Chapter 13

Type 1 Marsh Celiac Disease: Diagnosis and Response

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Abstract

The histological Marsh classification distinguishes three types of lesion, among which gluten-sensitive enteropathy with a Type 1 Marsh lesion is the most difficult to diagnose. Unlike Marsh 3 lesion, which almost always corresponds to celiac disease, Marsh 1 lesion has a wider differential diagnosis. This is further compounded by the absence of celiac disease-specific antibodies in up to 80% of the patients with a Marsh 1 lesion. For all these reasons, gluten-sensitive enteropathy diagnosis in Marsh type 1 lesions has become a challenge for clinicians. In recent years, new diagnostic techniques have emerged in order to distinguish gluten-dependent from non-gluten dependent Marsh 1 lesions. In this sense, the presence of transglutaminase IgA subepithelial deposits or increased intraepithelial lymphocytes expressing TCR gamma/delta in the duodenal mucosa strongly suggest the diagnosis of celiac disease. Another important issue is to determine which patients with a Marsh type 1 lesion should be treated. It should be noted that up to 50% of patients with minimal lesions present the same symptoms as those with Marsh 3 lesion, which suggests that they will benefit from a Gluten-Free Diet (GFD). Ultimately, the diagnosis of celiac disease cannot rely on the results of a single test and requires a good understanding of clinical, serological, genetic and histological criteria and of the GFD response.

1. Introduction

Celiac disease (CD) is an enteropathy caused by an immune reaction triggered by dietary gluten, a protein found in wheat, rye and barley, that manifests in genetically predisposed individuals. Since the first morphological lesion description by John Paulley in 1954, CD diagnosis was based precisely on the demonstration of the characteristic, gluten-dependent small intestinal lesion. This basic general concept is still valid. However, in recent decades, the discovery of accurate diagnostic methods (serological and genetic), through mass screening techniques or evaluating risk groups, has allowed the identification of large numbers of patients with silent or paucisymptomatic forms. This has afforded the knowledge that CD is not a rare disease, that its spectrum of clinical manifestations, both in type and severity, is very wide, and that there is not always a correlation between the severity of the histological lesion and intensity of the clinical manifestations. In this regard, an important change in CD diagnostic criteria has been the gradual acceptance that histologically mild enteropathy forms (type 1 Marsh lesions, also called lymphocytic enteritis, lymphocytic enteropathy or lymphocytic duodenosis) are also part of the CD spectrum and are to be treated as such when they produce clinically relevant symptoms or signs.

2. Histological Spectrum of Celiac Disease

In 1992 Michael N. Marsh published a classification scheme of histological lesion degrees based on the results of dynamic studies on gluten challenge which allowed to describe the whole histological injury spectrum.[1] This classification, subsequently modified by Oberhuber, Granditsch and Vogelsang, is the most widely accepted one among clinicians and patologists.[2] However, simpler schemes have been proposed, with fewer categories, allowing a greater degree of consistency and reproducibility between pathologists (Table 1).[3,4] In these more recent classifications type 2 or crypt hyperplasia has been eliminated, as this histological lesion phase is very unstable (fleetingly detected during lesion's progression towards atrophy)[1] and type 4 (related to CD refractory forms) which is usually diagnosed with cytometric and immunohistochemical techniques, showing an aberrant clonal expansion,[4] has also been eliminated.

In the most recent classification scheme, Ensari proposes maintaining Corazza's classification of lesion severity levels, but exchanges the term "degree" for "type", in order to avoid using a term which pathologists use for grading tumors.[4]

Thus, the most recent classification scheme foresees 3 levels of lesion severity:

Type 1: Preserved villous structure with increased intraepithelial lymphocytes (lymphocytic enteropathy, lymphocytic duodenosis or lymphocytic enteritis) and the few detected cases of crypt hyperplasia.
Type 2: Villi shortening (<3:1 or <2:1 in duodenal bulb) plus type 1 findings.
Type 3: Complete flattening of the villi plus type 1 findings.

