Gluten-Sensitive Enteropathy (Celiac Disease)

Controversies in Diagnosis and Classification

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Objectives.—To highlight the variations in clinical and pathologic presentation of gluten-sensitive enteropathy, to emphasize the importance of small-intestinal biopsy evaluation in the diagnosis, and to propose a new classification of mucosal pathology in gluten-sensitive enteropathy, in an effort to overcome the problems related to the classification systems currently available.

Data Sources.—A review of the literature on clinicopathologic features and the morphologic spectrum of gluten-sensitive enteropathy is presented.

Conclusions.—Considering that there are many entities in the differential diagnosis of gluten-sensitive enteropathy, because of the varied clinicopathologic spectrum of the disease, diagnosis depends on good clinicopathologic communication. The classification that is presented in this review is a simple and practical approach to improve clinicopathologic correlation in gluten-sensitive enteropathy.

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DIAGNOSIS

With the description of the spectrum of mucosal pathology and the availability of genetic and highly reliable serologic markers, a substantial change has occurred in the diagnosis of GSE. Most importantly, the presence of total flat mucosa, with no detectable villi on its surface, is no longer necessary provided other histologic features of GSE and specific antibodies are present in a patient with genetic susceptibility. Although diagnosis currently relies on clinicopathologic studies including mucosal biopsy, serologic tests, and the effects of a diet free of gluten on the symptoms, there is still a need for consensus guidelines set by the major scientific organizations associated with GSE. According to the revised criteria of the European Society for Paediatric Gastroenterology and Nutrition, an initial characteristic small-intestinal abnormality should be regarded as essential and there should be a clear-cut response to strict gluten-free diet (GFD) with clinical recovery within weeks. In asymptomatic patients, a second follow-up biopsy under a GFD is advised to demonstrate the histologic recovery of the mucosa, which usually does not develop before 6 months. The American Gastroenterological Association...
mandates a biopsy to confirm the diagnosis in people suspected of having GSE,\textsuperscript{10} whereas the US National Institutes of Health, in its recent consensus statement, recommends biopsies only after a positive serologic finding or when serologic results are nondiagnostic.\textsuperscript{11} In summary, the diagnostic scheme of GSE consists of the following:

- Clinical history and symptomatology
- Serologic testing (tissue transglutaminase, endomysial antibodies, and antigliadin antibodies)
- Histologic findings in proximal small-intestinal biopsy
- Clinical and serologic (optionally histologic) response to a GFD\textsuperscript{7,12,13}

**CLINICAL SPECTRUM OF GSE**

Symptomatology of GSE is not associated with the severity of the mucosal lesion but is mainly related to the length of the affected bowel.\textsuperscript{14–16} Clinical presentation varies from full-blown malabsorption with weight loss, diarrhea, and steatorrhea to more subtle symptoms such as folate- or iron-deficiency anemia, flatulence, episodic diarrhea, loose stools, neurologic problems, osteoporosis, and vitamin K and D deficiencies in as many as 50% of patients.\textsuperscript{2,3,17} It also varies with the age of the patient, the duration of the disease, and the presence of extraintestinal findings.\textsuperscript{12} In children, usually within few months of introducing the child to wheat-based foods, the classic syndrome of chronic diarrhea, steatorrhea, abdominal distension, and failure to thrive appears between 6 months and 2 years of age.\textsuperscript{14,18} Both weight (40% below 10th centile) and growth (25% below 10th centile) are affected in these children, although weight is affected more often.\textsuperscript{19} Other children with fewer symptoms may escape detection and will perhaps not be diagnosed until adulthood.\textsuperscript{20} If undiagnosed, however, GSE can lead to permanent short stature and pubertal delay as well as the sequelae of nutrient deficiency such as iron deficiency or megaloblastic anemia and rickets.\textsuperscript{7,19,20} Patients presenting with symptoms as adults may also have short stature and/or historical symptoms indicative of ongoing disease since childhood. Current estimates show that the incidence of GSE is 1:200 or higher in wheat-eating patients presenting with symptoms as adults may also have short stature and/or historical symptoms indicative of ongoing disease since childhood. Current estimates show that the incidence of GSE is 1:200 or higher in wheat-eating populations such as Western Europe and North America, while the incidence continues to rise in Eastern societies, possibly as a result of “western-style” eating habits.\textsuperscript{11} Prompt diagnosis and treatment of GSE not only eases symptoms and improves quality of life but also has the potential to decrease long-term risks for lymphoma, gastrointestinal cancers, dermatitis herpetiformis, osteoporosis, endocrine abnormalities, infertility, cardiomyopathy, and other autoimmune disorders.\textsuperscript{14,17,21}

