Quantitative assessment of the degree of villous atrophy in patients with coeliac disease

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ABSTRACT

Background: Endoscopy and biopsy are used to diagnose coeliac disease. There are, however, observer-dependent interpretations of the degree of villous atrophy in biopsies. A pilot study using quantitative image-processing procedures was performed to quantify the degree of villous atrophy in patients with coeliac disease.

Method: The degree of villous atrophy in duodenal biopsy images was quantified by calculating the ratio of villous edge-to-piecewise arc length (E/P ratio), and this value was compared with the blinded assessment of Marsh score for degree of villous atrophy.

Results: Mean E/P ratios for n = 31 biopsy images, 2.76 (SD 0.44) (Marsh IIIa), 1.91 (0.50) (Marsh IIIb) and 1.18 (0.22) (Marsh IIIc), were significantly different ($p = 0.006$). Based on non-parametric testing, the E/P ratios were inversely correlated with Marsh scores (Spearman coefficient $\rho = -0.798$, Kendall $\tau = -0.681$; $p<0.0001$).

Conclusions: Biopsy images quantified by image analysis correlated exceedingly well with the histopathological grade of villous atrophy. Since quantified measurements are real-numbered values and lack observer bias, measurement of villous atrophy based on image analysis lends itself to standardisation of histological grading.

Coeliac disease is an autoimmune disease that occurs in genetically predisposed individuals due to exposure to gluten, the storage protein of wheat and similar cereal grains.[1] Recent studies have demonstrated the disease to be common, occurring in about 1% of the population.[2 3]

While the diagnosis, established by endoscopy and duodenal biopsy, appears readily apparent in the majority of patients,[4] in some the diagnosis is difficult, and assessment of the grade of villous atrophy can be challenging. Difficulty in diagnosis often arises due to the fact that interpretation of biopsies is a subjective process with imperfect agreement among different pathologists.[5–7]

In order to overcome subjective observations, we have performed image quantification and enhancement techniques of intestinal biopsies. We hypothesised that quantification of the degree of villous atrophy can provide objective, real-numbered scores that correlate with the Marsh classification of villous atrophy.[8 9]

METHODS AND PATIENTS

Biopsy specimens from sequential patients (n = 31) who were undergoing endoscopy and biopsy for the diagnosis of coeliac disease and in whom villous atrophy was detected were identified. All patients had positive coeliac serological tests (tissue transglutaminase (IgA), or endomysial antibodies). Six biopsy specimens were obtained from the descending duodenum with standard forceps in each patient. Biopsies were fixed in formalin (10% neutral buffered formalin) and then embedded in paraffin, and the sections were stained with haematoxylin and eosin. Images of biopsy slides were obtained at 400× magnification using a digital camera mounted on an Olympus bright-field microscope and stored as high-resolution TIFF files for morphometric analysis.

The slides were blindly reviewed and the degree of villous atrophy classified using the scoring system for villous atrophy developed by Marsh,[8] and modified by Oberhuber;[10] the crypt-to-villous ratio was assessed in areas of the biopsies where at least three well-oriented villi could be identified. The degree of villous atrophy was classified as partial, subtotal and total villous atrophy corresponding to Marsh scores of IIIa, IIIb and IIIc (see fig 1).

Biopsy images were quantified as follows. Standard edge-detection methods were used to form a line representing the villous edge along its mucosal surface (fig 1).[11] The edge was detected by applying a Laplacian filter to find the sharp change in colour associated with the boundary between the villi and the lumen (fig 1A, red curved line). The length, E, of this edge was then calculated using ImageJ (Ver. 1.36b, National Institutes of Health, Bethesda, MD—public-domain Java image-processing program). It was used as a downloadable application on a PC-type computer running a Java virtual machine. The ImageJ program is capable of displaying, editing, analysing and processing digital images.

