often seen in the crypts; unlike CD, marked surface intraepithelial lymphocytosis is usually not present.[96] One of the hallmarks of AE is the presence of anti-enterocyte antibodies and some patients may also have anti–goblet cell, anti–parietal cell, and anti–smooth muscle antibodies. Anti-gliadin and anti-reticulin antibodies have also been described in AE.[7] Patients with anti–goblet cell antibodies, in some instances, show absence or reduced numbers of goblet cells; Paneth cells can be reduced as well on intestinal biopsy specimens, illustrated in Figure 3, A and B. However, it should be borne in mind that anti–goblet cell antibodies are not specific for this disorder. Patients with AE present with protracted secretory diarrhea, weight loss and malabsorption, unresponsiveness to a gluten-free diet, or total parental nutrition. Steroids and immunosuppressive therapy have been used with efficacy. Cases with overlapping autoimmune enteropathy and

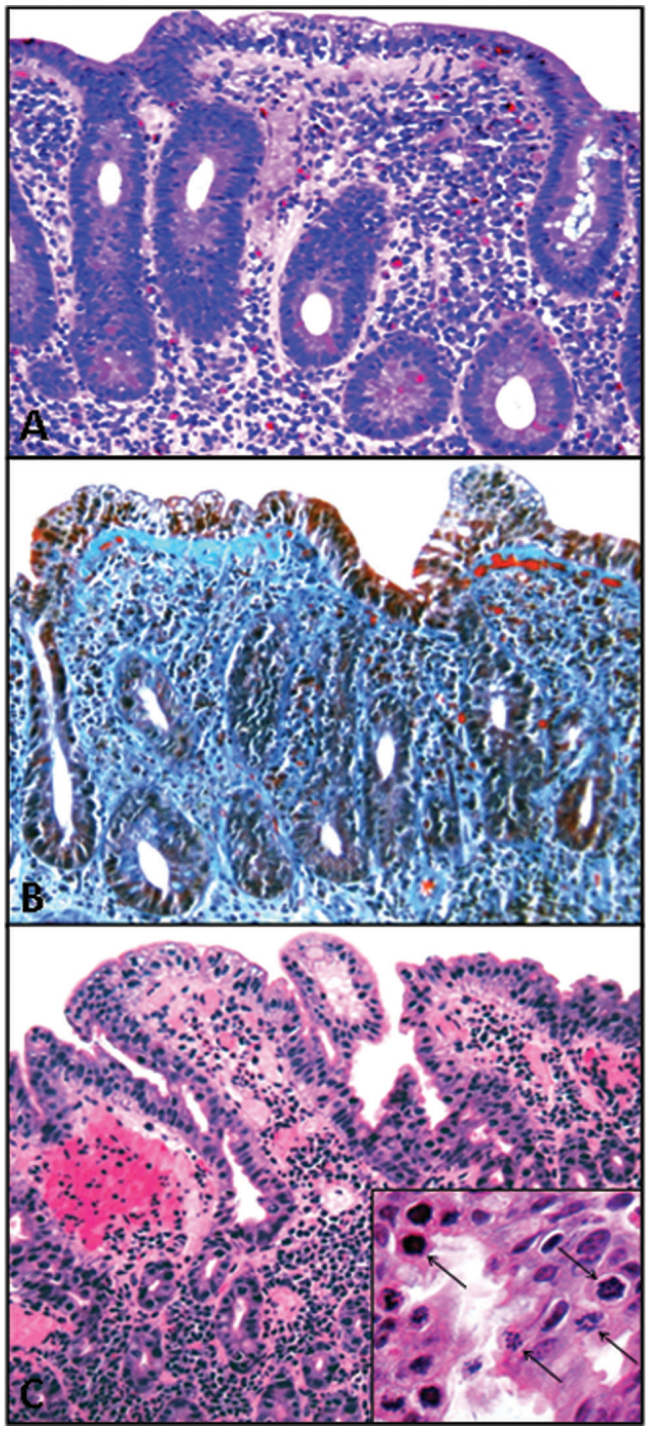

Figure 4. *Representative examples of collagenous sprue and colchicine-induced mucosal injury. A, Collagenous sprue with subepithelial fibrosis and intraepithelial lymphocytosis. B, Trichrome stain highlights moderate subepithelial fibrosis. C, Histopathologic features of colchicine toxicity in the duodenum with moderate villous atrophy and abundant mitosis and apoptosis in the crypts. Inset arrows indicate metaphase (ring-form) mitosis in the crypts (hematoxylin-eosin, original magnifications ×200 [A], ×100 [C], and ×400 [C, inset]; original magnification ×200 [B]).*