An essential aspect of anatomopathological diagnosis is to establish the normal limits of the intestinal mucosa, this is particularly important in lesions with preserved villous architecture. The generally accepted limit of normality for the amount intraepithelial lymphocytes is of 25 for each 100 epithelial cells[5-7] and it is advisable to systematically perform CD3 immunostaining which allows for a better differentiation between lymphocytes and epithelial cell nuclei.[4] To facilitate cell count it has been proposed to examine 20 enterocytes on 5 well-oriented villi considering the normal limit to be of less than 5 lymphocytes per each 20 enterocytes.[4]

Marsh 1992[1]	**Oberhuber et al. 1999[2]**	**Corazza & Villanaci 2005[3]**	**Ensari 2010[4]**
Type 1 Infiltrative lesion	Type 1 Infiltrative lesion	Grade A Infiltrative lesion	Type 1 Infiltrative lesion
Type 2 Crypt hyperplasia	Type 2 Crypt hyperplasia	Discarded Incorporated into Grade A	Discarded Incorporated into Type 1
Type 3 Atrophy	Type 3: Atrophy Type 3A: Partial Type 3B: Subtotal Type 3C: Total	Atrophy Grade B1 Grade B1 Grade B2	Atrophy Type 2 Type 2 Type 3
Type 4 Destructive lesion	Type 4 Destructive lesion	Obsolete	Obsolete

Table 1. Classification schemes for the histopathological evaluation of gluten-sensitive enteropathy.

3. Definition of Marsh Type 1 Lesions and Differential Diagnosis of Lymphocytic Enteropathy

The gluten-sensitive enteropathy spectrum of histopathological lesion is not pathognomonic to this entity, since other entities may produce indistinguishable microscopic lesions (Table 2).[4,8,9] The differential diagnosis is even broader for minimal lesions with conserved architecture than for villous atrophy. Lymphocytic enteropathy-type lesions can result from an unspecific and transient response of the intestine to multiple lesions (allergic, infectious, and toxic). In many cases the frequency of these alterations and their clinical relevance are not well established. However, in cases where there have been systematic studies to determine the frequency and severity of a lesion associated with specific agents, such as the parasite *Giardia lamblia*, it has been observed that atrophy and intraepithelial lymphocytosis are rarely produced by this parasite.[10]

Villous atrophy-causing diseases, besides CD, are generally too infrequent, such as microvillus inclusion disease, neonatal enteropathy or autoimmune enteropathy, which primarily affect children. In developed countries, gastrointestinal atrophy-causing infections are also much less frequent than in developing countries. On the other hand, the differential diagnosis with lymphocytic enteropathy is more difficult.[11-16] Lymphocytic enteropathy caused by *Helicobacter*

pylori is a challenging diagnosis and, in much the same way as that produced by gluten sensitivity, it may be clinically relevant. Therefore, reaching an etiologic diagnosis is essential. Other common lymphocytic enteropathy causes to be ruled out are NSAIDs lesion, food hypersensitivity in children, *Blastocystis hominis* parasitosis and Crohn's disease. Currently, an etiologic diagnosis can take a long time, since the response to sequential treatments must be determined and this requires a great deal of motivation, discipline and acceptance by both the patient and the physician.[15-17] Research in this field is nowadays focusing on finding cell markers (immunohistochemical and cytometric) and/or molecular which may allow the establishment of a baseline etiologic diagnosis without having to wait for the response to a specific treatment.

Intraepithelial lymphocytosis (Type 1)	**Atrophy (Types 2 and 3)**
• Gastroduodenitis caused by *H. pylori* • Hipersensitivity to food • Infections (viral, parasitic, bacterial) • Bacterial overgrowth • Pharmacological drugs (mainly NSAIDs) • IgA deficit • Common variable immunodeficiency • Crohn's disease	• Microvillus inclusion disease • Autoimmune enteropathy • Tropical sprue • Collagenous sprue • Refractory celiac disease (including enteropathy associated T cell lymphoma). • Lesions due to irradiation and/or chemotherapy. • Graft vs host disease • Nutritional deficits

Table 2. Histopathological differential diagnosis of gluten-sensitive enteropathy.[4,8,9,11-17]

4. Diagnostic Criteria for Celiac Disease Patients with Lymphocytic Enteropathy-Type Lesion

Recently, it has been considered that, to reach a CD diagnosis, 4 out of 5 of the diagnostic criteria described in Table 3 are needed. This is what has been called the "4 out of 5" rule.[18] According to these criteria, patients with type 1 Marsh lesions can be diagnosed with CD upon finding of typical CD serum antibodies (IgA anti-endomysium, IgA anti-transglutaminase or deamidated anti-gliadin) or, if there is negative serology, when subepithelial IgA transglutaminase deposits can be found. Recent ESPGHAN diagnostic criteria for CD in children and adolescents are plentiful in this sense.[19]