The length, P, of an open convex polygon[11] was determined by finding points of maximum distance from the convex villous edge to its approximate radial centre as shown in fig 1A (black line) and in more detail in the accompanying close-up (see fig 2). In geometry, a convex polygon has the following properties: (1) Every internal angle is less than 180°; (2) every line segment between two vertices of the polygon remains inside or on the boundary of the polygon. Based on the locations of the set of points meeting these criteria, a convex polygonal arc was drawn (solid black line; figs 1A, fig 2), and its length P was then calculated by ImageJ. Examples of alternative points that would not be used because they do not maintain the convex shape of the polygon are denoted as open circles in fig 2, and the lines they would form are denoted as dashed lines. The measured E and P lines and the calculated E/P ratio are shown in each image (fig 1A–D). The E/P ratio was 2.58 for the Marsh IIIa patient (fig 1A), 1.63 and 2.06 for the

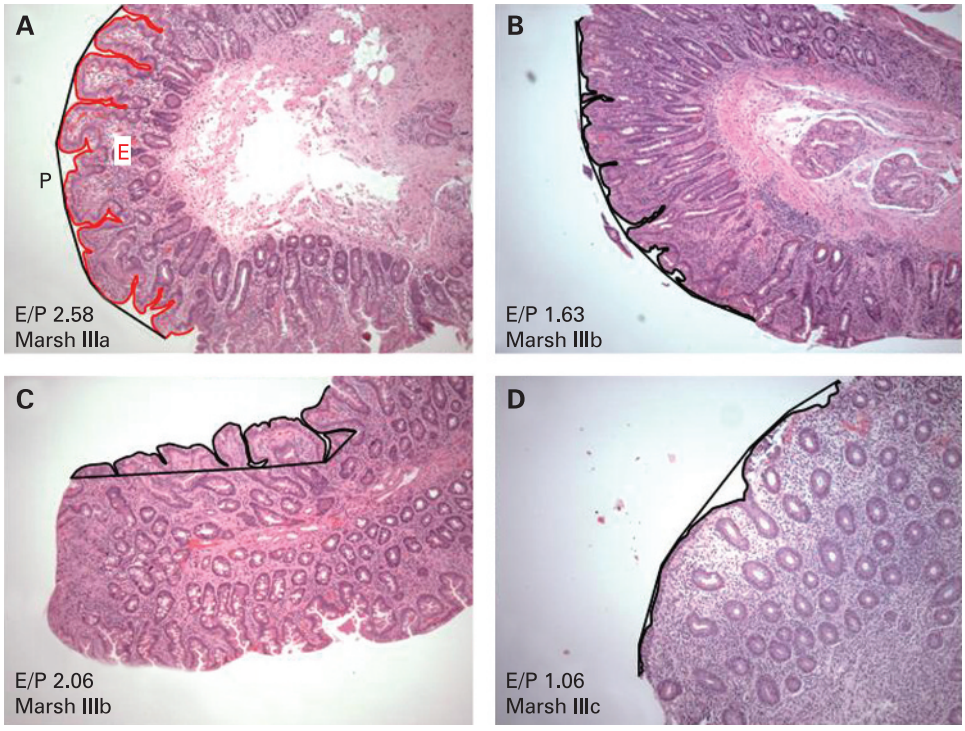

Figure 1 Biopsy images. Assessment of images according to Marsh criteria. Note the decreasing villous length from IIIa to IIIc. In (A), the villous edge (E) and piecewise arc length (P) are shown.