Table 3. Differential Diagnosis of Celiac Disease With or Without Villous Atrophy	
Normal Villous Architecture and Increased IEL Counts	**Villous Atrophy With/Without Increased IEL Counts**
Food hypersensitivity: cow's milk, soy, fish, rice, chicken, etc	Infections: tropical sprue
Peptic ulcer disease	Refractory sprue
Helicobacter pylori–associated gastroduodenitis	Collagenous sprue
Drugs: NSAIDs, proton-pump inhibitor	Immune dysregulation: autoimmune enteropathy
Infections: viral enteritis, *Giardia* organisms, *Cryptosporidium* organisms, etc	Immunodeficiency: common variable immune deficiency
	Graft-versus-host disease
Immune dysregulation: rheumatoid arthritis, Hashimoto thyroiditis, SLE, autoimmune enteropathy	Inflammatory bowel disease: Crohn disease
	Drugs: mycophenolate mofetil, colchicine
Immunodeficiency: common variable immune deficiency	Chemoradiation therapy
Graft-versus-host disease	Nutritional deficiency
Inflammatory bowel disease	Eosinophilic enteritis
Bacterial overgrowth	Bacterial overgrowth
Lymphocytic and collagenous colitis	Lymphoma
Irritable bowel syndrome	

Abbreviations: IEL, intraepithelial lymphocyte; NSAIDs, nonsteroidal anti-inflammatory drugs; SLE, systemic lupus erythematosus.

CD have been described.[98] However, further studies are required to determine the frequency of anti-enterocyte antibodies in patients with CD to determine whether the presence of such antibodies signifies a distinct subset of patients with unique features.

Common Variable Immunodeficiency

Common variable immunodeficiency is the most common primary immunodeficiency disorder after isolated IgA deficiency.[99] Common variable immunodeficiency is characterized by failure of terminal B-cell maturation into plasma cells, leading to decreased immunoglobulin levels in serum. Patients are prone to infections, especially giardiasis and bacterial overgrowth, and they have chronic gastrointestinal complaints including diarrhea and malabsorption.[99,100] Small-bowel biopsy specimens demonstrate a wide range of histologic changes, from increased IEL counts with variable degree of villous atrophy resembling CD, to nodular lymphoid hyperplasia and lymphoma in some cases.[101] A characteristic feature of CVID is the absence of, or the presence of only a few, plasma cells in the lamina propria as shown in Figure 3, C and D. An immunohistochemical stain for CD138 may be helpful in this regard. Common variable immunodeficiency can superficially resemble CD; however, the absence of plasma cells should alert the pathologist to the possibility of this disorder and the presence of hypogammaglobulinemia, as well as a lack of response to a gluten-free diet, should be confirmed by the gastroenterologist.

