REVIEW

Measuring Change In Small Intestinal Histology In Patients With Celiac Disease

Daniel C. Adelman, MD[1], Joseph Murray, MD[2], Tsung-Teh Wu, MD, PhD[3], Markku Mäki, MD, PhD[4], Peter H. Green, MD[5] and Ciarán P. Kelly, MD[6]

Small intestinal histologic abnormalities in celiac disease include atrophy of the intestinal villi, hypertrophy of the crypts and lymphocytic infiltration of intraepithelial spaces and lamina propria. These findings are central to diagnosis and their severity and change over time are valuable to monitor disease course and response to therapy. Subjective methods to grade celiac disease histological severity include the Marsh-Oberhuber and Corazza-Villanacci systems. Quantitative histology uses villus height (Vh), crypt depth (Cd), and intra-epithelial lymphocyte count (per 100 enterocytes) to provide objective measures of histologic changes including Vh:Cd ratio. Here we examine the available literature regarding these methodologies and support the use of quantitative histology as the preferred method for accurately and reproducibly demonstrating change of relevant histologic end points over time. We also propose a Quantitative-Mucosal Algorithmic Rules for Scoring Histology (Q-MARSH) system to partially align quantitative histology results with the traditional Marsh, Marsh-Oberhuber, and Corazza-Villanacci systems. Q-MARSH can provide a standardized, objective, and quantitative histology scoring system for use as a clinical or research application.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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INTRODUCTION

Celiac disease is an acquired chronic immune disorder that develops in genetically susceptible individuals upon exposure to ingested prolamin glycoproteins found in wheat (gliadins), barley (hordeins), and rye (secalins), collectively referred to as "gluten" (1). Innate and adaptive immunologic reactions to gluten result in chronic inflammatory responses in the small intestinal mucosa resulting in both structural and functional abnormalities.

There are several characteristics of disease activity that can serve as potential measures of response to therapeutic intervention in patients with celiac disease, including symptoms, small bowel histologic injury, and celiac-specific serologies. There can be wide variance in these measures, with some asymptomatic or minimally symptomatic patients exhibiting severe histologic changes, while other patients can experience severe symptoms and highly abnormal small bowel histologic changes with normal serologies.

For the patient whose symptoms and serologic test results suggest the possible diagnosis of celiac disease, establishing the diagnosis requires documentation of the characteristic histologic changes in the small bowel mucosa that include shortening of villus architecture, elongation or hypertrophy of the crypts, and lymphocytic infiltration of intraepithelial spaces and the lamina propria. In clinical practice these histologic features are most commonly qualitatively assessed; however, in the context of clinical research and therapeutics development, specific, reproducible, and quantitative measures need to be utilized to monitor responses over time and allow for clear measurement to assess efficacy end points.

The purpose of this state-of-the-art review is to examine the available methodologies to measure histologic response to gluten exposure in celiac disease. Our review supports the use of quantitative histology as the preferred method for accurately and reproducibly demonstrating change in celiac disease mucosal activity both for clinical disease management and for evaluating the efficacy of dietary and non-dietary interventions.

SMALL BOWEL HISTOLOGY IN THE DIAGNOSIS OF CELIAC DISEASE

Diagnosis of celiac disease

In current clinical practice the diagnosis of celiac disease is based on a combination of clinical, serologic, and histologic factors (2,3). The symptoms of celiac disease are widely varied. Some

[1]Department of Medicine, Division of Allergy and Immunology, University of California, San Francisco, San Francisco, CA, USA; [2]Department of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA; [3]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA; [4]Faculty of Medicine and Life Sciences, University of Tampere and Tampere University Hospital, Tampere, Finland; [5]Celiac Disease Center, Columbia University, New York, NY, USA; [6]Celiac Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA. **Correspondence:** Ciarán P. Kelly, MD, Celiac Center, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, DANA-601, Boston, MA 02215, USA. E-mail: ckelly2@bidmc.harvard.edu
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patients have no evident symptoms. When present symptoms generally include some combination of gastrointestinal (e.g., diarrhea and abdominal discomfort) and systemic manifestations (e.g., the dermatitis herpetiformis) (4). Nutritional deficiencies (e.g., iron deficiency) may also lead to symptoms (e.g., tiredness and fatigue) (5).

The usual initial test for diagnosis or exclusion of celiac disease is to assay for specific, serum, celiac-associated IgA (or in the case of IgA deficiency, IgG) antibodies directed to endomysium, tissue transglutaminase-2, and deamidated gliadin peptides (6). These serology tests used alone, or in combination, are highly sensitive and specific for the diagnosis of untreated celiac disease. In specific clinical circumstances, such as a patient presenting already on a strictly gluten-free diet (GFD) but without prior testing for celiac disease, evaluation for HLA DQ2 and DQ8 alleles can be useful, as a negative result will largely exclude celiac disease (7). Characteristic endoscopic features of celiac disease are also described (e.g., "mucosal scalloping", nodularity, flattening, or "decreased" folds) but their diagnostic accuracy is modest (8).

