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BASELINE QUANTITATIVE HISTOLOGY IN THERAPEUTICS TRIALS REVEALS VILLUS ATROPHY IN MOST PATIENTS WITH COELIAC DISEASE WHO APPEAR WELL-CONTROLLED ON GLUTEN-FREE DIET

Short Title: Quantitative histology and treated coeliac disease

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ABSTRACT

Background: The prevalence and severity of duodenal injury in coeliac disease patients controlled on a gluten-free diet is unclear.

Aims: To use quantitative histology to assess duodenal injury in treated coeliac disease.

Methods: Quantitative histology in pre-treatment duodenal biopsies collected in clinical trials assessing an investigational immunotherapy for coeliac disease were analysed. Morphometric readings were converted to Marsh classifications.

Results: 93 patients had duodenal biopsies. For well-oriented sections of second part biopsies, six (6%) patients were classified as Marsh 0 or 1, 30 (33%) as Marsh 2, and 56 (60%) as Marsh 3a or 3b. In second part biopsies from 78 seronegative patients on gluten-free diet >2 years, 27 (35%) were Marsh 2 and 45 (58%) were Marsh 3a or 3b. Distal compared to proximal duodenal biopsies had significantly higher villus height to crypt depth ratios (median, third part: 2.1 versus bulb: 1.4; $P<0.0001$) and higher intraepithelial lymphocyte density (44 versus 30; $P=0.0002$). The sum of paired villus height and crypt depth measurements was correlated strongly with villus height ($r_s = 0.82$, $P<10^{-10}$). At least one biopsy was graded Marsh 3a or worse in all 26 patients who had serial biopsies from the bulb, first, second and third part, but was overlooked in 20 patients by subjective histology.

Conclusions: Quantitative histology in well-oriented biopsy sections reveals villus atrophy in the majority of patients with coeliac disease who appear well-controlled on gluten-free diet. Standardisation of biopsy collection, processing and evaluation could substantially improve the value of follow-up biopsies in coeliac disease.

Key words: Coeliac disease, transglutaminase IgA, serology, quantitative histology, gluten-free diet.

Abbreviations: Human leukocyte antigen-DQ (HLA-DQ), transglutaminase 2 (TG2).

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INTRODUCTION

Persistent duodenal mucosal damage in patients with coeliac disease on gluten-free diet is associated with increased severe long-term complications (1). A recent meta-analysis found 31% of patients with coeliac disease had persistent villus atrophy on gluten-free diet, and only half of these patients had elevated transglutaminase 2 (TG2) IgA serology (2). A corollary of these findings is that about one in five patients apparently well controlled on gluten-free diet with normal TG2 serology has duodenal villus atrophy. These conclusions are, however, based on routine processing, and subjective or semi-quantitative analysis of biopsies, usually from the distal duodenum.

Quantitative histology provides accurate assessment of duodenal mucosal injury in coeliac disease by using highly reproducible measurements of villous height, crypt depth and intra-epithelial density in well-oriented distal duodenal biopsies (3, 4). With implementation of rigorous standard operating procedures, quantitative duodenal histology outperforms conventional qualitative histology using grouped classifications such as Marsh score (5-7), and is emerging as a preferred measure of efficacy in therapeutics trials for coeliac disease (5, 8-10). Perhaps surprisingly, quantitative histology has not yet, to our knowledge, been used to evaluate disease activity/duodenal injury in patients who appear "well-controlled" coeliac disease and are in serological remission.

Quantitative histology in baseline duodenal biopsies collected in phase 1 and 2 clinical trials assessing an investigational immunotherapy, Nexvax2®, for coeliac disease provided the opportunity to assess duodenal injury in patients with "well-controlled" coeliac disease on gluten-free diet (11-13). The primary objective of this study was to use quantitative histology to assess duodenal mucosal injury in treated coeliac disease, and in particular those patients who are well established on gluten-free diet, and in serological remission.

METHODS

Study design

The study analysed quantitative histology of duodenal biopsies collected during the pre-treatment periods of four clinical trials testing a novel investigational immunotherapy (Nexvax2®). Patients

were recruited at sites in Australia, New Zealand, and the United States. In each study, a subgroup of patients had biopsies collected as a safety assessment before and after the treatment period. Details of the four studies are summarized in Table 1. In the three phase 1 studies, which have been reported in detail elsewhere (11, 12), patients with coeliac disease were maintaining their usual gluten-free diet had no study interventions before their pre-treatment biopsy. In the phase 2 ("RESET CeD") trial, which has also been described elsewhere (13), patients with coeliac disease were maintaining their usual gluten-free diet, but had a single bolus gluten challenge (6 grams of gluten protein taken as 10 grams vital wheat gluten flour in water) three weeks before biopsy collection according to the protocol described by Tye-Din et al (14).