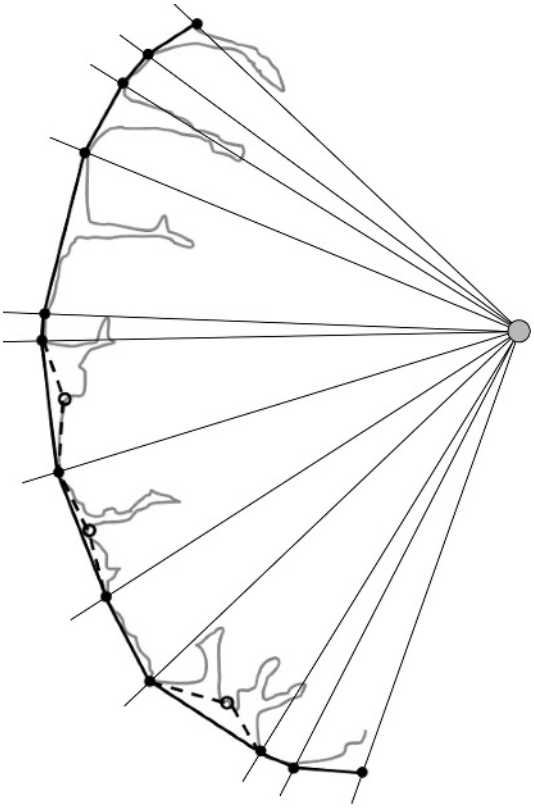

Figure 2 Close-up of the method used to determine the length and location of edge (E) and piecewise arc length (P) which are those delineated from fig 1A. The dark line denotes P (open convex polygon), and the grey line denotes E (villous edge). Points used to compute P are shown (closed circles). Alternative points (examples shown as open circles) are not used because they do not maintain the convex shape of the polygon.

Marsh IIIb patients (fig 1B,C) and 1.06 for the Marsh IIIc patient (fig 1D). Since the length of the villous profile E must be greater than or equal to the length of the convex polygon P, then by definition:

$$E/P \geqslant 1$$

When $E/P \approx 1$, it means that the villous profile and the open convex polygon formed from it are approximately coincident. The E/P ratio was calculated and used as the quantitative measure of villous atrophy. This value was compared with the Marsh score. The Holm–Sidak test for multiple comparisons was used to determine the statistical significance of the difference between the means of the E/P ratios after the images were binned based upon the grade of villous atrophy (PVA, STVA and TVA). Since the Marsh score is an ordinal categorical variable rather than a real number, the Spearman rank correlation coefficient (ρ) and Kendall τ were calculated to quantify non-parametrically the relationship between the Marsh score and E/P ratio. The graphs and statistical analyses were generated using Medcalc (ver. 9.5.2, Medcalc Software, Mariakerke, Belgium) and SigmaPlot (ver. 9.01, Systat Software, San Jose, CA). All measurements and calculations were double-blinded.

RESULTS

The histological grade of villous atrophy for the 31 patients was Marsh IIIa (n = 17), Marsh IIIb (n = 9) and Marsh IIIc (n = 6). The results of quantitative analysis of all biopsy images are shown in figs 3, 4 and table 1. Figure 3 shows the biopsy image measurements (n = 31). When the E/P ratios for these patients were binned according to the Marsh score, the mean quantitative values were 2.76 (SD 0.44) (IIIa), 1.91 (0.50) (IIIb) and 1.18 (0.22) (IIIc), as summarised in table 1. The difference between the means was statistically significant (p = 0.006). A scatter plot

Table 1 Comparison of Marsh score to edge-to-piecewise arc length ratio (E/P ratio)

Marsh score	Marsh IIIa	IIIb	IIIc
E/P ratio	2.90 (0.74)	1.91 (0.50)	1.18 (0.22)

The E/P ratio is given as mean (SE), which is unitless. The mean values are significantly different ($p = 0.006$).

of E/P ratio versus Marsh score from each biopsy slide is shown in fig 4 for comparison. There was a statistically significant correlation between Marsh score and E/P ratio based upon the Spearman rank correlation coefficient ($\rho = -0.798$) and Kendall tau ($\tau = -0.681$, $p<0.0001$). Thus, the E/P ratio decreases significantly as the Marsh score increases in the order IIIa to IIIb to IIIc.