Histologic evaluation of the small intestinal mucosa using endoscopic biopsy samples is the mainstay of diagnosis of celiac disease. However, similar findings may occur in other gastrointestinal disorders and should be suspected especially in patients presenting with villus atrophy but negative celiac-specific serology markers (9). This is another clinical scenario where testing for permissive HLA DQ2 and DQ8 alleles may be diagnostically helpful.

Most of the diagnostic features (e.g., clinical, serologic, and histologic) are expected to improve in most patients and in many, may normalize following the successful avoidance of gluten ingestion; the notable exception is HLA genotype. Hence, favorable response to the GFD is an additional and important component of diagnosis; whereas failure to respond to starting a GFD is an indication for additional evaluation (3,10).

Small intestinal mucosal histologic changes

Celiac disease primarily affects the superficial mucosa of the small intestine. Deeper layers are rarely affected. The mucosal lesion varies considerably in severity and extent but the most characteristic features are a loss of normal villus structure with a reduction in villus height (Vh), a marked enlargement of the crypts with an increase in crypt depth (Cd), and inflammatory cell infiltration with an increase in the density of intraepithelial lymphocytes (IELs) (11).

The lamina propria cellular infiltrate consists largely of plasma cells and lymphocytes. The number of plasma cells is increased two- to sixfold, but, as in normal mucosa, IgA-producing cells predominate (12). The number of IELs per unit area of absorptive epithelium (often reported as number of IEL per 100 enterocytes) is increased especially in the tips of the remaining villi (11,13). In the normal small intestinal mucosa, lamina propria T lymphocytes are predominantly CD4+ cells, whereas the IEL are mainly CD8+ cells. In untreated celiac disease, this distribution of lamina propria T cells is maintained, but the density of cells in both compartments is increased.

HISTOLOGIC CLASSIFICATION SYSTEMS IN CELIAC DISEASE

Marsh classification

The Marsh classification was originally created to help describe associations between gluten exposure, polymorphisms of the major histocompatibility complex, and mucosal pathology representative of the spectrum of mucosal appearances observed in response to gluten exposure (14); the classification was not intended for use in clinical practice. Marsh originally described five interrelated lesions, pre-infiltrative, infiltrative, hyperplastic, destructive, and hypoplastic. The classification was later modified by Oberhuber (15) and was intended to be used by clinical pathologists to assist in diagnosing celiac disease through assessing and categorizing the degrees of mucosal abnormality in small intestinal biopsies. The descriptive categories of the Marsh-Oberhuber classification include six diagnostic grades as presented in **Figure 1** and **Tables 1, 3, and 4**.

Limitations of the Marsh-Oberhuber classification

There are several practical limitations of the Marsh-Oberhuber classification (**Table 1**): it is descriptive, qualitative, categorical, and not sensitive to change (16). Although there is no established practice as to whether histologic assessment is based on the most severe changes, the least severe changes, or the average degree of change, the most common practice is to grade the histologic change on the most severe injury observed in the biopsy specimen (17). The lack of clearly defined criteria as well as reliance on judging the most severely affected areas contributes to the unreliability of the Marsh-Oberhuber classification to make accurate assessments of change over time. The classification relies on the subjective judgment of the pathologist and because of this, shows low interobserver agreement and therefore, reduced reproducibility (18,19). Corazza *et al.* evaluated the percent observed agreement between pathologists and showed mean kappa values ranging from 0.28 to 0.40 (low agreement), with an overall agreement kappa of 0.35 (18). Similar findings were reported by others (19,20). The agreement between pathologists by lesion (i.e., 0, I, II, IIIa, IIIb, and IIIc) is only moderate, with the highest for types 0 (normal, kappa=0.46), I (a nonspecific mild increase in IEL, kappa=0.23), and III lesions (kappa for IIIa, IIIb, and IIIc=0.19, 0.24, and 0.64, respectively) (18). This, in practice, reduces the scale to a functional binary scale (either "normal" or "abnormal") with inherently little ability to show small, but clinically significant changes in intermediate histology (19–21). Moreover, the low levels of agreement (even for Marsh 0, I, and III) means that even as a binary scale, the level of agreement is low to moderate, at best. Those features characterize the limitations of the Marsh-Oberhuber classification making it unsuitable for repeated measures to assess, over time, the effects of either a limited gluten exposure or an effective but non curative therapeutic intervention.

Corazza-Villanacci score

The Corazza-Villanacci score divides the spectrum of celiac disease into three grades.