**Latent Gluten Sensitivity**

The term latent gluten sensitivity is used to describe symptoms in patients with evidence of gluten sensitivity but without full-blown symptoms of sprue.\textsuperscript{22–25} Terms that have been applied to such cases include subclinical, silent, or occult celiac disease; gluten-sensitive disease with mild enteropathy; low-grade enteropathy; minimally symptomatic enteropathy; and potential celiac disease.\textsuperscript{26–29} This form of GSE is characterized by the presence of no or only mild changes in the proximal small-intestinal mucosa, subtle symptoms when investigated carefully, and isolated positivity of serologic tests. These cases prove to be difficult to diagnose because of the atypical symptoms that do not immediately suggest a gastrointestinal cause. Indeed, almost half of individuals with latent gluten sensitivity may be apparently well, while others may have a sense of malaise that is difficult to define. Such cases require vigorous histopathologic evaluation, as the findings may occur along a spectrum of normal mucosa with increased numbers of intraepithelial lymphocytes (IELs) to flat mucosa, and they should not be missed since patients still remain predisposed to cancer or lymphoma by virtue of chronic gluten ingestion.\textsuperscript{25,26,27,29}

**Serologic Tests**

Widespread availability of serologic tests has permitted physicians to test their patients for GSE without further aid from pathology. Serologic tests are based on the use of immunoglobulin (Ig) A isotypes and include antigliadin antibodies as well as connective tissue antibodies such as reticulin antibodies, endomysial antibodies (EMAs), and tissue transglutaminase (tTG) antibodies.\textsuperscript{12} In most patients, serologic positivity supports the diagnosis and these tests are very useful for screening and follow-up.\textsuperscript{7,22–24} The enzyme-linked immunosorbent assay–based clinical test for IgA anti-tTG antibodies has a high sensitivity and specificity and has recently become the serologic test of choice for GSE, largely replacing other antibody tests.\textsuperscript{30,31} While EMA is as sensitive and specific as tTG, the immunofluorescent test used for detection of EMA is time-consuming and more subjectively interpreted than the tTG test.\textsuperscript{32,33} Serologic positivity usually correlates with the degree of mucosal damage, while minimal histologic lesion of intraepithelial lymphocytosis (IELosis) often presents with normal serologic findings.\textsuperscript{7,25–28} In parallel with this view, a previous study\textsuperscript{34} demonstrated that increasing titers of tTG predicted higher levels of villous flattening. Similar findings were observed in pediatric patients who had tTG levels above 100 units and showed advanced (Marsh type 3) lesions in their biopsy specimens.\textsuperscript{34,38} Taken together, these findings indicate that a negative serologic result is not sufficient to rule out GSE with a Marsh type 1 lesion.

Human leukocyte antigen (HLA) testing to detect susceptible HLA subtypes is also used in the routine diagnostic workup of GSE.\textsuperscript{7,30} While HLA-DQ2 is found in 90% to 95% of patients with GSE, most of the remaining cases are associated with HLA-DQ8. Since these HLA alleles are found in up to 40% of the general population, their presence does not aid the diagnosis directly, but the absence of DQ2 or DQ8 virtually excludes GSE.\textsuperscript{7} It is therefore justified to state that, despite the development of new serologic tests and genetic analysis, small-intestinal biopsy continues to be the gold standard for the diagnosis of GSE, particularly in less severely affected patients with mild mucosal abnormality.

**Small-Intestinal Biopsy: Site and Number?**

Similar to its wide variation in clinical manifestations, GSE has a wide spectrum of histologic abnormalities, which makes interpretation of small-intestinal biopsy specimens problematic for the pathologist. The damage to the small-intestinal mucosa classically involves the proximal small intestine including duodenum and upper jejunum and extends distally for a variable length into the ileum.\textsuperscript{14,40,41} Healing of the small-bowel mucosa, on the other hand, takes place in a distal to proximal direction.\textsuperscript{45} This may take at least 6 months and may even be prolonged
Because of the slow tempo of IEL (γδ subtype in particular) loss from the epithelium and entry after gluten ingestion, IEL count may be the last feature to return to normal after a GFD.\textsuperscript{41,43}

