Abstract

We studied small bowel biopsy specimens with architecturally normal villi from 78 adult patients with potential gluten sensitivity (GS) and correlated them with outcome to characterize morphologic features that would allow a pathologist to suggest GS. No patient had a previous GS diagnosis. Twelve study patients had GS. The mean number of intraepithelial lymphocytes (IELs) per 20 enterocytes from the tips of 5 random villi was significantly greater in GS than non-GS biopsy samples, but the groups overlapped significantly, making the number diagnostically useful only when markedly increased. Crypt mitoses counts had similar relationships. Twelve patients had an even distribution of IELs along villus sides and over tips (3/66 [5%] non-GS patients, 9/12 [75%] GS patients). Non-GS patients had a decrescendo pattern of IELs along the sides of villi. Architecturally normal small bowel biopsy specimens with an appreciable, continuous, even distribution of IELs along the sides and tips of villi and a mean of 12 or more IELs in the tips of several villi are suggestive of GS. Pathologists should be watchful for these morphologic features in small bowel biopsy specimens to suggest GS.

Gluten sensitivity can cause a broad range of signs and symptoms, including none, one, or a few nonclassic symptoms to full-blown celiac sprue–type malnutrition, weight loss, diarrhea, and steatorrhea. It is estimated that nonclassic manifestations in adults, such as microcytic anemia, folate deficiency, mild diarrhea, flatulence, loose stools, neurologic complaints, and severe osteoporosis, constitute the predominant complaint in 20% to 50% of gluten-sensitive (GS) patients. Because of the large number of GS patients with nonclassic signs and symptoms, it is widely agreed that a large number of adult GS patients in the United States go undiagnosed. One recommended clinical approach to remedy this deficiency is to explore the distal duodenum or jejunum during upper endoscopy in all patients.

Similar to its protean clinical manifestations, GS can produce a spectrum of morphologic abnormalities within the proximal small bowel mucosa, ranging from architecturally normal villi to complete flattening. It does not seem to be common knowledge among United States pathologists that clinically significant GS can be associated with architecturally normal proximal small bowel villi. No morphologic descriptions or discussions of the minimally abnormal small bowel biopsy specimen from untreated GS patients are mentioned in the major gastrointestinal pathology and general surgical pathology textbooks. Morphologic criteria that raise the possibility of GS in the context of an architecturally normal small bowel biopsy specimen would be useful to avoid overlooking the diagnosis in this subset of GS patients. Identification of GS is important because treatment is thought to reduce or prevent the development of malignant neoplasms, chronic iron deficiency anemia, and osteoporosis.
We studied 78 patients with architecturally normal villi in which GS was a consideration but had not been investigated completely. The goal of our study was to identify the morphologic features associated with GS in the context of small bowel mucosa with architecturally normal villi.

Methods

From January 1994 through February 2000, 727 patients older than 16 years underwent upper endoscopy with distal duodenal or jejunal biopsy and had an antigliadin serum antibody test (IgA or IgG) at William Beaumont Hospital (Royal Oak, MI). For the 78 patients who constituted the study group, clinical follow-up information and IgA antiendomysium and IgA antigliadin serum antibody titers were available; they had no previous diagnosis of GS and no small bowel biopsy specimens that revealed partial, subtotal, or complete villus flattening.

A control group of 24 untreated patients with gastroesophageal reflux disease (GERD) with distal small bowel biopsy specimens procured at the time of upper endoscopy were selected from a large pool of patients with GERD to produce a group that was age- and sex-matched with the study group patients. For all GERD control patients, intraepithelial neutrophils and eosinophils were present in their esophageal squamous mucosa, and antral biopsy specimens were normal and devoid of gastritis.

The morphologic features quantified from each small bowel biopsy were as follows:
1. Number of small bowel tissue fragments.
2. Villus tip intraepithelial lymphocyte (IEL) score calculated by counting the number of IELs per 20 enterocytes from 5 randomly chosen villus tips and then computing the mean value of the 5 villus tip IEL counts.
3. Mean number of mitoses per 20 crypt profiles.
4. Pattern and distribution of villus IELs. Normal patterns of villus IELs were either an insufficient number of IELs to produce an appreciable, even distribution of IELs along the sides and over the tips of villi or a sparse IEL infiltrate in the basal portion of villi that decreased to no appreciable pattern (decrescendo-like) in the middle and upper regions of villi. An abnormal IEL distribution pattern was an appreciable, sparse to dense IEL infiltrate along the length of the villus and over its tip (see the "Results" section for a detailed description). The evaluation of IEL distribution was easiest using intermediate magnification, at which the majority of a villus could be evaluated within a single microscopic field. This feature became difficult to judge when the magnification was such that evaluation of a villus required several movements of the slide.