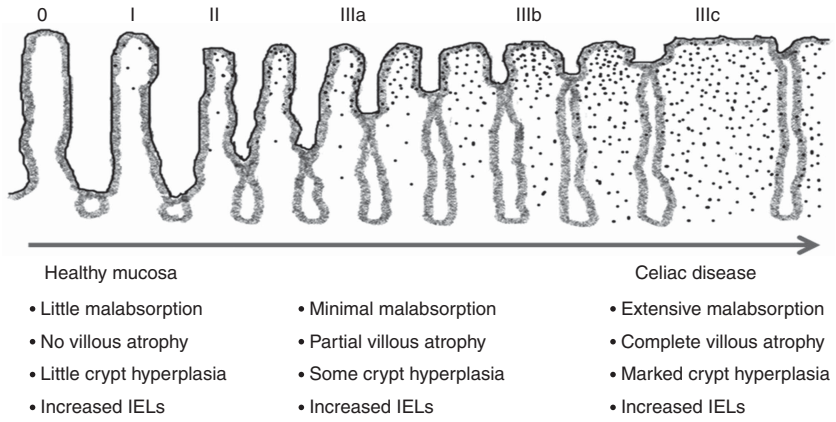

Figure 1. Schematic depiction of Marsh-Oberhuber grades I–IIIc. Figure courtesy of M.M.; text adapted from Rostrom *et al.* (17). Footnote: due to sampling variation histologic changes in small intestinal biopsy samples do not fully reflect the totality of small intestinal histologic changes and also may not correlate closely with clinical malabsorption.

Table 1. Marsh-Oberhuber histologic grading in celiac disease

Grade	Description and components
0	Normal—normal appearing villus architecture
I	Infiltrative—normal mucosal and villus architecture; increased numbers of IEL
II	Hyperplastic—similar to I with enlarged crypts and increased crypt cell division
IIIa	Partial villus atrophy—shortened, blunt villi; mild lymphocyte infiltration, enlarged hyperplastic crypts
IIIb	Subtotal villus atrophy—clearly atrophic villi, enlarged crypts whose immature epithelial cells are generated at an increased rate, influx of inflammatory cells
IIIc	Hypoplastic—total villus atrophy, complete loss of villi, severe crypt hyperplasia, infiltrative inflammatory lesion

(Modified from (ref. 17)).

Grade A, so-called non-atrophic, where there are normal crypts and villus architecture but increased IELs (utilizing a threshold of 25 IELs per 100 enterocytes).

Grade B consists of grades B1 and B2. Grade B1 is atrophic with Vh:Cd ratio of <3:1 but where villi are still clearly detectable and IELs are increased. Grade B2 is atrophic to the extent that villi are flat and not detectable. There is also crypt hyperplasia and increased intraepithelial lymphocytosis (18,22). The Corazza-Villanacci score has less variability and greater agreement between pathologists (18). It may also approximate what often is used in clinical practice, separating biopsy findings into just three groups: architecturally intact; partial villus atrophy; or total villus atrophy.

Biopsy histology reporting in current clinical practice

Of the histopathologic features of celiac disease summarized above, three key elements are the most commonly noted as a part of routine, clinical pathology reporting: Vh, which is most often referred to in terms of a reduction (i.e., villus "blunting", "flattening", or "atrophy"), crypt enlargement (often using terms such as "elongation" or "hyperplasia"), and an increase in IEL density, especially at the tip of the villus. At one of our institutions a template has been developed for use by all members of the pathology department to reduce variability in reporting (23). This includes number of biopsy pieces, degree of orientation, degree of inflammation, IELs, and Vh:Cd. This template allows the clinician to assess the value of the pathological assessment as it includes information on multiple factors, including the adequacy of specimen orientationed.

In the CeliAction Study (Alvine Pharmaceuticals, San Carlos, CA), patients entering screening were required to have a documented diagnosis of celiac disease in the medical history (24). Clinical pathology reports from academic university-based medical centers and community-based practices were reviewed to determine eligibility to enter into the screening stage of the study. **Table 2** provides a summary of the histopathologic elements reported from 137 randomly selected diagnostic clinical pathology reports. These data reveal that in practice, clinical reporting of pathology findings are almost entirely descriptive, are seldom comprehensive, and rarely cite Marsh grade (9%) or use any type of scoring system (either descriptive or quantitative). Similar findings were reported by Arguelles-Grande *et al.* (20) on review of pathology reports from pathologists in different practice setting.

Table 2. Small bowel histologic descriptions cited in clinical pathology reports

Described element	All centers, *n* (%) Total *n*=137	University medical centers, *n* (%) Total *n*=11	Community-based centers, *n* (%) Total *n*=126
Villus	128 (93)	10 (91)	118 (94)
IEL	123 (90)	8 (73)	115 (91)
Crypt	41 (30)	4 (36)	37 (29)
Marsh Grade[a]	12 (9)	2 (18)	10 (8)
Vh:Cd	5 (3)	2 (18)	3 (2)

IEL, intraepithelial lymphocyte; Vh:Cd, villus height to crypt depth ratio.
[a]Marsh grades reported (*n*): Marsh 3 (3c=3, 3b=6, and 3a=1); Marsh 2 (*n*=1); Marsh 1 (*n*=1).