The number of small-intestinal biopsies has substantially increased over the years, partly because of the increased awareness by clinicians of the atypical forms of GSE, as well as the increased use of upper gastrointestinal endoscopy. Previously, small-intestinal biopsies were always taken by suction capsule, conventionally positioned at the distal duodenum, duodeno-jejunal junction (ligament of Treitz), or proximal jejunum under fluoroscopic control.\textsuperscript{12,14,16} Although, capsule biopsies are usually bigger and easier to orientate, swallowing the capsule is discomforting for the patient and is more labor-intensive and time-consuming for the physician. In the past decade, duodenal biopsies have almost entirely replaced capsule biopsies of jejunal mucosa for the diagnosis of GSE in most gastroenterology units, essentially because endoscopy has the advantages of saving time and reducing the risk of failure and false-negative findings.\textsuperscript{12,44–48} Also, multiple targeted biopsies can be taken during endoscopy because, in most patients with flat mucosa, the duodenum shows typical endoscopic features described as mosaic appearance, scalloping, or reduction of duodenal folds.\textsuperscript{12,14,45,46}

Newer endoscopic methods, such as push enteroscopy and double-balloon enteroscopy, allow access to the entire length of small bowel,\textsuperscript{15} but are more timely and costly compared with upper gastrointestinal endoscopy. Obtaining biopsy samples of adequate size from across a circular mucosal fold is as important as the biopsy site itself.\textsuperscript{12,14,15} Pathologists should be aware that biopsy forceps can crush and destroy tissue, causing hemorrhage in the sample and thus making the evaluation of specimen difficult. Superficial biopsy samples lacking muscularis mucosa can cause separation of the villous bases, resulting in shorter and thicker villi that can easily be misinterpreted in favor of a diagnosis of GSE.

Conflicting reports exist in the literature regarding the distribution pattern of mucosal pathologic features along the small bowel.\textsuperscript{16,49–51} Although it was widely accepted in the past that villous flattening rarely coexisted with histologically normal mucosa, currently, many investigators believe that GSE can exhibit a patchy distribution, that is, areas with villous flattening may occur in proximity to areas with mild villous shortening and also in areas with normal histologic features, particularly in pediatric patients.\textsuperscript{50,51} This notion may lead to false-negative diagnosis, particularly when there is inadequate sampling. However, the optimal number of biopsy specimens necessary to confirm the diagnosis of GSE is still not known. There are no recommendations in the guidelines of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition,\textsuperscript{52} while the American Gastroenterology Association has recommended 6 biopsy specimens as necessary for the diagnosis of GSE.\textsuperscript{53} In practice, it seems reasonable to suggest that at least 4 endoscopic biopsy samples must be taken from distal duodenum with 2 samples from the bulbus to detect patchy and subtle mucosal lesions in GSE. Biopsy specimens from the bulbus, however, should be interpreted with caution because this area is susceptible to peptic injury and contains prominent Brunner glands, which can lead to obliteration of the villi, with an artifically flat mucosal appearance.\textsuperscript{15,47,54}

**What Is “Normal”?**

Pathologists should be capable of recognizing normal features of small-intestinal mucosa so as to interpret the abnormalities associated with GSE correctly. It is generally accepted that the presence of at least 3 or 4 consecutive villi with a normal villous to crypt ratio in a biopsy sample is sufficient to consider as normal.\textsuperscript{12,15} Normal small-intestinal mucosa has long, slender villi, with this ratio ranging between 3:1 to 5:1 depending on the site of the biopsy; shorter villi are found in the duodenum, whereas the height of the villi increases distally from jejunum to ileum (Figure 1). Normal distribution of IELs along the villi shows a characteristic decrease from the base of the villus toward the villous tip and resembles the musical “decrescendo” sign.\textsuperscript{55} However, the pathologist should be aware of the possible patchiness of mucosal lesions when making a decision of normal mucosa and ruling out GSE.

For accurate histopathologic assessment of villus to crypt ratio, it is essential that the biopsy specimen be

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**Figure 1.** Normal duodenal mucosa with 3 to 4 long villi with a villous to crypt ratio of >3:1 and normal number of intraepithelial lymphocytes (hematoxylin-eosin, original magnification ×200).

**Figure 2.** Vertical orientation of the villi to the muscularis mucosae (hematoxylin-eosin, original magnification ×100).
properly oriented with the luminal surface upwards such that the villi should be vertical to the muscularis mucosae (Figure 2).

Specimens can be oriented on a supporting medium (eg, a strip of filter paper, a piece of tissue, or dental wax) with the naked eye or with the assistance of a dissecting microscope; embedded in wax; and cut through vertical plan. In tangentially cut sections, artificial shortening of the villi and falsely increased lymphocytes in the surface epithelium can occur. The approach to biopsy specimen by the pathologist is as important as the correct orientation and involves low-power microscopic examination for architectural abnormalities, as well as cellular distribution, and is followed by a high-power view to assess cellular content and epithelia. Although the exact numbers are not known, few inflammatory cells comprising plasma cells, lymphocytes, eosinophils, and macrophages are found in the lower one-third of the lamina propria, while villous lamina propria is considered as ‘‘empty’’ in normal small-intestinal mucosa. In the presence of inflammation, however, inflammatory cells, including polymorphonuclear leukocytes, infiltrate upper parts of the lamina propria and cause obliteration of the villi.