However, it is well known that celiac serology is often negative in the milder forms of CD: in 30% of the patients with partial villus atrophy and up to 80% of those with Marsh 1 lesions.[20,21] Gluten challenges have been performed in these patients in order to determine if this tends to worsen the histologic lesion or if antibodies become positive[15,22], which would lead to a CD diagnosis. Furthermore, the presence of subepithelial IgA transglutaminase deposits or increased intraepithelial lymphocytes expressing gamma/delta TCR has been considered suggestive of celiac disease.[19,23,24] To benefit from these new diagnostic techniques it is necessary to obtain duodenal mucosa samples which must be immediately frozen in liquid nitrogen and processed by

immunofluorescence under confocal microscopy to determine subepithelial deposits or by means of immunohistochemistry for TCR gamma/delta.

•	Typical celiac disease symptoms[*1]
•	High titers of IgA-class celiac disease serum antibodies[*2]
•	HLA-DQ2 o DQ8 haplotypes[*3]
•	Celiac type enteropathy in small intestinal biopsy[*4]
•	Response to the GFD[*5]

[*1]Examples: chronic diarrhea, growth delay in children or weight loss in adults, iron deficit anemia.
[*2]10 x times the normal value (IgG-class in subjects with IgA deficit).
[*3]Also with only half the heterodimer (positive HLA-DQB1*02).
[*4]Including Marsh 1 to 3 lesions associated with positive celiac serology with high/low titers and Marsh 1 to 3 lesions associated to IgA subepithelial deposits.
[*5]Clinical and histologic response in patients with negative serology.

Table 3. Celiac disease diagnostic criteria: "4 out of 5" rule.[18]

Response to the GFD is an important diagnostic criterion in patients with type Marsh 1 lesions and it is still essential to document the histological response in patients with negative serology for proper diagnosis of CD. In research studies our group has used the following criteria in order to define whether a complete or partial histological response to the GFD is occurring:[25] a) Complete response: Evolution of Marsh-Oberhuber types 3, 2 and 1 to type 0, or, in type 1, at least a reduction of over 50% in the number of intraepithelial lymphocytes in relation to a baseline biopsy; b) Partial response: Improvement of the atrophy degree (Marsh-Oberhuber type 3C to 3B-3A or Ensari type 3 to type 2) and in the case of patients with a type 1 baseline biopsy, at least an intraepithelial lymphocyte reduction of 25% to 50% in relation to the baseline biopsy. Given the possible existence of a patchy lesion and to properly assess the response it is necessary to clearly identify the location (bulb, distal duodenum or jejunum) for the taking of samples in both the basal biopsy and in posterior control biopsies. These criteria may be useful and applicable in routine clinical practice.

The adequate time to carry out the follow-up biopsy after starting the gluten-free diet has not yet been well established, even in patients with villous atrophy. In a recent systematic review of the literature it has been recommended not to perform it before 1-2 years have elapsed after beginning of the diet.[26] If there is mucosal healing, there is no justification for further biopsies, except for the appearance of changes in clinical status. If histological improvement is incomplete, it would probably be necessary to perform a new control 1-2 years later.

4.1. Usefulness of Intraepithelial γδ+ Determination

The γδ+ intraepithelial lymphocyte determination is considered useful in doubtful or difficult cases.[27] In CD patients these γδ+ T cells are increased in all stages of the disease, both in untreated CD and under the gluten-free diet.[27] It has also seen that they are increased both in potential and latent CD.[28,29] This increase in γδ+ T cells has not been observed in other common

intestinal diseases, thus it is possible to affirm that CD is the only disease in which they are systematically, permanently and intensely increased.[27]

An increase in this type of cells has been detected in most patients with mild enteropathy.[30] Therefore, their determination may be useful in the differential diagnosis of lymphocytic enteropathy.