Patients and interventions

All patients gave written, informed consent prior to undergoing any trial-related procedures. Eligibility criteria for each clinical trial have been reported fully elsewhere (11-13). In brief, for all four studies, patients who had biopsies collected had met initial screening criteria, which included being aged between 18 to 70 years, having documented duodenal villus atrophy while consuming gluten, being positive for *HLA-DQA1*05* and *DQB1*02* ("HLA DQ2.5"), and having maintained a gluten-free diet for at least one year. Patients with uncontrolled complications of celiac disease including refractory coeliac disease were excluded. In phase 1 studies, additional study-specific eligibility criteria were intended to select for patients entering the treatment period who were strictly maintaining a gluten-free diet. In the first and second phase 1 studies, patients who had a known gluten exposure within two months prior to screening were excluded, and patients were discontinued if subjective assessment of Marsh class for any pretreatment biopsy was more severe than 1 (raised intra-epithelial lymphocytes with normal villous architecture). In the third phase 1 study, patients were excluded if both TG2 IgA and deamidated gliadin peptide IgG serology were elevated, or if their score in the Celiac Dietary Adherence Test suggested non-compliance to gluten-free diet. In the phase 2 study, patients in the biopsy subgroup had met the inclusion criteria that experienced worsening digestive symptoms on the day of screening gluten food challenge three weeks earlier.

Quantitative duodenal histology

Biopsy collection, processing and quantitative histology methods in the first and second phase 1 studies have been described in detail elsewhere (3, 11, 12). Briefly, biopsies were collected from four sites: the duodenal bulb, and from the first, second, and third parts of the duodenum. Two biopsies were obtained at each site with a single biopsy per pass of the forceps, and placed in formalin fixative. Both biopsies from each site were processed together; they were embedded in paraffin, sectioned, and stained with haemotoxylin and eosin, and by anti-CD3 immunochemistry. The pathologist at the central laboratory made a subjective assessment of whether a patient had any biopsy consistent with a Marsh classification above 1. Subsequently the same histology slides as used to assess the initial Marsh score were batched and sent to the University of Tampere for quantitative histology. If sections from a biopsy were considered poorly oriented and prevented accurate assessment, the central pathology laboratory was requested to re-cut tissue sections. Two observers (JT, AP) analysed all slides independently and were unaware of the clinical data or laboratory findings of the patients. Replicate measurements of villus height, crypt depth and frequency of intraepithelial CD3+ lymphocytes per 100 villus enterocytes (IELs) with well-oriented villus-crypt units were determined according to published protocols (3). Final Marsh classifications for formalin-fixed biopsies were based on average villus height to crypt depth ratios according to the previously reported conversion scale: villus height to crypt depth ratio ≥2.8 for Marsh (M)0 if intraepithelial CD3+ lymphocyte density per 100 enterocytes <25, or M1 if ≥25; villus height to crypt depth ratio 2.0 - 2.7 for M2; villus height to crypt depth ratio 1.2 - 1.9 for M3a; villus height to crypt depth ratio 0.5 - 1.1 for M3b; and villus height to crypt depth ratio 0.0-0.4 for M3c (15).

In the third phase 1 study and phase 2 study, biopsy collection was limited to the second part of the duodenum, PAXgene tissue fixative (QIAGEN, Hilden, Germany) was used, and biopsies were sent directly to Jilab Inc. (Tampere, Finland) for processing and quantitative histology. Biopsy collection and handling, and the protocol for quantitative histology are described elsewhere (3, 12, 16). Paxgene fixative facilitated a wider repertoire of immunohistochemical, immunofluorescence assessments, and genome-wide transcriptome analysis in the same tissue sample (16). In brief, after collection, biopsies were placed one per compartment of a multi-compartment tissue cassette. The cassette was immersed in PAXgene fixative for 1-4 hours, and then transferred to the proprietary storage solution in

PAXgene dual-chamber fixative containers (Qiagen #765112, Venlo, Netherlands). Samples in fixative were shipped at ambient temperature and then stored at +4°C until processing. Each biopsy was embedded in a separate paraffin block under a dissection microscope and aimed for a cutting plane perpendicular to the mucosal lumen surface to orientate the specimens correctly. As for the earlier studies, the slides were stained with haematoxylin and eosin and by anti-CD3 immunochemistry. De-identified slides were scanned as whole-slide images using a SlideStrider scanner at a resolution of 0.28 μm per pixel (Jilab Inc., Tampere, Finland). Images were stored as JPEG2000 files in the image server and viewed over the Internet with web-based client software developed for this study (Celiac Slide Analyzer). The same two observers (JT, AP) analysed all slides independently and were unaware of the clinical data or laboratory findings of the patients. Final Marsh classifications for PAXgene-fixed biopsies were based on average villus height to crypt depth ratios according to the previously reported conversion scale: villus height to crypt depth ratio ≥2.3 for Marsh (M)0 if intraepithelial CD3+ lymphocyte density per 100 enterocytes <25, or M1 if ≥25; villus height to crypt depth ratio 1.8 - 2.3 for M2; villus height to crypt depth ratio 1.1 - 1.7 for M3a; villus height to crypt depth ratio 0.5 - 1.0 for M3b; and villus height to crypt depth ratio 0.0-0.4 for M3c (16).