THE MORPHOLOGIC SPECTRUM OF GSE

Histologic evidence of GSE depends on abnormalities in either architecture (villous shortening and crypt hyperplasia) or the number of IELs or both. In its classical form, GSE results in shortened, widened villi or even totally flat mucosa with hyperplastic crypts. Overall thickness of the mucosa remains relatively unchanged, but villous to crypt ratio (normally 3:1 in distal duodenum and 2:1 in bulbus) decreases as the villi become shortened. These architectural changes are preceded by an increase in the number of IELs as compared to normal numbers, corresponding to the cell-mediated immune nature of the disorder. It is this group of cases that should be actively sought in biopsy specimens in which GSE is part of the clinical differential diagnosis.

Marsh was the first to describe the morphologic continuum of GSE, which ranges from normal villous architecture with IEL increase as the only abnormality to flat mucosa with crypt hyperplasia, increase in the number of IELs, epithelial destruction, and increased lamina propria inflammation. Marsh classification will be discussed in detail in association with other classification schemes in the following sections.

Intraepithelial Lymphocytes

Intraepithelial lymphocytes have been considered to be responsible for the epithelial damage observed in GSE, although the exact mechanism is still not known. Most IELs are T lymphocytes, which are mostly cytotoxic T cells expressing $\beta$2 T-cell receptor (TCR) on their surface. The population specifically expanded in GSE is the CD3+ CD4+ CD8+ y6 TCR-bearing IELs, which is only $5\%$ of the total in normal mucosa.

Over the years, the cutoff value, and thus the normal number of IELs, has been subject to major variation and a significant reduction has occurred in the highest value for normal. The trend toward a lower normal number of IELs is reflected by the decreasing numbers of IELs, including 25, 22, or even 20 IELs per 100 epithelial cells, incorporated into revised classification schemes. The reasons for this can partly be attributed to the change in biopsy site (see previous sections)—normal jejunal mucosa has a higher number of IELs than duodenal mucosa—and also to the decrease in section thickness over the years and to the application of immunohistochemistry to define IELs. Currently, the normal upper limit of IELs is accepted as 20 lymphocytes per 100 enterocytes (a ratio of 1 IEL per 5 enterocytes) in hematoxylin-eosin sections, whereas 25 IELs per 100 enterocytes (or a ratio of 1:4) is considered the upper limit of normal in CD3-immunostained slides.

It remains, however, to be determined whether lowering the upper limit for IELs will adversely affect the specificity of small-bowel biopsy in the diagnosis of GSE, since it may cause overlaps with other causes of IELosis. At this point, it would be wise to state that the greater the number of duodenal biopsy samples seen by the pathologist, the more accurately will be defined the normal range of IELs.

To Count or Not To Count?

Although there are various ways of enumerating IELs, almost all are impractical for incorporation into the routine pathology practice. Counting IELs per 100 enterocytes (usually in a total of 300–500 epithelial cells) has been the most widely used method, either with the aid of CD3 immunostaining or without. Recently, an alternative method of screening for GSE has been proposed in which IEL counts in villous tips (5 well-oriented villi, 20 enterocytes at the tip of each) can be used for a rapid assessment. The normal average number of IELs according to the ‘‘villous-tip’’ method is less than 5 per 20 enterocytes, while counts between 6 to 12 per 20 epithelial cells are considered suggestive of GSE. Routine application of CD3 immunohistochemistry has been suggested as a better means of evaluating the number and distribution of IELs when there is normal villous architecture in a biopsy specimen. In my experience, immunohistochemistry may, indeed, be very useful when there is a suspected, rather than a definite increase in IELs. In practice, however, I strongly believe that the distribution pattern of IELs within the epithelium is more valuable than the actual counts, since accurate quantification can be difficult because of nuclear overlap and resemblance of IELs to enterocyte nuclei and granulocytes. Patients with GSE, including those with normal villi in their biopsy specimen, lack the normal decrescendo pattern as a result of increased density of IELs in the distal tips of the villi, thus causing a diffuse infiltration of the villous epithelium. Therefore, a practical approach would be to scan the villi and look for the loss of normal decrescendo pattern or the presence of a diffuse and uniform infiltration. This approach would also serve to correctly interpret cases with mild increases in IEL numbers that may overlap with those of healthy individuals. It should be stressed, however, that the loss of normal distribution pattern is not a diagnostic, but a suggestive feature of GSE. In my experience, as well as that of others, immunohistochemical staining with CD3 helps to highlight the distribution pattern of IEL within the villous epithelium, though there are some contradictory reports in the literature (Figure 3, A and B).