Refractory Celiac Disease

A subgroup of patients with CD (approximately 5%) may develop refractory CD (RCD), a condition that has also been referred to as *refractory sprue* in the past, which is characterized by persistent symptoms and severe villous atrophy not responding to a gluten-free diet for at least 6 months.[17] Both primary and secondary forms of the disease have been described. The diagnosis of RCD is made after exclusion of other small-bowel diseases, such as tropical sprue, CVID, or AE. Furthermore, RCD may be subdivided into 2 subtypes (RCD type 1 and type 2). Cases of RCD type 1 display a normal IEL phenotype, that is, $CD3^+$ and $CD8^+$. In individuals with RCD type 2, the IELs show an aberrant phenotype in that intracytoplasmic CD3 expression is noted, but surface CD3, and often CD8, are not present; additionally, a clonal T-cell receptor gene rearrangement is detected.[41] Refractory CD type 1 often responds to therapy with immunomodulatory agents and patients have a low risk of progression to lymphoma, whereas RCD type 2 either has a transient response or is refractory to immunosuppressive agents, and patients often manifest ulcerative jejunitis and are at an increased risk for enteropathy-associated T cell lymphoma.[41,102] RCD type 2 is also considered by some to represent a ''cryptic'' or a lower-grade variant of enteropathy-associated T cell lymphoma.[41,103] Patients with type 2 RCD have very poor prognosis with the 5-year survival rate being less than 50%.[41]

Collagenous Sprue

Collagenous sprue (CS) is a rare type of small-bowel enteropathy associated with chronic diarrhea and severe malabsorption, typically affecting middle-aged or elderly females.[104,105] Histologically, CS is characterized by villous atrophy, crypt hyperplasia, and a thick subepithelial collagen band (>10 μm) that entraps small capillaries and cellular elements in the lamina propria,[106] as illustrated in Figure 4, A and B. The occurrence of CS has been reported in individuals with CD, tropical sprue, CVID, and malignancy-related paraneoplastic syndromes.[104] A significant percentage (40%–86%) of patients with CS have CD, especially RCD.[41,104,107] However, a clear etiology of this disorder in some cases remains undetermined. Patients with CS were thought to have a uniformly poor prognosis with high morbidity (due to severe malnutrition) and mortality, but recent studies have shown that with current therapeutic management strategies, including active monitoring for adherence to a GFD and/or use of immunomodulatory drugs, patients with CS can have good clinical outcomes.[104,105]

TEMPLATED PATHOLOGY REPORTING

A checklist-based, templated pathology report can be beneficial to ensure capturing and reporting all relevant histopathologic features. Such reports should include the following:

1. Site and number of biopsy specimens, with a comment on specimen orientation.
2. Villous to crypt ratio: Normal (3:1 to 5:1) or abnormal.
3. Presence and degree of villous atrophy: Normal or atrophic—mild (partial), moderate (subtotal), or severe (total).

4. Increase in IEL counts with use of immunohistochemistry for CD3 in equivocal cases:
   - Normal: Fewer than 25 IELs per 100 enterocytes
   - Borderline increased: Twenty-five to 29 IELs per 100 enterocytes
   - Definitely increased: At least 30 IELs per 100 enterocytes
5. Presence/absence of surface epithelium damage.
6. Presence/absence of subepithelial collagen.
7. Lamina propria inflammation: Type and degree.
8. Other: Clinical information and serology results; descriptive diagnosis, including differential diagnoses deemed relevant; and histopathologic impression consistent with or suggestive of CD. Classification of the lesion (Marsh-Oberhuber and/or Corazza-Villanacci classification) is useful for cross comparison of cases from other institutes and for investigative work.

CONCLUSIONS

Celiac disease is a chronic systemic immune-mediated disorder associated with variable degrees of small-intestinal mucosal injury caused by gluten ingestion in genetically predisposed individuals. It is a common disorder affecting individuals worldwide, with recent studies suggesting an increase in prevalence. Small-bowel biopsy currently remains the gold standard for diagnosing CD. The improved sensitivity and specificity of serologic testing and growing awareness among gastroenterologists regarding the utility of sampling multiple sites, and ensuring an adequate number of biopsy specimens, should increase the diagnosis of CD. Because of the changing presentation of disease, as well as the recognition of a number of potential histopathologic mimics, communication between pathologists and gastroenterologists is essential for appropriate interpretation of small-bowel biopsy specimens. The clinical, histologic, and laboratory data need to be assessed to provide an explanation for atypical manifestations of CD and possible differential diagnoses. The intent of this review is to provide an update on diagnostic testing and assessment, as well as the reporting, of histopathologic features of CD with the hope of promoting standardization and consensus in diagnosing CD by pathologists and gastroenterologists.

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