4.2. Diagnostic Utility of Tissue IgA Transglutaminase Subepithelial Deposits

It has been shown that the production of CD autoantibodies happens locally in the small intestinal mucosa, where they pass into to the bloodstream. However, besides being detectable in the bloodstream, these autoantibodies remain sequestered in the place where they have been produced. In untreated CD it is possible to detect tTG IgA deposits in the intestinal mucosa subepithelially and around blood vessels.[31] Interestingly, these deposits can be detected in patients with positive EMA and without villous atrophy[30,32,33] and even in patients with negative serology and Marsh type 1-3 lesions.[34-36]

In a recent series of studies on untreated CD it was demonstrated that 100% of 261 patients with villous atrophy had subepithelial IgA tTG deposits (9% had negative serum EMA), 90% had moderate to strong intensity. In contrast, 18% of the controls had deposits of minor intensity. After the gluten-free diet, there was a gradual decrease in the intensity of these deposits, which remained positive, in the long term, in 56% of the patients. The sensitivity and specificity of these deposits for CD diagnosis was of 100% and 82%, however, serology sensitivity and specificity were of 91% and 100% respectively.[36]

In a study on children with positive EMA or tTG and positive genetics (HLA-DQ2 or DQ8) but no villous atrophy, IgA tTG deposits were detected in 85% of 39 patients. Similarly, a study on another group of children revealed negative serology and Marsh type I lesions, with increased gamma/delta intraepithelial lymphocytes, allowing the detection of IgA tTG deposits in 66% of 18 patients. Instead such deposits were detected in 9% of 34 children with normal intestinal mucosa and absence of gluten sensitivity markers.[35]

4.3. Emerging Diagnostic Tools: Intraepithelial CD3+TCR$\gamma\delta$+ and CD3- Determination by Flow Cytometry

Flow cytometry is a powerful analytical tool for the study of intraepithelial lymphocytes (IEL) compared to immunohistochemistry. It allows fast, sensitive, reproducible and objective semi-quantitative results. Since an increase of CD3+TCRγδ+ and a decrease in CD3- IEL has been previously described as a characteristic flow cytometric pattern (FCP) of CD with atrophy,[37-39] a recent study[40] has evaluated the usefulness of this technique for diagnosing lymphocytic enteritis due to CD. In this recent study 205 patients (144 females) who underwent duodenal biopsy for clinical suspicion of CD and positive celiac genetics were evaluated. Fifty had villous atrophy, 70 lymphocytic enteritis, and 85 normal histology. Eight patients with non-celiac atrophy and 15 with lymphocytic enteritis secondary to Helicobacter pylori acted as control group. Duodenal biopsies were obtained to assess two typical flow cytometric patterns (FCP): complete CD FCP,

was defined when TCRγδ+ ≥8.5% and CD3- ≤10% were detected, and incomplete CD FCP was defined when an isolated TCR γδ+ increase (≥8.5%) was detected. Moreover, anti-TG2 IgA subepithelial deposit analysis (CD IF pattern) was also assessed. Sensitivity of IF pattern, and complete and incomplete cytometric patterns for CD diagnosis in patients with positive serology (Marsh 1+3) was 92%, 85 and 97% respectively, but only the complete cytometric pattern had 100% specificity. CD cytometric pattern showed a better diagnostic performance than both IF pattern and serology for CD diagnosis in lymphocytic enteritis at baseline (95% vs 60% vs 60%, p=0.039). Thus, IEL flow cytometric pattern seems to be an accurate method for identifying CD in the initial diagnostic biopsy of patients presenting with lymphocytic enteritis, even in seronegative patients, and seems also to be better than anti-TG2 intestinal deposits.

5. Relationship between Clinical Manifestations and Degree of Histological Lesion

It was formerly thought that type 1 Marsh lesions were not associated with symptoms or signs of malabsoption.[39] However, recent studies suggest otherwise. In a multicenter study on first-degree relatives, using a diagnostic method consisting of genetic testing followed by intestinal biopsy in positive cases, we observed that a similar percentage of relatives with type 1 and 3 lesions had symptoms when compared with relatives with normal intestinal mucosa (56% and 54% vs 21%, p=0.002) (Table 4).[40] It is important to note that, in this study, relatives with lymphocytic enteropathy were diagnosed by screening within this risk group and not by their symptoms, yielding, therefore, the actual frequency of symptomatic patients in this group.

Symptoms (%)	Normal mucosa	Type 1 lesion	Type 2-3 lesion	p value
Abdominal pain	23	41	38.5	0.20
Diarrrhea	22	41	38.5	0.14
Flatulence	39	69	57	0.02
Bloating	22	56	57	0.003
Asthenia	16	47	46	0.002
Hypertransaminasemia	1.5	9	7	0.11
Osteoporosis/ Osteopenia	–	37	44	0.76

Table 4. Frequency of symptoms in first-degree relatives depending on the type of histological lesion (Modified from Esteve et al.[40]).