Causes of IELosis

Intraepithelial lymphocytes are active components of the mucosal immune system, which undergoes threat from luminal antigens such as gluten, microorganisms, drugs, and other toxic molecules, all of which can cause an increase in IEL numbers. Although IELosis in a normal mucosa is an increasingly reported pathologic feature in GSE, it is by no means diagnostic, since overlap in the IEL
counts occurs between patients with and without GSE. Various pathologic processes, including food allergies other than GSE, *Helicobacter pylori*-associated duodenitis, giardiasis, graft-versus-host disease, tropical sprue, viral enteritis, injury caused by nonsteroidal anti-inflammatory drugs, chemoradiotherapy-induced enteritis, autoimmune enteropathy, immunodeficiencies, and Crohn disease can induce IELosis with or without associated architectural changes. Some of these entities are discussed in the differential diagnosis section and are listed in Table 1.

### Lamina Propria

Although considerable amount of research has focused mainly on IELs, lamina propria seems to have an even more important part in the pathogenesis of GSE. The presentation of gliadin peptides by antigen-presenting cells bearing DQ2 or DQ8 to lamina propria helper T cells leads to T cell activation and secretion of various cytokines. These events take place in the lamina propria and are considered to precede IEL infiltration. Because of the immunopathologic basis of the disease, the pathologists encounter a complex and heterogenous population of inflammatory cells in the lamina propria comprising plasma cells that locally produce antigliadin and EMAs in addition to T cells that include predominantly helper T cells as well as a few cytotoxic cells. Also, neutrophils, eosinophils, and mast cells may be found in varying numbers, although grading this inflammatory reaction is difficult and impractical. Since none of these changes is specific to GSE, IELosis and architectural changes affecting villous to crypt ratio remain as the main diagnostic parameters of pathologic evaluation.

### CLASSIFICATION OF GSE

On the basis of substantial amount of clinical research, including a sequence of dynamic studies, Marsh first introduced the morphologic continuum of gluten sensitivity. Marsh classification is composed of 4 consecutive states of mucosal damage (types 1–4) as follows.

**Infiltrative lesion:** Increased numbers of IELs in the villus epithelium in an otherwise normal mucosa with normal villous to crypt ratio (Marsh type 1). A type 1 lesion may be observed in patients with GSE who follow a GFD and indicate that minimal amounts of gliadin are still ingested or that the patient is not yet in full remission. More importantly, it is found in latent GSE, in family members of patients with GSE (potential candidates for celiac disease), and in some individuals with dermatitis herpetiformis. An increase in the number of IELs is the first and most sensitive index of gluten effect on the mucosa and, although not specific, is the most characteristic histologic feature of GSE. Gluten sensitivity has also been known to affect other parts of the gastrointestinal tract, including esophagus, stomach, and large intestine and, interestingly enough, IELs are also increased in these areas. In accordance with this information, rectal biopsy, performed before and after a rectal gluten challenge, was proposed as a simpler means of diagnosing GSE. However, it has not gained international acceptance as a useful clinical tool and thus, pathologists are currently unlikely to receive rectal biopsy specimens taken for the diagnosis of GSE other than for research purposes.

**Hyperplastic lesion:** Crypt hyperplasia with normal villi showing increased numbers of IELs (Marsh type 2). The importance of this lesion is that it was first described by...
Mowat and Ferguson in a neonatal mouse model of graft-versus-host disease. It therefore clearly represents another distinctive, T-cell–mediated immune response of intestinal mucosa. Type 2 lesion is very rarely, if ever, encountered in the biopsy samples of patients with GSE and has mainly been observed under experimental conditions or time-dose–related gluten challenge studies. The results of these studies demonstrated that crypt hyperplasia was the first architectural change in the evolution of the mucosal lesion, initiated by the increase in IELs. Crypt hyperplasia or elongation of the length of the crypt is a process that precedes villous shortening despite the general misconception that it is a compensatory feature occurring in response to villous shortening. Elongation may be caused by the interaction of stromal cells with the epithelium through the secretion of various cytokines and influx of inflammatory cells releasing growth factors. In the advanced stages of the disease, matrix metalloproteinases and their tissue inhibitors may also play a role in the development of mucosal architectural changes through their degradative action on the stromal cells of the lamina propria.