Quantitative histology

Quantitative histology typically measures Vh, Cd, and IEL density per 100 epithelial cells (**Figure 2**) (16,18,25). In contrast to assessments using the Marsh-Oberhuber classification, all three elements are quantitatively measured to allow for a continuous measure of changes in the mucosa over time.

The primary architectural changes seen in celiac disease (i.e., reduced Vh and increased Cd; **Figure 2d–f**) as well as the most characteristic element of inflammatory cell infiltration (increased IEL density; **Figure 2b**) are used to obtain objective, continuous measurements of the severity of the celiac lesion in a particular biopsy. Quantitative histology follows well-established methodologies and has been used as a tool in celiac disease clinical research for many decades. Dissatisfaction with the descriptive and categorical histologic classification standards first established in the 1960s and 1970s led pediatric celiac disease researchers to first develop quantitative morphometric measures (26). As early as 1984, almost 10 years before the Marsh classification was first proposed, Ciclitira *et al.* used quantitative histology in adult patients with celiac disease to follow the histologic changes that occurred following the intraduodenal infusions of unfractionated gliadin and of α-, β-, γ-, and ω-gliadin subfractions in serial jejunal biopsies obtained using a peroral biopsy capsule (27).

Quantitative histology has been extensively used by clinical researchers for decades (16,18,25,26,28,29). The basic quantitative histologic methods used by Ciclitira *et al.* in the 1980s continue to be used today with modifications and refinements. Standardized operating procedures were implemented by the Tampere celiac disease study group and the Mayo Clinic Core Histology Laboratory to perform quantitative histology for a large clinical study recently concluded (16,24). These included measurements of Vh and Cd from 5 to 12 villus–crypt units from at least four biopsy specimens taken from the distal portion of the second part or from the third part of the duodenum; all paired (blinded to baseline or post-treatment sequence) samples were read by the same pathologist to minimize observer variability. A light microscope with a calibrated micrometer was used to measure individual Vh and Cd (in μm) from each well-oriented villus–crypt unit and those measurements are recorded. The Vh:Cd of each villus–crypt unit was calculated; the reported ratio of Vh to Cd was then derived from the average of the Vh:Cd values from the individual villus–crypt units. This differs from the traditional approach of scoring the single worst area of the biopsy when using histology to confirm a diagnosis of celiac disease. This methodology was used to reduce sampling bias by obtaining multiple measures of each element within a single biopsy and across the multiple biopsies that were obtained during a single diagnostic procedure.

The ratio of Vh to Cd is commonly used as a single value (Vh:Cd) to encompass two of the main elements of the mucosal architectural change of celiac disease. Because measurements of Vh and Cd are obtained independently and each can be used as an individual measure (as can the quantification of IELs per 100 enterocytes), quantitative histology provides three distinct and highly quantifiable and relevant measures of small bowel mucosal changes over time in response to therapeutic intervention.

Limitations of quantitative histology

Proper tissue orientation of the biopsy sections (perpendicular to the luminal surface) is required for accurate quantification of Vh and Cd; quantification of IEL is not affected by orientation of tissue blocks (16). This requirement is not unique to quantitative histology; to assess the qualitative Marsh classification proper orientation of the small bowel mucosa is required also for suitable readings (16,20).

The basic techniques of quantitative histology are not difficult for a gastrointestinal pathologist to master, access to a suitable microscope with a calibrated micrometer to acquire accurate Vh and Cd lengths and well oriented sections, are all that is required. The main limitations of quantitative histology, and the primary reasons that it is not used more widely in clinical practice are lack of awareness and the fact that it is time consuming. For example, at the Mayo Clinic Core Histology Laboratory, it takes ~5 min for a clinical pathologist to provide descriptive reporting of small intestinal biopsy samples, whereas acquiring accurate quantitative measurements from properly oriented tissue specimens of Vh, Cd and IEL counts takes approximately 30 min per patient (T.T. Wu, personal communication).