Statistical analyses

No formal power calculation was undertaken for these exploratory, post hoc analyses. Non-parametric tests were used to assess the significance of differences in paired (Wilcoxon signed rank test) or unpaired (Mann-Whitney test) observations, and to assess correlations (Spearman's coefficient). All statistical tests were 2-tailed. Summary and significance statistics were computed using Graphpad Prism V7.0d.

RESULTS

Patient characteristics

Table 1 shows the characteristics of the 93 patients who had pre-treatment duodenal biopsies collected across three phase 1 trials and one phase 2 study investigating Nexvax2 in patients with coeliac disease. Disposition of patients in the phase 1 studies who had biopsies has been reported previously,

(11, 12) and is shown in Figure S1 for patients in the phase 2 study. Patient characteristics were similar for the four studies. The median period since diagnosis of coeliac disease was six years. Three (4%) of the 85 patients who had serology evaluated had elevated levels of transglutaminase IgA. At screening, 30 (75%) patients in phase 1 studies and 48 (91%) patients in the phase 2 study had been diagnosed and managed with a gluten-free diet for at least two years, and were confirmed sero-negative for TG2-specific IgA.

Quantitative histology in second part duodenal biopsies

Overall, among all 93 patients assessed, Table 2 shows six (6%) had second part duodenal biopsies classified as Marsh class 0 or 1, 30 (33%) had Marsh 2, and 56 (60%) had Marsh 3a or 3b. The proportion of patients with second part duodenal histology graded as Marsh 3 was similar for each study. There were non-significant trends for Marsh 3a and 3b classification to be less prevalent in patients confirmed as seronegative for transglutaminase 2 IgA who had been diagnosed for at least two years (58%) compared to other patients (78%), and more prevalent in patients subjectively assessed during screening in phase 1 studies as having Marsh class worse than 1 (84%) than those subjectively assessed as Marsh 0 or 1 (68%). Table 3 shows that there was no difference in the distribution of Marsh grades between patients recruited in Australia, New Zealand, and the United States.

Proximal to distal changes in duodenal quantitative histology

For all 26 patients having pre-treatment biopsies in the first and second phase 1 Nexvax2 clinical trials, two biopsies were collected from each of the bulb, first, second and third part of the duodenum. Figure 1 shows there were no significant differences in quantitative histology between the bulb and first part, but there were differences in villus height to crypt depth ratio and intra-epithelial lymphocyte density between proximal and distal sites that were progressive and statistically significant. Median villus height to crypt depth ratio increased from 1.4 (Marsh 3a) in the bulb to 2.1 (Marsh 2) in the third part, and median intra-epithelial lymphocyte density increased modestly from 30 in the bulb to 44 in the third part (normal <25). Intra-epithelial lymphocyte density in second part biopsies was significantly correlated with the bulb (Spearman $r_s = 0.70$, P = 0.0001), first part ($r_s =$

0.66, P = 0.0003) and third part (r_s = 0.76, P < 0.0001) in the same patient. Villous height to crypt depth ratio in second part biopsies was significantly correlated with the first part (r_s = 0.35, P = 0.044) and third part (r_s = 0.41, P = 0.021) in the same patient.

Quantitative histology identifies mucosal injury overlooked by subjective assessment

It is well known that most small bowel mucosal biopsies are currently processed without orientation in the endoscopy or pathology laboratory, and that poor orientation may compromise interpretation of villus-crypt architecture (17). In PAXgene fixed biopsies in the present study, Marsh class changed with level of cutting in the same biopsy block, as presented in Figure 2. For example, the section shown in Figure 2A was classified as Marsh 1 (tall villi and increased intra-epithelial lymphocytes) becomes a crypt hyperplastic lesion classified as Marsh 3a in Figure 2F, and Figure 2C shows to the right, where the cutting is perpendicular to the luminal surface, a crypt hyperplastic lesion classified as Marsh 3c. When measuring all readable villus-crypt units in the six histology sections presented, the global mean villus height to crypt depth ratio is 0.85, i.e. Marsh class 3b according to Taavela et al (16). Hence, to improve orientation of tissue sections prepared by the central laboratory and allow accurate assessment by quantitative histology, 40% of biopsies from the first and second phase 1 studies were re-cut. Overall, 26 (100%) patients had biopsies from at least one location graded as Marsh class 3a, 3b, or 3c (median 3b) by quantitative histology, which contrasted with the subjective assessment during screening that 20 (77%) had duodenal histology no worse than Marsh 1.