Although I believe that the original type 2 (hyperplastic) lesion clearly exists within the spectrum of gluten sensitivity, in routine practice we do not see these lesions in the form that they were originally described by Marsh. In his schematic drawings, the type 2 lesion has always been illustrated as normal villi with elongated crypts. In practice, however, when there is crypt hyperplasia, it is always accompanied by shortened villi. Similarly, for treated patients with celiac disease, one observes restoration of villi (still short, though) together with crypt hyperplasia, which again is different from Marsh’s original type 2 lesion. This led to a confusion among pathologists who have (naturally) interpreted Marsh type 2 lesion as hyperplasia, which again is different from Marsh’s original lesion. The photographs illustrating Marsh type 2 lesions are those of mucosa with shortened villi and hyperplastic crypts, in contrast to Marsh’s original definition. The type of lesion occurs only in strictly time-dose–dependent gluten challenge studies, where it is possible to witness mucosal abnormalities in a more dynamic manner. In one such study, in which jejunal biopsy was performed before and after 4 years, has been proposed after 4 years, has been classified in a neonatal mouse model of GSE.

The second revision in this classification was the subgrouping of type 3 lesion (flat mucosa) into 3 grades with regard to the severity of villous shortening (‘‘atrophy’’ in the original report). The authors have classified Marsh type 3 lesions as follows:

- Type 3A: Mild villous atrophy. Mild villous flattening indicates minor or moderate degree of shortening and blunting of villi.
- Type 3B: Marked villous atrophy. Marked villous flattening indicates that only short tentlike remnants of the villi are present.
- Type 3C: Completely flat mucosa. Total villous flattening or flat mucosa indicates that no more villi can be recognized and that the surface is flat.

Although the Marsh classification, as modified by Oberhuber et al, is used by many pathologists, both for diagnosis and also to assess the response to therapy, this classification has the potential to cause significant reproducibility problems leading to increased intraobserver and interobserver variations because of the greater number of diagnostic categories. Moreover, definitions of type 3A lesions (as mild or moderate villous shortening) and type 3B lesions (as marked villous shortening) lack objective criteria with respect to villous to crypt ratio, thereby increasing the subjectivity of interpretation.

Recently, a new and relatively simpler classification was proposed by Corazza and Villanaci. Their 3-grade classification maintains the original type 1 (infiltrative) lesion as grade A, while type 2 (hyperplastic) lesion is totally left out by the authors, stating that it has no additional use for diagnosing cases that would already be identified by the increased IEL counts. Oberhuber type 3A and 3B lesions are grouped into a single grade as grade B1,
whereas type 3C, the flat mucosa, is maintained as grade B2. Marsh type 4 (atrophic) lesion has been made obsolete by the recent finding that refractory sprue, ulcerative jejunitis, and EITCL are all characterized by aberrant clonal T-cell expansions, as demonstrated by immunohistochemical and molecular techniques.12,68-69 In summary, Corazza and Villanaci24 have proposed that lesions characterizing GSE should be classified as nonatrophic (grade A) and atrophic (grade B) and that grade B lesions should be split into (1) grade B1, characterized by a villous to crypt ratio of less than 3 to 1, and (2) grade B2, characterized by completely flat mucosa with no detectable villi. Although there are no obvious problems with this proposal, I would object to the use of the term grade to classify the mucosal lesions, since the severity of mucosal lesion does not necessarily indicate clinical severity. Moreover, the term grade is used for tumor differentiation, thereby, making it inappropriate for classifying the mucosal pathology of GSE. I would personally suggest that we retain the term type, not only because it is accepted both by the gastroenterologists and pathologists, but also as a tribute to Marsh. I would also object to the use of the term atrophy to define villous architectural changes, as dynamic studies in the past have shown that the mucosa does not at all undergo a process of atrophy, but rather it demonstrates a hyperplastic state characterized by elongation of the crypts and widening of the lamina propria by inflammation. This observation is also supported by the finding that the height of the mucosa is not changed when an infiltrative lesion progresses to a flat lesion.27-40 Highlighting the term atrophy as the major feature of immunologically driven pathologic processes that are induced by gluten fails to include the condition of most patients, who often present with milder lesions devoid of atrophy, or more appropriately, villous flattening.35,50

The “New” Version of an “Old” Classification

The above discussion highlights the problems regarding the classification systems currently used for GSE. The growing number of duodenal biopsies taken for suspected GSE, however, potentiates the pathologist’s need for a more reproducible and standardized classification, which is equally appealing to clinicians. At this point, I would like to propose an updated version of the original Marsh classification, which, from a pathologist’s point of view, is much simpler and user-friendly. In the proposed classification, mucosal pathologic features will be defined in 3 groups, mainly depending on the degree of cellular and architectural abnormalities.

Type 1: Normal villi with IELosis (Figure 4, A). This type corresponds to Marsh type 1, also present in the Oberhuber classification, and to grade A in Corazza and Villanaci’s proposal.