A variation of quantitative histology methodology involving the derivation of mucosal volume and mucosal surface-to-volume ratios has been proposed for assessing responses over time (30). This technique is rarely used, even in the research setting, as it represents an even greater time burden and ideally requires

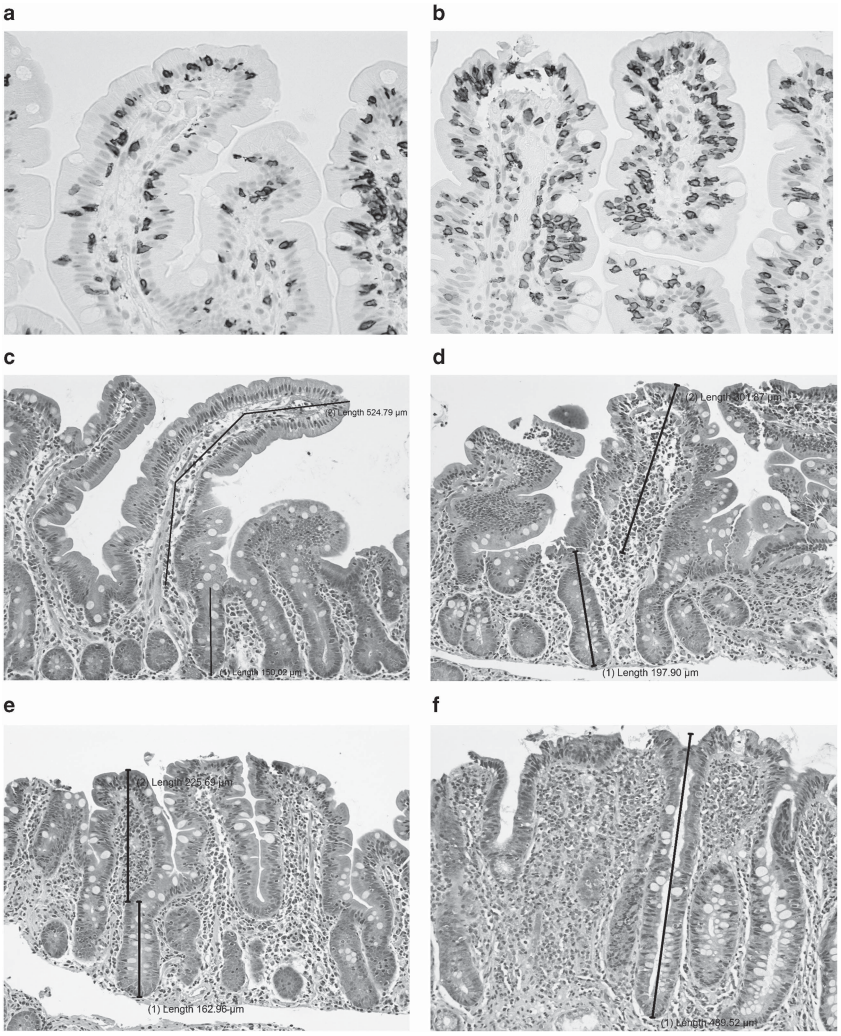

Figure 2. Quantitative histology: evaluating intraepithelial lymphocyte (IEL) numbers and villus height to crypt depth (Vh:Cd) ratios. Mucosal sections at high-power magnifications stained for CD3+ lymphocytes, (**a**) normal numbers and (**b**) increased IELs especially at the tip of the villus (>30 IELs per 100 enterocytes). Mucosal sections cut perpendicular to the luminal surface from (**c**) a patient with well-treated celiac disease on a gluten-free diet (GFD; Vh:Cd=3.50; Vh 301.87 µm, Cd 197.90 µm), (**d**, **e**) patients on a GFD with incompletely treated celiac disease and varying degrees of architectural abnormality (**d**: Vh:Cd=1.53; Vh 524.79 µm, Cd 150.02 µm and **e**: Vh:Cd=1.38; Vh 225.69 µm, Cd 162.96 µm), and (**f**) total villus atrophy in a patient with newly diagnosed celiac disease; the villi are flat and the crypts are elongated (Vh:Cd=0).

three-dimensional digital reconstruction of the mucosa using multiple stacked sections to obtain accurate results.

It is important to note that intestinal changes in celiac disease vary from the proximal to distal small intestine and are often focal or patchy. Hence, apparent histologic changes in small intestinal biopsy samples do not fully reflect the totality of small intestinal histologic changes and may not correlate closely with clinical malabsorption. This important sampling limitation remains despite expert and even quantitative histologic evaluation of mucosal biopsies.

Advantages of quantitative histology

Quantitative histology is the preferred methodology for assessing outcomes in clinical research and therapeutics development. This preference is based on the quality of the data that are derived by assessing the three independent and biologically relevant elements, Vh, Cd, and IEL densities. Furthermore, multiple measurements can be obtained within a single biopsy and from multiple biopsy specimens to reduce sampling error.