Villus height is linked to variability in duodenal villus-crypt length

The height of the villus-crypt axis (i.e. the sum of villus height and crypt depth) has been depicted as a constant, which implies that there is a reciprocal relationship between villus height and crypt depth (4). Figure 3 shows the relationships between measurements of duodenal villus height and crypt depth, and their ratio or sum for the pooled set of 169 biopsy assessments from 93 patients. As expected, villus height and crypt depth are significantly, but rather weakly correlated (Figure 3A), and that the sum of paired values of villus height and crypt depth are relatively constant with a median of 533 μm (interquartile range: 482.5 - 575.5) (Figure 3B). The ratio between villus height and crypt depth is, as expected, correlated strongly positively with villus height (Spearman r_s = 0.78, P $<10^{-10}$),

and negatively with crypt depth ($r_s = 0.74$, $P < 10^{-10}$) (Figure 3C). However, villus height increases about four-times more rapidly than crypt depth decreases as the sum of villus height and crypt depth increases (Figure 3D), and the sum of villus height and crypt depth is correlated strongly positively with villus height ($r_s = 0.82$, $P < 10^{-10}$) and weakly negatively, but significantly, with crypt depth ($r_s = -0.22$, $P = 0.004$). When analysed separately, the correlation between the sum of villus height and crypt depth and villus height was statistically significant for each study and for each region of the duodenum (Table S1). Collectively, these findings provide support for a model of mucosal behavior, healing and deterioration that involves not just reciprocal changes in villus height and crypt depth, but also includes increasing villus-crypt length as villus height increases in patients with coeliac disease on gluten-free diet (Figure 4).

DISCUSSION

This study used quantitative histology to assess duodenal mucosal injury in 93 patients with treated coeliac disease who enrolled in clinical trials of an investigational immunotherapy. Eligibility criteria for these clinical trials were intended to select for patients who were well-controlled on gluten-free diet, and consequently, transglutaminase IgA serology was elevated in fewer than 5% of participants tested. Altogether, quantitative histology of biopsies from the second part of the duodenum indicated mucosal "healing" (Marsh 0) in only one case, mucosal recovery (Marsh 0 – 2) in 36 (38%), and persistent villus atrophy (Marsh 3a, 3b or 3c) in 57 (61%) cases. Mucosal recovery (42%) and persistent villus atrophy (58%) were similar for the subgroup of 78 (84%) patients we defined as "well-controlled", who were sero-negative for transglutaminase antibodies and had been established on gluten-free diet for at least two years. Severity of mucosal injury was similar across the three countries where patients were recruited, Australia, New Zealand and the United States. Persistent villus atrophy revealed by quantitative histology in our cohort of "well-controlled" patients is, in fact, higher than the 38% recently reported for 1,345 patients with self-reported moderate or severe coeliac disease-associated symptoms (4). Even though it is well-known that persistent duodenal villus atrophy is not efficiently detected by transglutaminase serology in patients on gluten-free diet (2), our findings suggest that standard processing and subjective evaluation of duodenal biopsies also fails to detect

villus atrophy in many patients with treated coeliac disease. For example, in the first phase 1 study of Nexvax2 immunotherapy, subjective histology identified 14 of 16 patients as Marsh 0 or 1, but all 16 had Marsh 3 lesions in at least one biopsy collected from four sites across the duodenum. Inadequate orientation of tissue sections and simply evaluating biopsies bases on villi only where sections show no crypts cut longitudinally appeared to contribute to unrecognized mucosal injury in our study, and has been highlighted in recent pathology guidelines (17). A novel aspect of our study was to assess quantitative histology at four duodenal sites from the bulb to third part. Villus flattening was significantly more severe in the proximal duodenum, but intraepithelial lymphocyte density was modestly higher in the distal duodenum. An unexpected finding was that the combined length of the duodenal villus-crypt unit can vary by as much as 50%, and this can be attributed to relative increases in villus height being four times as much as decreases crypt depth. These findings provide support for a model of mucosal behavior, healing and deterioration that involves not just reciprocal changes in villus height and crypt depth, but also includes increasing villus-crypt length as villus height increases in patients with coeliac disease on gluten-free diet (Figure 4).