Type 2: Shortened villi (<3:1 or <2:1 in bulbus) with IELosis and crypt hyperplasia (Figure 4, B). This type corresponds to types 3A and 3B in the Oberhuber classification and to grade B1 in Corazza and Villanaci’s proposal.

Type 3: Completely flat mucosa with IELosis and crypt hyperplasia (Figure 4, C). This type corresponds to Marsh type 3, and also to type 3C in the Oberhuber classification, and to grade B2 in Corazza and Villanaci’s proposal.

While types 1 and 3 are exactly identical to their counterparts in the original Marsh classification, type 2, in the current proposal, comprises cases with crypt hyperplasia and any degree of villous shortening but not flattening (ie, villi can still be identified). As already stressed, villous shortening must be evaluated according to the biopsy site, since the normal villous to crypt ratio varies throughout the small intestine. Thus, types 2 and 4 of the original Marsh classification have been made obsolete in this proposal because of the reasons already discussed in the previous paragraphs. The similarity of this new version to the original Marsh classification may help to gain a wider acceptance from both pathologists and clinicians; however, further validation through clinicopathologic studies is necessary. Comparison of all 4 classifications is presented in Table 2.

VARIANTS OF GSE

Patients with incomplete or no response to GFD are considered within the context of refractory sprue. Diagnosis of refractory sprue is rarely made and should be strictly limited to patients with clinical features of GSE not responding to GFD for at least 6 months. It can be either primary, as lack of initial response to diet, or secondary, as unresponsiveness to diet in the form of a relapse. Primary refractory sprue can include many different pathologic conditions mimicking GSE, comprising collagenous sprue, ulcerative jejunitis, and EITCL.68,69 Histopathologically, most cases with refractory sprue show flat or near flat mucosa with a dense mononuclear infiltrate of mainly plasma cells and lymphocytes in the lamina propria and a massive increase in IELs (Figure 5, A). The lymphocytes in the villous, crypt epithelium, and lamina propria are normal to medium in size, with a normal cytologic appearance (Figure 5, B). However, most cases of refractory sprue have abnormal T-cell phenotypes (CD103+ and CD4−/CD8−/TCR-) showing clonal expansions as proved by monoclonal rearrangements of the TCR γ gene. These features have led to the idea that such cases represent a form of “in situ” T-cell lymphoma.12,15,65-90

Collagenous sprue refers to a variant entity having a thick collagen table beneath the surface epithelium of a mucosa, which also has other typical features of GSE, including IELosis.12,14,15,91 Patients with long-standing malabsorption can develop EITCL as the most fearsome complication of GSE. They usually suffer from severe malabsorption refractory to GFD, causing weight loss and complications such as perforations and bleeding due to ulcerative lesions. Histopathologically, the mucosa is flat with ulcerations. An atypical population of neoplastic lymphocytes infiltrate the mucosa. Lymphoma cells are pleomorphic large cells that are double-negative (CD8− and CD4−) for cell surface markers, but almost always positive for CD30, which is also associated with poor prognosis.55-90

DIFFERENTIAL DIAGNOSIS

Differential diagnosis of GSE involves a great variety of disorders with architectural abnormalities and/or IELosis (see Table 1). Among these, only conditions that are likely to be encountered by the pathologist in routine duodenal biopsies will be briefly mentioned here.

Chronic H pylori–associated duodenitis: Helicobacter pylori gastritis can cause an increase in IELs in duodenal bulb, which causes a major diagnostic confusion with GSE. This is particularly a problem when distal duodenum is not biopsied, since it is usually normal in H pylori infection.54 Helicobacter pylori colonizes the duodenal...
mucosa only in areas of gastric foveolar metaplasia, which can lead to active duodenitis.\textsuperscript{92–94} Features useful in distinguishing this condition from GSE are the heavy neutrophilic infiltration of the lamina propria, as well as the surface epithelium; relatively less architectural damage in terms of villous shortening; and the presence of foveolar metaplasia, though the latter may also be seen in GSE if the biopsy is taken from the bulbus affected by \textit{H. pylori} (personal observation).