One of the most important advantages of quantitative histology, particularly in the context of clinical research and drug development applications, is the sensitivity to assessing change. Lähdeaho *et al.* used quantitative histologic methods to demonstrate the absolute change in Vh:Cd and IEL density in well-treated patients with celiac disease (seronegative and without celiac-related symptoms) undergoing a 6-week gluten challenge with varying doses of gluten. Because of the ability and sensitivity of quantitative histology to show continuous change in mucosal architecture and

Table 3. Comparison of main elements of histology measure in celiac disease

	Marsh-Oberhuber grade						Quantitative histology
	0	I	II	IIIa	IIIb	IIIc	Measures
Descriptor		Infiltrative	Hyperplastic	Partial villus atrophy	Subtotal villus atrophy	Total villus atrophy	NA
Villus architecture	Normal	Normal	Normal	Shortened, blunt	Clearly atrophic	Complete loss	Continuous range of villus height (μm)
Crypt architecture	Normal	Normal	Enlarged	Enlarged	Enlarged	Severe hyperplasia	Continuous range of crypt depth (μm)
IEL	Normal	Increased	Increased	Mild infiltration	Influx	Infiltrative	Lymphocyte count per 100 enterocytes
Interobserver reproducibility[a,b]	Fair κ=0.46	Low κ=0.23	Very low κ=0.04	Low κ=0.19	Low κ=0.24	Good κ=0.64	Excellent ICC=0.978 Intraobserver ICC=0.983

ICC, intraclass correlation coefficient; IEL, intraepithelial lymphocyte; NA, not applicable; Vh:Cd, villus height to crypt depth ratio.
[a]Kappa for Marsh-Oberhuber (18).
[b]Intraclass correlation coefficient for quantitative histology (16).

IEL densities, the authors were able to demonstrate a linear dose–response curve at daily gluten doses of 1.5, 3.0, and 6.0 g (**Supplementary Figure F1** online) (28).

Quantitative histology measurements of Vh:Cd and IEL have been shown to be both reliable and reproducible continuous measures (interclass correlation coefficients for intra- and interobserver Vh:Cd 0.983 and 0.978, respectively, and for intra- and interobserver IEL measures (0.961 and 0.842, respectively) (18). Thus, quantitative histology allows for the most objectively derived, quantitative and continuous measures and show superior reproducibility to the Marsh-Oberhuber classification.

APPLICATION OF QUANTITATIVE HISTOLOGIC MEASURES IN CLINICAL RESEARCH AND THERAPEUTICS DEVELOPMENT

Quantitative histology has been used in celiac disease clinical research for over three decades (26,27). The advantages of quantitative histology include, objective, accurate, and highly reproducible measurements (16), which have been used by researchers when reliable serial assessment of the small intestinal mucosa over time are needed to identify small or transient changes. Typically, these studies have examined the dose response and kinetics of the responses to gluten exposure and withdrawal. There is a rich medical literature of reports detailing the use of quantitative histology in celiac disease research to evaluate mucosal changes between study groups and over time (**Table 3**). The findings and relevance of selected studies listed in **Table 3** are described in **Supplementary Table S1** and **Supplementary Text**.

It must be noted that the studies of quantitative histological evaluation of duodenal biopsies to date have been limited to assessing biopsies of the more distal duodenum and not biopsies of the duodenal bulb that have been proposed to confer increased sensitivity but also perhaps reduced specificity for diagnosis (3,31–33).

QUANTITATIVE-MUCOSAL ALGORITHMIC RULE FOR SCORING HISTOLOGY

On the basis of the information summarized above we propose a Quantitative-Mucosal Algorithmic Rule for Scoring Histology (Q-MARSH) in celiac disease. This can act as a quantitative refinement of the qualitative and categorical Marsh, Marsh-Oberhuber and Corazza-Villanacci Scales. In the CeliAction Study 1,345 patients with celiac disease following a GFD and who still complained of moderate or severe symptoms associated with celiac disease underwent upper gastrointestinal endoscopy with duodenal mucosal biopsy (24). **Figure 3** shows the frequency distribution of the Vh:Cd in these patients at baseline assessment. Of note, 38% showed a Vh:Cd value of ≤2.0, values that in the proper clinical context would be confirmatory of a diagnosis of active celiac disease. In contrast, only 8% of patients had a Vh:Cd of ≥3.0, which is consider to be "normal" (34,35). The majority of patients with celiac disease (54%) on a GFD in this study had Vh:Cd values between 2.1 and 2.9, demonstrating that in a "real-world" setting the GFD alone is not sufficient to allow healing and normalization of the mucosa in many patients. On the basis of these data, anchored on quantitative histologic criteria, a more quantitative categorical classification could be constructed to assist clinical practitioners in the diagnosis of patients and to facilitate the evaluation of changes in mucosal injury over time for patient management and for the evaluation of novel therapeutic interventions. Such a system, called the Q-MARSH is presented in **Figure 3** and **Table 4**.