DISCUSSION

We used quantitative image manipulation techniques to grade the degree of villous atrophy by determining the ratio of villous length to arc length of digitised biopsy slides. Since image processing is used for quantification, these measures are objective and are not susceptible to subjective impressions or interobserver variability. Moreover, they provide real-numbered scores, which potentially reflect the state of the intestinal mucosa more precisely. Moreover, the entire quantification process could be automated for more rapid and accurate assessment of villous atrophy in patients suspected of having coeliac disease.

However, in this preliminary study, we used images from patients with coeliac disease that all had villous atrophy—partial, subtotal or total (Marsh grade III). No patients with Marsh I or II lesions, characterised by the absence of villous atrophy and having only intraepithelial lymphocytosis, with or without crypt hyperplasia, were studied.

Our study demonstrates that quantitative techniques can be used for histopathological assessment of biopsy specimens. Not all pathology services have dedicated gastrointestinal pathologists available to interpret the enormous number of biopsies submitted from an endoscopy unit. However, this technique may be used by pathology services that lack dedicated gastrointestinal pathologists, or can be used as a quality-assurance tool for experienced gastrointestinal pathologists.

From biopsy slides, the E/P ratio does not actually take into account the crypt depth (or the crypt-to-villous ratio). E/P is a measure of total villous length per arc length, P. It is interesting that the original Marsh criteria did not use a crypt-to-villous ratio, merely the actual length of the villi.[8] Most pathologists assess the crypt-to-villous ratio as a parameter of villous atrophy, which is a modification of the Marsh criteria.[9 10] Since E/P is correlated to the Marsh score (fig 4), it suggests that villous length normalised by the arc length is by itself an accurate measure of villous atrophy. The inverse correlation