Food allergy: Hypersensitivity to food antigens other than gluten, including cow’s milk, soy protein, fish, rice, and chicken may also be associated with increased numbers of IELs, as well as architectural changes in the form of patchy or diffuse disease in any part of the gastrointestinal tract in affected individuals. Lymphoid hyperplasia in the bulbus is a common feature of food allergy together with eosinophilic infiltration of the lamina propria and IELosis, especially in children, while villous shortening is only rarely encountered.\textsuperscript{14,95,96}

Infections: A wide variety of infectious agents including viruses, parasites, and bacteria can affect small-intestinal mucosa. Since it is beyond the scope of this review to discuss these in detail, a general view will be given. Most infections cause only mild and nonspecific alterations in the intestinal mucosa including IELosis, lamina propria inflammation, and rarely, architectural changes, whereas some, such as giardiasis; Whipple disease; opportunistic infections including microsporidiosis, cryptosporidiosis, and CMV can be diagnosed by identifying the microorganism in biopsy specimens. Mucosal histologic features in bacterial overgrowth, either due to gastric hypochlorhydria or dysmotility, may be normal but often show architectural changes as well as increased IEL counts in a patchy fashion.\textsuperscript{12,14,97,98}

![Figure 4](image1.png)

\textbf{Figure 4.} New proposal for classification of mucosal pathology in gluten-sensitive enteropathy. \textbf{A}, Type 1 lesion showing normal mucosa with diffuse increase in intraepithelial lymphocytes (IELs). \textbf{B}, Type 2 lesion with villous shortening and diffuse increase in IELs. \textbf{C}, Type 3 lesion with completely flat mucosa and diffuse increase in IELs (anti-CD3 immunostain, streptavidin-biotin-px, original magnification $\times$200 \textbf{[A]}; hematoxylin-eosin, original magnifications $\times$200 \textbf{[B} and \textbf{C}]).

![Figure 5](image2.png)

\textbf{Figure 5.} \textbf{A}, Flat mucosa with crypt hypoplasia and diffuse lymphoid infiltration in the lamina propria. \textbf{B}, Medium-sized lymphocytes infiltrating lamina propria and surface epithelium (hematoxylin-eosin, original magnifications $\times$200 \textbf{[A]} and $\times$400 \textbf{[B]}).
Autoimmune enteropathy: This condition, caused by autoantibodies to enterocytes, primarily affects children. Histologic findings of villous flattening and crypt hyperplasia are similar to GSE, but the resultant secretory diarrhea is unresponsive to GFD or total parenteral nutrition. There may be some degree of IEL increase, but neutrophils are more prominent in the surface epithelium. 12,14,15,90

Crohn disease: Although the exact frequency of upper gastrointestinal involvement in Crohn disease is not known, it can represent up to 30% to 50% of cases in some centers. Granulomas are uncommon, but when found, they are usually more often in the stomach than the duodenum and can be helpful in the differential diagnosis. 12,14,100,101

REPORTING

Histopathologically, the manifestations of GSE display a range in severity. Also, a long list of entities cause a pathologic picture similar to that of GSE, thereby complicating the histopathologic diagnosis and the pathologic reporting. I believe that the small-intestinal biopsy is essential in the diagnosis of GSE and that a biopsy specimen is not properly oriented. An IEL count of more than 20 per 100 enterocytes in hematoxylin-eosin sections (>25 in CD3 immunostaining) should be considered a definite increase and reported as “compatible with a diagnosis of GSE” if it is diffusely distributed over the villi.

As our understanding of the disease has changed over the years, a need for modification of the original classification of mucosal pathology has emerged. A simpler and clarified version, comprising identical counterparts of original Marsh types 1 and 3 lesions, and the “new” type 2 lesion, which is redefined as crypt hyperplasia with shortened but not flattened villi, is proposed in this review. This proposal is based not only on the vast amount of information available in the literature but also on the research data from Marsh’s laboratory and from almost 20 years of personal experience in small-intestinal pathology.

CONCLUSIONS

Widely varying clinical presentations and mucosal pathologic features of GSE make the diagnosis difficult for the pathologist. Although considered as characteristic, IELosis can be observed in a number of other diseases affecting the small intestine, thereby complicating the diagnostic picture as there is no cutoff for IEL counts for distinguishing GSE from other causes of IELosis. Therefore, in routine workup, the pathologist should not feel obliged to enumerate IELs, but rather should scan through the biopsy pieces to see whether there is a definite increase in IELs and whether it is diffuse or absent from villous tips. CD3 immunostaining is recommended to highlight the distribution pattern of IELs. Counting is useful when there is no serologic correlation. It may also help when there is no obvious increase in IEL counts or when the biopsy specimen is not properly oriented. An IEL count of more than 20 per 100 enterocytes in hematoxylin-eosin sections (>25 in CD3 immunostaining) should be considered a definite increase and reported as “compatible with a diagnosis of GSE” if it is diffusely distributed over the villi.

REFERENCES


Table 2. Classification Schemes For Pathologic Evaluation of Gluten-Sensitive Enteropathy

<table>
<thead>
<tr>
<th>Marsh,14,15,90</th>
<th>Oberhuber et al,12,22,23,24,34</th>
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