Another recent study compared the clinical features and analytical alterations between 1249 atrophy patients and 159 with mild enteropathy.[41] Gastrointestinal manifestations (70% vs 70%) and extraintestinal (66% vs 57%) appeared with similar frequencies in both groups.

These and similar studies have unequivocally established that patients with mild histological forms of celiac enteropathy do not suffer from a mild disease and can benefit from the GFD as well as those with atrophy.[25,42]

Although it is unknown whether individuals with lymphocytic enteropathy have the same risk of malignancy and autoimmune diseases than patients with atrophy, indirect evidence suggests that it is probably not so.[43] Therefore, the GFD is recommended for patients with lymphocytic enteropathy only if they are symptomatic (anemia, osteoporosis or both intestinal and extraintestinal bowel symptoms), mainly if the symptoms are serious and affect the quality of life. Moreover, and as already mentioned, in patients with lymphocytic enteropathy it is very important to make a correct differential diagnosis. The gluten-free diet is indicated only in symptomatic cases in which there is an unequivocal demonstration of the relationship between histological lesions and gluten intake.

6. Proposed Diagnostic Algorithm

Symptoms **S**	**Antibodies** **A**	**Genotype** **G**	**Endoscopy/ Histology** **E**	**Score Points**
Malabsorption syndrome	EmA+ and/or anti-TG2 >10xULN	×	Marsh 3b o 3c	2
Relevant CD symptoms or type 1 diabetes or first-degree relatives	Anti-TG2+ <10xULN or only anti-DGP+	Full HLA-DQ2 and/or DQ8 heterodimer	Marsh 2 or 3a or Marsh 0-1 with anti-TG2 deposits and/or an increase in lymphocytes expressing TCR gamma/delta	1
Asymptomatic	No serology available	No HLA results or only half of DQ2 (DQB1*0202)	No available histology or Marsh 0-1	0
×	All CD antibodies negative	Negative DQ2/DQ8	×	-1

Table 5. Celiac disease diagnostic algorithm: SAGE score (modified from Husby et al.[19]; the presence of gamma/delta T cells+ has been added to 0-1 Marsh histology as suggested in the literature (see corresponding section).

Recently a diagnostic algorithm has been proposed which is based on using a point scale ranging from -1 to 2 to rate symptoms, celiac antibodies, celiac genotype and suggestive endoscopic and histological changes which allows the CD diagnosis without referring to the response to the GFD (table 5).[19] The CD diagnosis becomes firm with a final score of 4 points or more. To diagnose CD when this score is lower than 4, which generally occurs in patients with negative celiac serology, it is necessary to consider the response to the GFD. In patients with suspected type 1 CD it is always necessary to assess the clinical and histological response to the GFD.

7. Difficult Cases: Overlap with Non-Celiac Gluten Hypersensitivity

Recent studies, including a placebo-controlled clinical trial have shown the existence of an entity known as non-celiac gluten-sensitivity.[44-46] This entity appears in patients who, having no duodenal histological lesion nor genetic predisposition to CD, have symptoms triggered by gluten consumption. There are still important problems in defining these patients since many authors consider that the definition encompasses those who have positive celiac disease genetics (40% of these patients are HLA-DQ2 positive) and lymphocytic duodenal infiltration. Therefore, the overlap between patients with non-celiac gluten sensitivity and celiac disease patients with type I Marsh lesion becomes evident and differential diagnosis quite difficult. It is possible that, in the future, the availability of cellular or molecular markers may help in the differential diagnosis.

8. Conclusions

In conclusion, all studies and data reviewed here demonstrate that CD diagnosis cannot rely on one single test. Collaboration between clinicians, immunologists and pathologists is essential to integrate clinical, serological, genetic and histological criteria, as well as the response to the GFD. In other words, although in many patients the presumptive diagnosis, with a high probability of success, can be performed with fewer data ("4 out of 5" rule),[13-15] it is necessary to have as much information as possible whenever possible ("5 out of 5"rule). And this is not only important for the initial diagnosis as it also is for management during the follow-up, as it is common for diagnostic doubts to arise when basal key points are not well known, and it is of relevance when there is an inadequate patient evolution. In the case of type 1 lesions, the requirement to obtain as much information as possible is even more accentuated, being necessary to frequently employ new diagnostic tools such as counting intraepithelial lymphocytes which express gamma/delta TCR or the study of IgA tTG subepithelial deposits.

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