Our study had several limitations. Biopsies evaluated were from patients in subgroups of those enrolled in four clinical trials testing an experimental immunotherapy. These patients may not be representative of randomly selected patients in Australia, New Zealand, or the United States because the eligibility criteria were intended to ensure only patients well-controlled on gluten-free diet were enrolled. Notably, the prevalence of elevated transglutaminase IgA serology in our study patients was similar to that reported in recent follow-up studies from Norway, Finland and Australia that reported persistent villus atrophy in 4% to 15% of adults on gluten-free diet for more than five years (18-20). Although the patients in phase 1 studies had no per protocol gluten exposure before collection of biopsies, those in the phase 2 study had consumed a bolus of six grams of gluten three weeks before biopsy collection. Consequently, biopsies in the phase 2 study may have shown residual effects of this gluten exposure, even though Marsh classes in these patients were similar to milder than those from phase 1 study patients. Although an important practical consideration for future studies, this study did not systematically compare evaluation of mucosal injury by the local pathologist with subsequent quantitative histology. Under-reporting the severity of mucosal injury in follow-up biopsies is

concerning for patients and treating physicians, but in this study was rectified by re-cutting biopsies with poorly orientated tissue sections. A further limitation of our findings might be that we did not consider potential drug-induced enteropathy to occur in our study subjects, for example subjects using Olmesartan (21), proton pump inhibitors, non-steroidal anti-inflammatory drugs or selective serotonin reuptake inhibitors (22).

This study had four principal findings. We confirm that quantitative histology demonstrates persistent duodenal mucosal injury in the majority of patients with coeliac disease on gluten-free diet (4), and extend this observation to patients who are sero-negative and would be considered "well-controlled". Second, villus height to crypt depth ratio, and intra-epithelial lymphocytosis increase progressively from the proximal to distal duodenum. This finding highlights the importance of standardizing anatomical sites where biopsies are collected during clinical follow-up and in clinical trials. Third, there is a strong positive correlation between villus height and overall crypt-villus length, which may provide additional information regarding healing of the mucosa. Finally, there is a marked discrepancy between subjective histology and quantitative histology when evaluating duodenal mucosal injury in patients on gluten-free diet. While quantitative histology is unlikely to be practical in routine laboratory assessment of duodenal biopsies, our findings suggest that the clinical value of follow-up histology could be improved by adhering to published guidelines (17), which could more accurately reveal previously unrecognised mucosal injury in patients with coeliac disease on gluten-free diet.

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Figure Legends

Figure 1. A shows villous height to crypt depth ratios, and **B** shows intraepithelial lymphocyte (IEL) densities in formalin-fixed biopsies from the proximal and distal duodenum. Two biopsies were collected from the bulb, 1st, 2nd and 3rd part of the duodenum in 26 patients enrolled in the first and second phase 1 studies of Nexvax2. Medians and interquartile ranges are shown; p values by Wilcoxon test. Data for biopsies with well-oriented villus-crypt units is shown; data for biopsies from the bulb for two patients are missing as neither had any well-oriented villus-crypt units observed.

Figure 2: A-F a series of hematoxylin eosin-stained cuttings of the same endoscopy forceps biopsy specimen fixed in PAXgene and embedded in paraffin. **A** shows the cutting that was graded as Marsh 1 because of tall normal looking villi and an increased density of intraepithelial lymphocytes. The hallmark for tangential cutting is evident, no crypts are cut longitudinally, only cross sections of crypts are seen. Morphometry cannot be performed, and neither can a Marsh class can be given. **B** shows a section from the same biopsy block re-cut after tilting. **C-F** show four further sections resulting from further tilting between each re-cut. Crypt hyperplasia is evident and measurable villus-crypt units in the 6 sections give a global mean villus height crypt depth ratio of 0.85, i.e Marsh class 3b according to Taavela et al (16)

Figure 3. Relationship between measurements of duodenal villus height and crypt depth, and their ratio or sum for the pooled set of 169 biopsy assessments from 93 patients. **A** shows villus height and crypt depth are significantly, but weakly correlated, (Spearman correlation coefficient and associated P-value). **B** shows the individual sums of paired values of villus height and crypt depth measurements in each clinical trial for each site sampled. The median (interquartile range) for all 169 samples was 533 μm (482.5 - 575.5). No significant differences were found between biopsies from the 2nd part compared to biopsies from other sites in Study 1 and 2 (formalin-fixed), or 2nd part biopsies in Study 3 and 4 (PAXgene-fixed). **C** shows the expected strong correlations between villus height to crypt depth ratio and villus height, and to crypt depth. **D** shows villus height increases more about four-

times more rapidly than crypt depth decreases as the sum of villus height and crypt depth increases (VH+CrD = 0.81*VH+273, and CrD = -019*VH+273).