Establishing a broadly accepted and objective celiac disease activity measure will be valuable in facilitating development of non-dietary interventional therapeutics for the treatment of celiac disease. As discussed by Dr Janet Woodcock at a Critical Path Institute presentation, an accepted disease measure can reduce or eliminate second guessing during therapeutics development regarding the validity and interpretation of the meaning of the measure (http://www.fda.gov/downloads/AboutFDA/CentersOf-

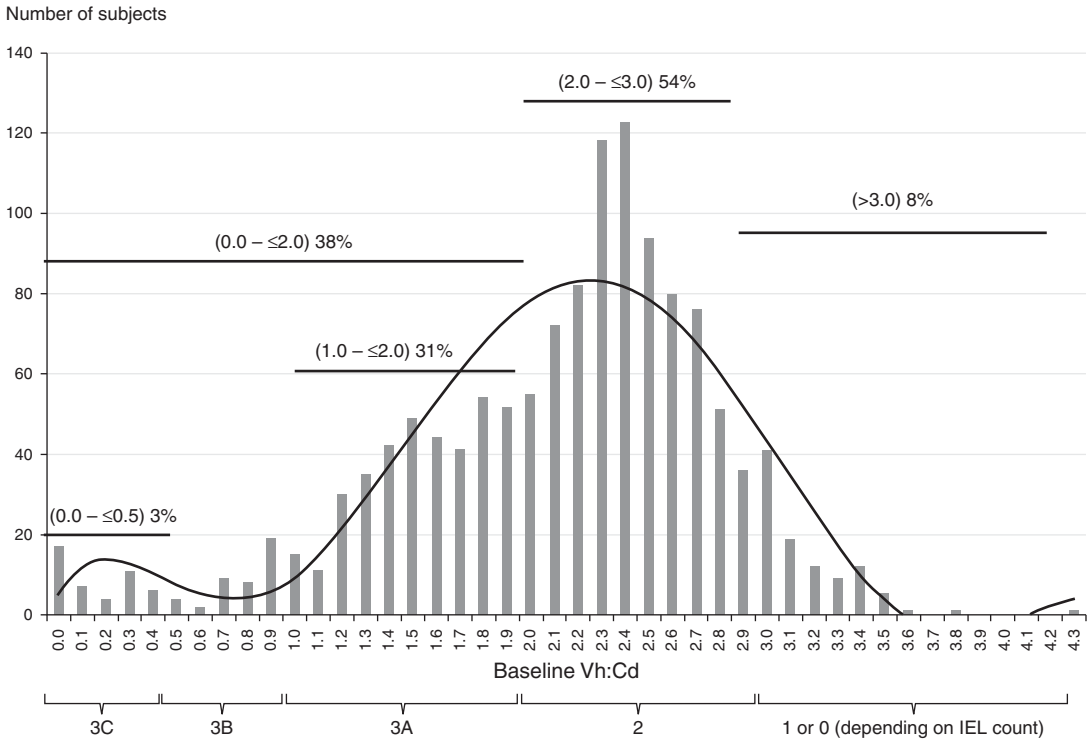

Figure 3. Villus height to crypt depth (Vh:Cd) frequency distribution of 1,345 patients with self-reported moderate or severe celiac disease-associated symptoms. Frequency distribution of Vh:Cd in duodenal biopsies taken from 1,348 patient with celiac disease on treatment with a gluten-free diet but with continuing moderate or severe celiac-associated symptoms (from CeliAction Study) (24). Proposed Quantitative-Mucosal Algorithmic Rules for Scoring Histology scores are illustrated.

Table 4. Q-MARSH and proposed comparisons with Marsh, Marsh-Oberhuber, and Corazza-Villanacci systems

Q-MARSH		Marsh (14)	Marsh-Oberhuber (15)	Corazza-Villanacci (18)
IELs	**Vh:Cd**			
Normal	≥3.0	0	0	Normal
Increased[a]	≥3.0	1	1	Grade A
Increased	≥2.0–<3.0	2[b]	2[b]	
Increased	≥1.0–<2.0	3[c]	3a[d]	Grade B1[e]
Increased	≥0.5–<1.0		3b[d]	
Increased	>0.5		3c[d]	Grade B2[f]

IEL, intraepithelial lymphocyte; Vh:Cd, villus height to crypt depth ratio; Q-MARSH, Quantitative-Mucosal Algorithmic Rules for Scoring Histology.
[a]Varies between studies but circa 30 per 100 enterocytes.
[b]Crypt hyperplasia or elongation. Vh:Cd in Q-MARSH does not discriminate between crypt elongation and shortening of the villi.
[c]Villus atrophy.
[d]Increasing villus atrophy.
[e]Partial villus atrophy.
[f]Complete villus atrophy.

fices/OfficeofMedicalProductsandTobacco/CDER/UCM352761.pdf). At present an accepted measure widely used in practice by clinical researchers, therapeutic development scientists, clinical pathologists, and practitioners does not exist; however, quantitative histology has been used for decades and is widely accepted by clinical researchers and therapeutics developers as a reliable measure of mucosal histology (24,25,27–29,36,37).