All small bowel biopsy specimens were Hollandes fixed and paraffin embedded. Three groups of section ribbons, separated by approximately 50 µm, were cut from each block. Each group of 3 or 4 section ribbons was placed on a separate slide. All slides were stained with H&E.

Clinical factors recorded from the endoscopic report were as follows:
1. Patient sex and age at initial upper endoscopy.
2. Preendoscopic symptoms.
3. Date and status of GS-related serologic tests, including IgA antigliadin and IgA antiendomysium antibody titers. A positive antigliadin titer was greater than 19 U/mL, and a positive IgA antiendomysium titer was greater than 1:5.
4. Date of last contact.
5. Disease status at last contact date, categorized as having GS or not having GS. A patient was gluten sensitive based on response to a gluten-free diet, antibody status, and morphologic features of subsequent small bowel biopsy specimens.

The SAS, version 8.0, statistics program (SAS, Cary, NC) was used for the Fisher exact test, linear regression, and logistic regression analyses. Follow-up and other clinical information was procured predominantly from the William Beaumont health care system information system.

Morphologic features initially were quantified without the knowledge of the clinical outcome of the patient. All biopsy specimens were reevaluated after the diagnostic codes were broken, keeping the results of the blind evaluation in mind. The goal of the second slide review was to evaluate whether the morphologic features that were significantly associated with GS could be used in daily practice.

Results

Clinical Findings

Of the 78 study patients, 51 (65%) were women. The overall mean age at initial endoscopy was 35.9 years, and the mean follow-up period after initial endoscopy was 15.4 months. Twenty-nine patients (37%) had only irritable bowel syndrome or irritable bowel syndrome and dyspepsia, 27 patients (35%) had microcytic anemia, 14 patients (18%) had dyspepsia only, and 8 patients (10%) had steatorrhea.

Twelve (15%) of 78 study patients had gluten sensitivity, 8 of whom were women. The mean patient age of this group was 35.2 years, and the mean follow-up period after initial endoscopy was 17.1 months. A GS diagnosis was established in 10 patients based on symptom improvement while on a gluten free diet; of these 10 patients, 8 also had subsequent positive serum endomysium antibody titers, 5
had subsequent positive serum IgA gliadin antibody titers, and 4 had subsequent endoscopy and small bowel biopsy specimens that showed classic, full-blown celiac sprue lesions (Marsh stage IV lesions) with complete villus flattening. The GS diagnosis was established in 2 patients by subsequent endoscopies and small bowel biopsies that showed classic, full-blown celiac sprue lesions (Marsh stage IV lesions) with complete villous flattening. Both patients also had subsequent positive serum endomysium antibody titers and serum IgA gliadin antibody titers.

The mean age of the 66 study non-GS patients was 36.1 years. Forty-nine (74%) of the patients were female. The mean follow-up period after the initial endoscopy was 15.1 months. Three patients had positive serum antiendomysium titer results. All 3 underwent a gluten-free diet trial but had no improvement of microcytic anemia (2 patients) or irritable bowel syndrome–related (1 patient) complaints. Thirteen of the 66 non-GS study patients had positive serum antigliadin titer results, all of whom had negative serum antiendomysium titer results. Of these 13 serum antigliadin titer–positive patients, 5 underwent a subsequent endoscopy that showed normal small bowel histologic features. Of the 13 patients, 10 had Helicobacter pylori–positive antral gastritis and or GERD changes in the lower esophageal squamous mucosa.

The 24 age- and sex-matched control subjects with GERD had a mean age of 34.5 years, and 18 (75%) were women.

There were no statistical differences in the mean patient ages between the study and control groups ($P = .63$) and the study GS and study non-GS groups ($P = .84$). The mean number of biopsy fragments was similar in the 3 groups ($P = .361$ and $P = .447$, respectively). The mean follow-up periods were similar between study GS and study non-GS patients ($P = .678$).

**Pathologic Features**

The mean villus tip IEL scores were 11.6, 4.3, and 2.2 in study GS, study non-GS, and control groups, respectively, and the median villus tip IEL scores for these 3 groups were

**Image 1**

A Normal villus. A few intraepithelial lymphocytes (IELs) are present along the sides and over the villus tip, but no linear pattern can be detected (H&E, ×50).

B Normal villus; higher magnification of Image 1A. A slightly greater number of IELs can be seen in the villus base than along the upper third. No IEL distribution pattern can be detected (H&E, ×160).