Figure 4. Schematic representation of the relationship between villus height and crypt depth measurements in the duodenal mucosa of patients with treated coeliac disease in the present study.

Table 1. Study details and patient characteristics

Study:	**1**	**2**	**3**	**4**	**Total**
Study details					
Nexvax2 trial	1st Phase 1	2nd Phase 1	3rd Phase 1	Phase 2	
Biopsy locations in duodenum	Bulb, 1st-3rd	Bulb, 1st-3rd	2nd	2nd	
Biopsies per site	2	2	4	6	
Start date	8Apr13	17Apr14	4Jan16	7Sept18	
End date	10Sept13	31Jul14	20Sept16	25Mar19	
Patient characteristics, number (%) of patients unless indicated					
Number of patients having biopsies	16	10	14	53	93
Females	12 (75)	7 (70)	7 (50)	40 (75)	66 (71)
Age, years †	44 (33-57)	40 (25-55)	44 (33-57)	39 (33-51)	40 (32-51)
Years after diagnosis CeD †	6 (5-7)	3 (2-7)	6 (3-10)	7 (4-11)	6 (3-11)
<2 y after diagnosis CeD	1 (6)	0	1 (7)	3 (6)	5 (5)
2 copies *HLA-DQB1*02*	7 (44)	4 (40)	5 (36)	9 (17)	25 (27)
TG2 IgA‡ serology elevated	1 (6)	0	0	2 (4)	3 (3)
TG2 IgA‡ serology not done	2 (13)	4 (40)	2 (14)	0	8 (8)
Screening histology Marsh 0 or 1 ††	14 (88)	6 (60)	ND	ND	
TG2 IgA‡ negative & diagnosis ≥2 y	13 (81)	6 (60)	11 (79)	48 (91)	78 (84)

† Median (interquartile range). ‡TG2 IgA, INOVA rh transglutaminase 2 IgA. †† Modified Marsh score assessed by local pathologist on initial cuts of biopsies before re-cutting and quantitative histology was performed. GFD, gluten-free diet. ND, not done.

Table 2. Number (%) of patients with well-controlled coeliac disease (1^O)†, and other coeliac disease patients, with indicated Marsh (M) grading based on quantitative histology of 2nd part duodenal biopsies.

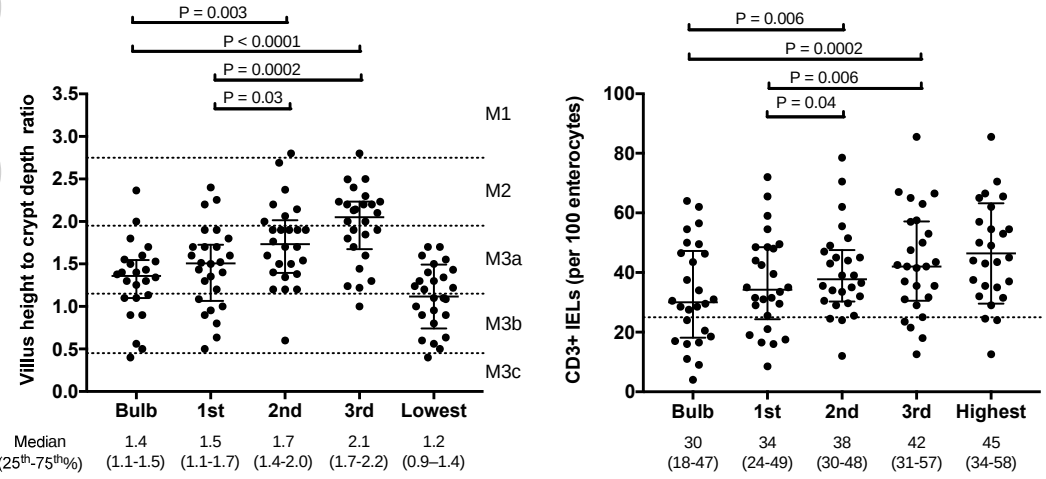
Study:	**1 & 2**			**3**			**4**			**Total**		
Patients:	**1^O**	**Other**	**All**	**1^O**	**Other**	**All**	**1^O**	**Other**	**All**	**1^O**	**Other**	**All**
M0	0	0	0	1 (9)	0	1 (7)	0	0	0	1 (1)	0	1 (1)
M1	1 (5)	0	1 (4)	1 (9)	0	1 (7)	3 (6)	0	3 (6)	5 (6)	0	5 (5)
M2	5 (26)	1 (14)	6 (23)	3 (27)	0	3 (21)	19 (40)	2 (40)	21 (40)	27 (35)	3 (20)	30 (32)
M3a	12 (63)	6 (86)	18 (69)	6 (55)	2 (67)	8 (57)	25 (52)	2 (40)	27 (51)	43 (55)	10 (67)	53 (57)
M3b	1 (5)	0	1 (4)	0	1 (33)	1 (7)	1 (2)	1 (20)	2 (4)	2 (3)	2 (13)	4 (4)
M3c	0	0	0	0	0	0	0	0	0	0	0	0
Total	19	7	26	11	3	14	48	5	53	78	15	93

†Patients with TG2 IgA serology in the normal range and on gluten-free diet for >2 years are in the primary analysis population indicated by 1^O, patients who had positive TG2 serology or it was positive, or were on GFD for <2 years are not in the primary analysis group are indicated by "Other".