The reasons for preferring quantitative histology become evident when comparing the two main methodologies (**Table 3**). Both methods take into consideration the key elements of

mucosal architecture (i.e., Vh, Cd, and IEL density); however, the Marsh-Oberhuber classification incorporates subjective, non-quantitative evaluation of the key mucosal elements into an assigned categorical grading of the appearance of the mucosa. In contrast, quantitative histology methodology relies on objective, quantitative, and continuous measures of the key mucosal structural elements. Rather than pooling the key elements into one qualitative assessment (Marsh-Oberhuber), quantitative histology can objectively and accurately measure the key mucosal elements to assess changes over time. Quantitative histology is sensitive to change over time, reproducible, and demonstrates excellent intra- and interobserver agreement. In practice, for over two decades quantitative histology has been used by clinical investigators and drug developers to define the underlying pathophysiology of celiac disease in terms of response to specific gliadin peptides, as well as the dose–response and time course to graded gluten exposure (18,28). The inability to achieve uniformity in the application of the Marsh-Oberhuber classification is the greatest limitation of a non-quantitative interpretation of histology. This certainly calls into question the value of this methodology for accurately assessing mucosal histologic change over time in response to therapeutic intervention. As a future, alternative approach computerized assessment of biopsy slides using quantitative sophisticated image-processing technology has been suggested as an automated approach that lacks human observer bias and easily lends itself to standardization (38).

As potential therapeutic interventions are being brought through the development process, it is critically important to be able to identify clinically meaningful improvements, or a lack thereof, in both symptoms and quantifiable histology measures of disease activity. This necessitates the use of reliable, reproducible, and accurate measures that are sensitive to change; full and complete normalization of histology (i.e., moving from Marsh-Oberhuber grade III to grade 0 lesions) is unlikely to be necessary for a potential drug in development to be, in fact, clinically beneficial to patients with celiac disease.

We believe that descriptive reporting of small intestinal mucosal histopathology, with or without Marsh-Oberhuber or Corazza-Villanacci grading, is adequate in the majority of clinical circumstances for initial diagnosis of celiac disease. However, quantitative histology is required to adequately measure change in histopathology over time. This is relevant to two main clinical circumstances. First, although not universal, many physicians recommend repeat biopsy after 6–24 months of treatment with a GFD both to confirm the diagnosis of a gluten-sensitive enteropathy and to evaluate the degree of response to therapy (3,39). These goals are best achieved by a quantitative comparison of current to initial (diagnostic) biopsies.

Second, the examination of biopsy histopathology and its comparison to prior biopsies is a pivotal point in the differential diagnosis and management of non-responsive celiac disease. Current guidelines use this assessment of histopathologic response to determine the course of further investigation and treatment (3,39). This important evaluation is best achieved by quantitative measurement of the relevant biopsy specimens in order to provide clinicians with the best possible information as to the degree of mucosal improvement or deterioration.

A third, less prevalent, clinical situation that is best served by quantitative histopathology is the finding of non-celiac villous atrophy. A reduction in villous height to Cd ratio that is not associated with a substantial increase in IELs can point to an alternative diagnosis such as peptic duodenitis or auto-immune enteropathy. Quantitative histology highlights this important distinction.

Quantitative histology is, at present, the best research tool to measure mucosal immune response in celiac disease. It also has the potential to become a valuable and accepted disease measure for routine clinical application as outlined above. It can provide very valuable summary measures of celiac disease activity (e.g., in the form of Vh:Cd ratio and IEL count) for evaluating response to therapy over time. Thus, we propose a set of quantitative-based rules for scoring histology in celiac disease, called the Q-MARSH. A hypothetic alignment of Q-MARSH scores against the more traditional, subjective scoring systems of Marsh, Marsh-Oberhuber, and Corazza-Villanacci is proposed in **Table 4**. Accordingly, as shown in **Figure 3**, Vh:Cd values ≥3.0 without increased IEL would be category 0 (normal) (34,35); Vh:Cd ≥3.0 with elevated IEL, category 1; values ≥2.0–<3.0 with elevated IEL, category 2; values ≥1.0–<2.0 with elevated IEL, category 3A; ≥0.5–<1.0 with elevated IEL, category 3B; and <0.5 with elevated IEL, category 3C. Hence the Q-MARSH can be considered a parallel, alternative approach that can provide a standardized quantitative histology scoring system for use as a clinical or research application.

CONFLICT OF INTEREST

Guarantor of the article: Ciarán P. Kelly, MD.

Specific author contributions: Daniel C. Adelman: conceived article, co-wrote early drafts, edited and finalized text, and developed figures. Joseph Murray, Tsung-Teh Wu, Markku Mäki, and Peter H. Green: edited and finalized text, and developed figures. Ciarán P. Kelly: conceived article, co-wrote early drafts, edited and finalized text, and developed figures.

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