**Image 2**

Normal villus with an intraepithelial lymphocyte (IEL) decrescendo pattern. Increased IELs along the basal half of the villus produce an appreciable pattern of increased IELs. However, this pattern disappears (decrescendos) to scattered IELs along the upper third of the side and over the tip (H&E, ×36).
The SDs for villus tip IEL scores were large in both the GS and the non-GS study groups. Many of the non-GS patients and half of the GS patients had similar villus tip IEL scores. On review, only the GS biopsy specimens with villus tip IEL scores substantially greater than the median value were recognized as abnormal.

The mean crypt mitotic counts were 18.8, 12.6, and 12.7 in GS, study non-GS, and control groups, respectively. Identical to villus tip IEL scores, there was substantial overlap in the mitotic counts among the 2 study groups and the control group. On re-review, only the biopsy specimens in which the mitotic count approached the upper end of the GS patient range could be identified as probably abnormal.

Twelve biopsy specimens had an appreciable, even IEL distribution along the lengths of most villi. Of these 12 patients, 9 (75%) had GS and the other 3 were study non-GS patients. The even IEL distribution and lack of an IEL decrescendo pattern along the sides of villi were conspicuous in biopsy specimens with large mean villus tip IEL scores (Images 3 and 4). All 6 GS patients with villus tip IEL scores above the median value for GS patients had an even distribution of villus IELs.

The even IEL distribution pattern could be seen in 6 cases with villus tip IEL scores that were within the range of, or close to, that for control subjects. These 6 patients included 3 study GS patients and 3 study non-GS patients. The latter 3 patients had villus tip IEL scores of 5.1, 6.7, and 8.2, respectively. All 6 biopsy specimens had a sparse but even distribution of IELs along the sides of villi and over their tips. The distribution of the IELs along the upper third and over the villus tips were similar to the IEL pattern in the lower third of the villus and as it emerged from the adjacent flat mucosa.

Two of the 3 biopsy specimens from GS patients that displayed a normal decrescendo pattern in IEL density had villus tip IEL scores of 3 and 4.8, respectively, and the third had a score of 10.9.

**Feature Comparison**

Positive IgA gliadin and IgA endomysial serum antibodies were associated with GS ($P = .024$ and $P < .01$, respectively).

Mean villus tip IEL scores and crypt mitoses were significantly greater in study GS patients than in study non-GS patients ($P < .01$ and $P < .01$, respectively). The mean
villus tip IEL scores also were significantly greater in study non-GS patients than in control subjects ($P < .01$). An even IEL distribution pattern along villi was present significantly more often in biopsy specimens from study GS than study non-GS patients ($P < .01$).

Of the histologic features, only an even IEL distribution along villi was associated independently with a positive serum antibody result ($P < .01$) on multivariate analysis.

**Discussion**

Gluten sensitivity can produce a spectrum of villus architectural abnormalities of the proximal small bowel mucosa, ranging from normal to flat.$^{17,29-32,39-42}$ Marsh and others$^{17,30-32,39-46}$ characterized these abnormalities and classified the biopsy specimens with architecturally normal villi and increased IELs as stage I infiltrative lesions. Terms that
have been applied to GS patients with architecturally normal small bowel mucosa include subclinical or silent celiac sprue, gluten-sensitive disease with mild enteropathy, low-grade enteropathy, latent celiac sprue, minimally symptomatic enteropathy or celiac sprue, high-density IEL enteropathy, and potential celiac disease.6,13,20,37,40,47-52 Marsh recommended, and we agree with, the term gluten sensitivity rather than celiac sprue, which reflects increased immunologic sensitivity to gluten in genetically susceptible people.17,29,30,32 Regardless of the name, many authors have recognized that considerable GS symptoms can be associated with architecturally normal small bowel mucosa with increased IELs.6,13,17,32,37,39,40,43-67 The present study focused on morphologic features suggestive of GS in small bowel biopsy specimens with architecturally normal villi, which constitutes a major problem in the diagnosis of GS in many patients.30-32,37,51 Identification of these patients is important because treatment of GS is thought to reduce or prevent the development of malignant neoplasms, chronic iron deficiency anemia, and osteoporosis.29-32,36-38

We found that an appreciable pattern of an even distribution of IELs along villi was the most sensitive morphologic feature for GS. Of the GS biopsy specimens, 75% (9/12) displayed this feature compared with 4% (3/68) of study non-GS and 0% (0/24) of control specimens. This pattern also could be appreciated in 3 (25%) of the GS cases in which there was no quantitative increase in villus tip IELs. However, the even IEL distribution pattern as a diagnostic marker of GS seems limited because of its specificity; 25% of the patients with this feature did not have GS. We view this morphologic pattern as a flag for the potential presence of GS.