Table 3. Number (%) of patients† with indicated Marsh (M) grading based on quantitative histology of 2nd part duodenal biopsies according to country of recruitment.

Location:	**Australia**				**New Zealand**			**USA**	
Study:	**1&2**	**3**	**4**	**Total**	**1&2**	**4**	**Total**	**4**	**Total**
M0	0	1 (9)	0	**1 (3)**	0	0	**0**	**0**	**1 (1)**
M1	0	1 (9)	2 (11)	**3 (8)**	1 (10)	1 (13)	**2 (11)**	**0**	**5 (6)**
M2	3 (33)	3 (27)	7 (37)	**13 (33)**	2 (20)	2 (25)	**4 (22)**	**10 (48)**	**27 (35)**
M3a	6 (67)	6 (55)	9 (47)	**21 (54)**	6 (60)	5 (63)	**11 (61)**	**11 (52)**	**43 (55)**
M3b	0	0	1 (5)	**1 (3)**	1 (10)	0	**1 (6)**	**0**	**2 (3)**
M3c	0	0	0	**0**	0	0	**0**	**0**	**0**
Total	9	11	19	**39**	10	8	**18**	**21**	**78**

†Limited to patients in the primary analysis population: TG2 IgA serology in the normal range and on gluten-free diet for >2 years



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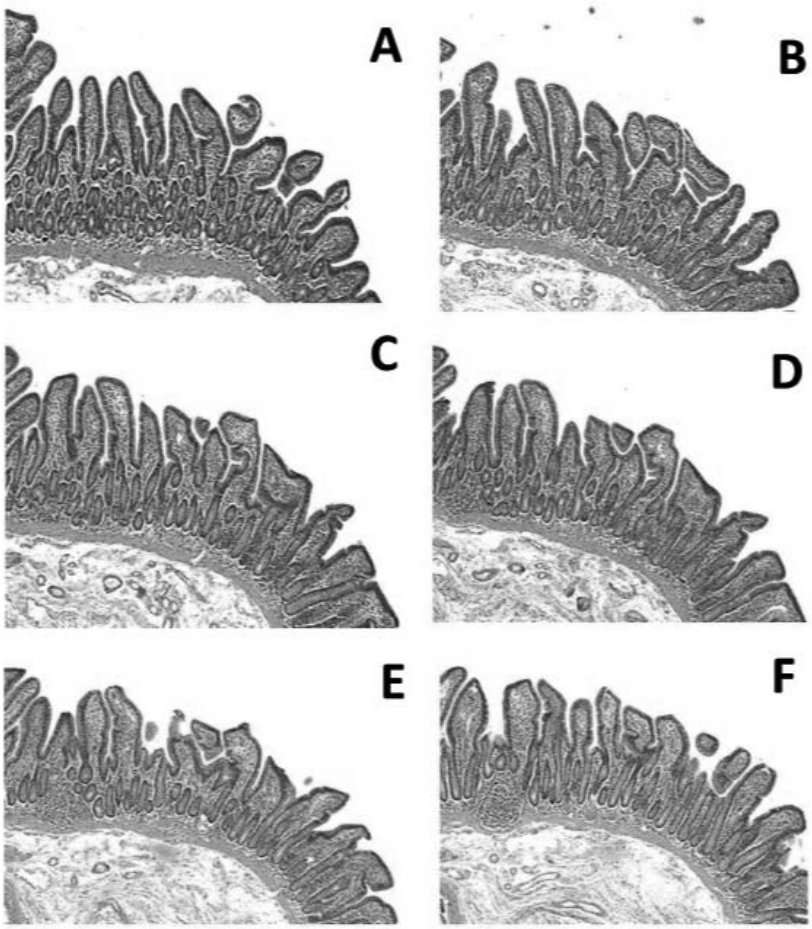