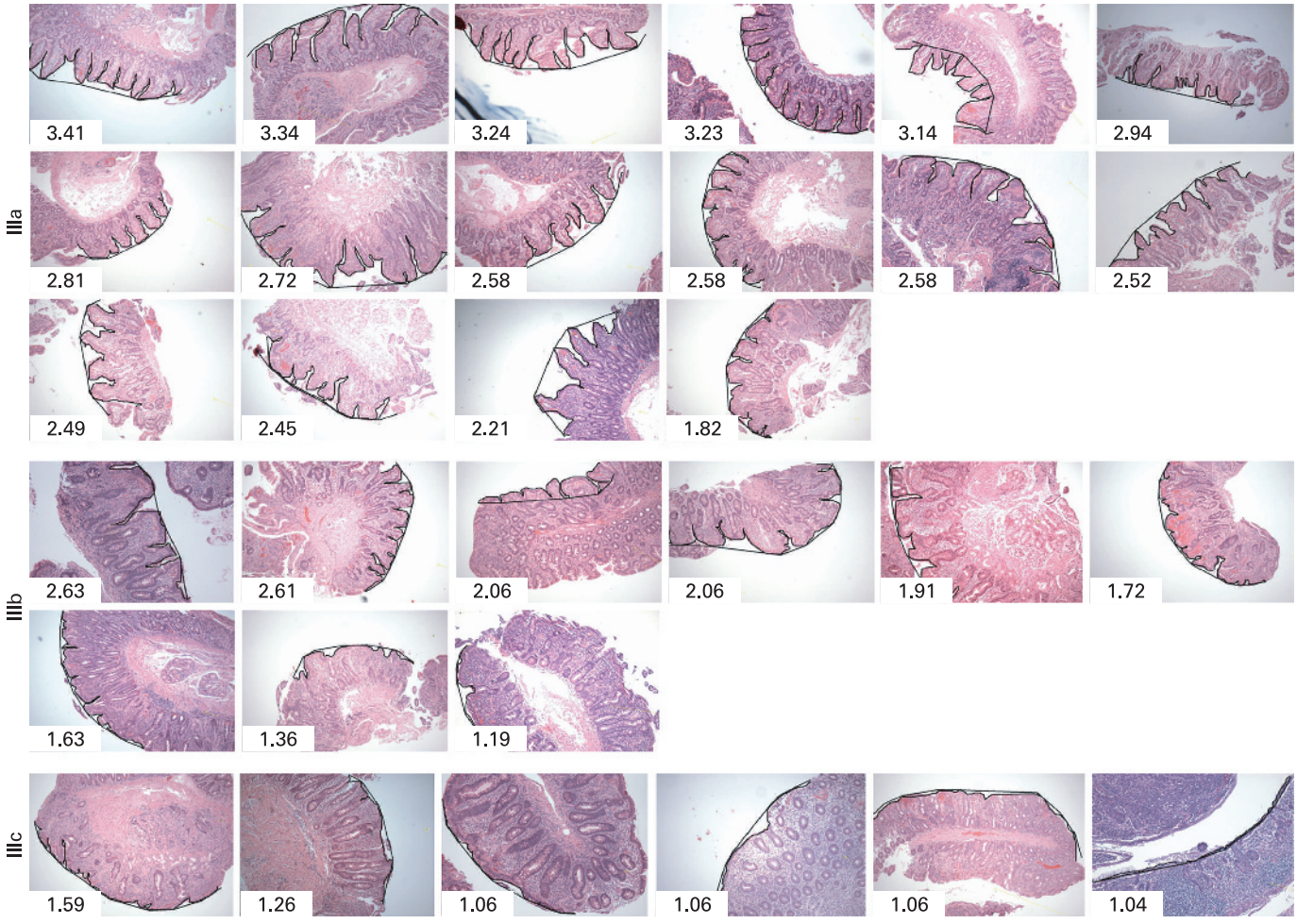

Figure 3 Quantitative results for measurement of edge-to-piecewise arc length (E/P ratio). In each panel the length of the villous edge (E) and the piecewise arc length (P) are detected, and the E/P ratio is given. The E/P ratio progressively decreases from a score of Marsh IIIa to IIIb, to IIIc.

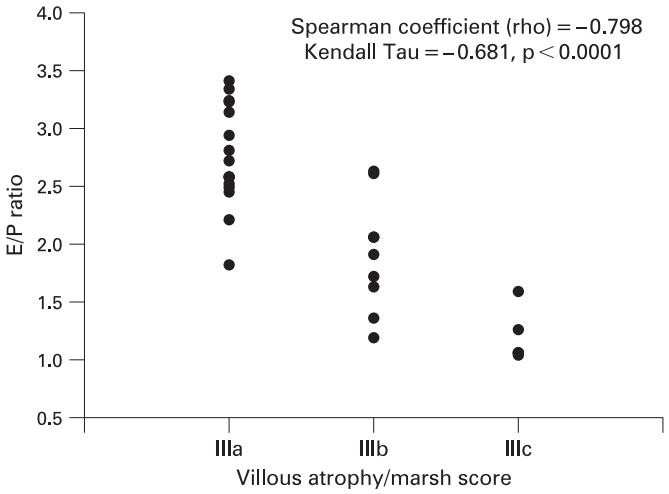

Figure 4 Scatter plot of E/P ratio versus Marsh score for comparison of the variables. There is a significant inverse correlation between the Marsh score and the quantitative measurement E/P based upon non-parametric tests ($p<0.0001$).

between the Marsh score and E/P ratio (fig 4) quantitatively reflects the presence of progressively shorter villi in the order Marsh IIIa to IIIb to IIIc biopsy specimens.

In conclusion, we have presented a novel quantitative technique to assess villous atrophy in pathology samples. Once standardised, this technique avoids interobserver or subjective interpretations. Applications of such techniques are multiple, including ensuring quality pathological interpretation and diagnostic techniques when expertise is not available, as well as serving as a quality-assurance tool for experienced gastrointestinal pathologists, and improving the possibility that low-level villous atrophy will be detected.

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Competing interests: None.

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