An increased mean villus tip IEL score also was associated with GS. This result replicates the findings of other authors.40,54,56,68,69 An increased number of IELs is one of the earliest morphologic features of GS in response to gluten ingestion, occurring before villus flattening.37,40,44,58,63,70 In practice, however, we believe, similar to other authors, that formal IEL counts have limited use unless the count is markedly increased because of an extensive overlap in the number of IELs between GS and non-GS study patients.48

We chose to quantify the number of IELs per 20 enterocytes over 5 villi because it was fast and simple. Our intention was not to create a novel IEL quantification method to replace existing systems. Rather, our goal was to create a system that would detect marked elevations in IELs and, therefore, could be used in routine practice by busy surgical pathologists. Twenty villus tip enterocytes was used as the

<table>
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<th>Villus Tip IEL Score</th>
<th>IEL Distribution Along Villi</th>
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<tbody>
<tr>
<td>3</td>
<td>Normal/decrescendo</td>
</tr>
<tr>
<td>4.8</td>
<td>Normal/decrescendo</td>
</tr>
<tr>
<td>6.6</td>
<td>Abnormal/even</td>
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<td>8.9</td>
<td>Abnormal/even</td>
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<tr>
<td>9.0</td>
<td>Abnormal/even</td>
</tr>
<tr>
<td>10.9</td>
<td>Normal/decrescendo</td>
</tr>
<tr>
<td>&gt;11.3 (n = 6) (median)</td>
<td>Abnormal/even (n = 6)</td>
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</tbody>
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IEL, intraepithelial lymphocyte.
denominator because it is a round number and is the approximate number of enterocytes that cover the tips of most villi on cross-section. We believe that quantifying IELs per 1,000 enterocytes is too onerous and computer-drive methods are too laborious to be used in daily practice.

Similar to the IEL distribution pattern, increased villus tip IELs are suggestive, but not diagnostic, of GS because diseases other than GS can have increased IELs. T-cell receptor (TCR) gamma/delta–positive cells account for the majority of IELs found in untreated GS, and the increased IELs in other diseases are usually, but not always, cells other than the TCR gamma/delta–positive subset. Morphologic TCR analysis currently can be evaluated only on fresh frozen tissue. Antibodies to TCRs that work in formalin-fixed tissue are not available.

Several issues require elaboration. First, biopsy findings for patients with nonclassic GS symptoms should not be misconstrued as a reflection of architecturally normal villi. The length of injured small bowel, rather than the amount of villus flattening, determines the severity of malabsorptive symptoms. GS patients with Marsh stage IV morphologic features of complete villus flattening can be asymptomatic if the length of involved proximal small bowel is sufficiently short. Second, some GS patients in the study initially did not have positive GS-related antibodies, which reaffirms the findings of most studies of a small percentage of false-negative and false-positive test results. Serum antiendomysium titers are more specific than antigliadin titers for GS. However, serum antiendomysium may not be optimally sensitive in patients with architecturally normal villi because its presence in the serum is associated with villus injury or flat mucosa. A small bowel biopsy specimen with the morphologic features diagnostic of GS therefore remains a requisite criterion before a diagnosis of GS is made. Third, the patients in the present study do not represent a cross-section of patients undergoing upper endoscopy at our institution. There was a strong bias toward GS patients in this small selected study group, evidenced by a 15% GS prevalence, compared with an estimated prevalence of less than 1% in the general population. Fourth, the morphologic features associated with GS described herein are intended to suggest the possibility of GS, especially when only one morphologic feature is present. We do not believe they are definitive morphologic features of GS. Last, we chose not to study villus shape and density of lamina propria lymphoplasmacytic inflammation because of the variation that can be found in these features. The shape of villi can vary within a single biopsy fragment, with long and thin, leaf-shaped and clubbed, and occasionally blunted and broad-based villi all being described in healthy patients. A great amount of this variation can be attributed to the presence or absence of an attached muscularis mucosa, for its absence alters the villus shape. Similarly, the number of lamina propria lymphocytes and plasma cells can be so variable that it defies the establishment of normal parameters.

Distal duodenal or jejunal biopsy specimens with a continuous, even distribution of IELs along the sides and tips of villi are suggestive of GS but are a less frequent finding. Pathologists should be watchful for these morphologic features in small bowel biopsy specimens in order to raise the possibility of GS.

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References

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