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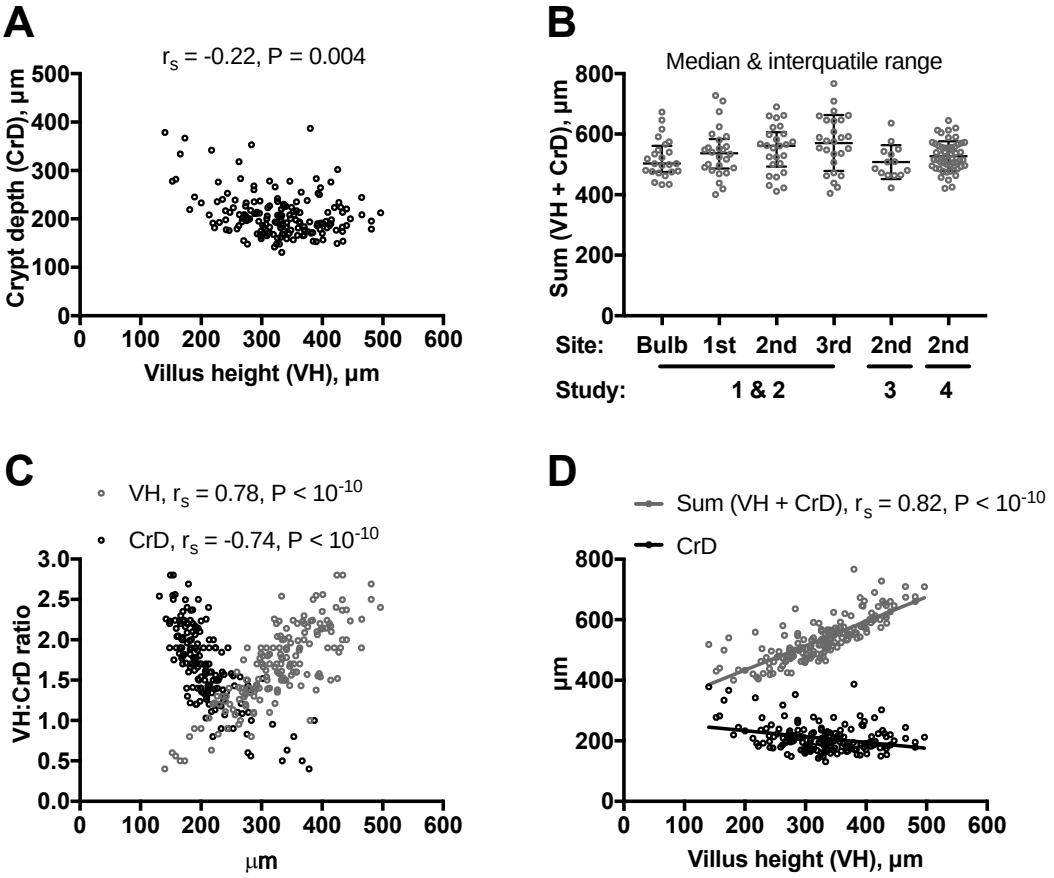

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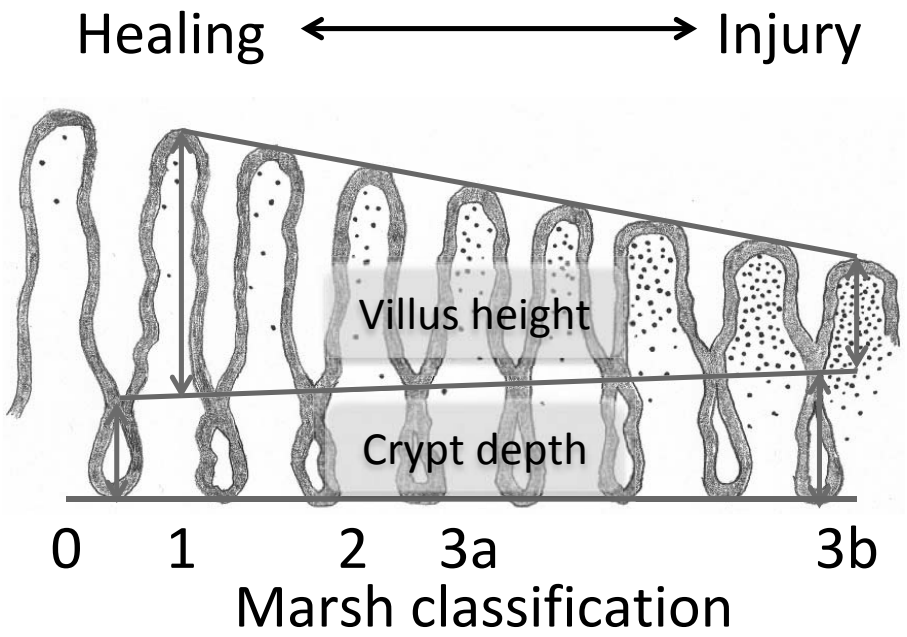

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