A Composite Morphometric Duodenal Biopsy Mucosal Scale for Celiac Disease Encompassing Both Morphology and Inflammation

Jack A. Syage,[1] Markku Mäki,[2] Daniel A. Leffler,[3] Jocelyn A. Silvester,[3] Jennifer A. Sealey-Voyksner,[1] Tsung-Teh Wu,[4] and Joseph A. Murray[4]

[1]ImmunogenX, Inc, Newport Beach, California; [2]Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland; [3]Celiac Disease Research Program, Harvard Medical School, Boston, Massachusetts; and [4]Mayo Clinic, Rochester, Minnesota

BACKGROUND & AIMS: Villus height to crypt depth ratio (Vh:Cd) and intraepithelial lymphocytes (IEL) are key measures of histology of the small intestine in celiac disease. Although the field of celiac disease has advanced, there remains no broadly accepted measure of mucosal injury. We assessed whether a composite Vh:Cd and IEL scale (VCIEL) can improve accuracy and statistical precision for assessing histology, compared with individual measures.

METHODS: The formulation of the VCIEL composite histologic scale was based on combining the Vh:Cd and IEL measurements for individual patients with equal weighting, by converting each scale to a fraction of their standard deviation and summing the results. The VCIEL formula was applied to several clinical trials and the results for Vh:Cd and IEL were compared with those for VCIEL with regards to clinical significance (effect size) and statistical significance.

RESULTS: For the ALV003-1021 trial, we observed an effect size and *P* value (analysis of covariance) of 1.37 and 0.038 for ΔVh:Cd, 1.17 and 0.005 for ΔIEL, and 1.86 and 0.004 for ΔVCIEL. For the similar gluten-challenge IMGX003-NCCIH-1721 trial, the corresponding results were 0.76 and 0.057 for ΔVh:Cd, 0.98 and 0.018 for ΔIEL, and 1.14 and 0.007 for ΔVCIEL. Similar improvements with the use of VCIEL over individual Vh:Cd and IEL measures were observed for other studies, including a nontherapeutic gluten challenge study.

CONCLUSIONS: The composite VCIEL scale combining Vh:Cd and IEL values seems to improve accuracy and statistical precision compared with either component alone.

Keywords: Small Intestinal Histology; Mucosal Scale; Celiac Disease; Morphology; Inflammation.

See editorial on page 1188.

Celiac disease (CeD) is a chronic enteropathy for which the only available treatment is lifelong strict adherence to a gluten-free diet (GFD).[1] CeD affects about 1% of the world's population.[2,3] Among many studies, Lanzini et al[4] demonstrated that after a median of 16 months on a GFD, only 8% of patients experienced histologic normalization and 26% had no improvement at all. Recent evidence indicates that, despite GFD adherence, patients with CeD on average consume unsafe levels of gluten on a GFD, which may account for persistence of histologic inflammation and residual symptoms.[5,6] Low levels of gluten exposure can also lead to inflammation and morphologic changes that can increase the risk of complications including lymphoma, bowel cancer, osteoporosis, anemia, and malnutrition.[7,8] Despite strict adherence to GFD, about half of patients with CeD show evidence of persistent small intestinal mucosal injury (Marsh grade II-III).[9,10]

In 1960, Rubin et al[11,12] and Thurlbeck et al[13] set the basis for correct small intestinal biopsy processing and reading in CeD diagnostics, showing that a key feature in CeD was increase in length of the crypts accompanied by shortening of the villi. In 1982, Kuitunen et al[14] published a paper establishing modern morphometry, which includes quantitative assessment of the gluten-dependent small intestinal mucosal morphologic (villus height [Vh]

and crypt depth [Cd]) and inflammatory changes (density of intraepithelial lymphocytes [IELs]). Standard operating procedures for processing of duodenal biopsies and validation of interobserver and intraobserver morphometric analyses of biopsy readouts in CeD have been reported.[15] Quantitative histopathology measurements have extensively been used to assess CeD activity.[10,16–22]

In the early 1990s, the Marsh classification of gluten-induced mucosal lesions was introduced, including these grades: the preinfiltrative type 0, infiltrative type 1, hyperplastic type 2, destructive type 3, and hypoplastic type 4.[23] The injury classification was modified by Oberhuber et al[24] and further subdivided the type 3 lesion into subgroups a-c. The Marsh-Oberhuber (M-O) groups M0, M1, M2, M3a, M3b, and M3c have since gained popularity in some clinical settings. The groups M1 to M3c include increased density of IELs.

A review by Hindryckx et al[25] assessed acceptable outcome measures of disease activity for use in CeD clinical trials. Quantitative morphometric histologic analyses had better reliability and responsiveness compared with qualitative scales, but were thought to be time-consuming and highly dependent on optimal orientation of the specimen. Interobserver variability between pathologists has been at best fair to moderate, mostly because of variability in the use of M-O classification.[26–29] These concerns have also been expressed in the Food and Drug Administration–sponsored 3rd and 6th Gastroenterology Regulatory Endpoints and Advancement of Therapeutics Workshop (GREAT III and VI).[30,31]

There are weaknesses in the quantitative and qualitative ways of measuring gluten-induced mucosal injury in patients with CeD. Morphometry measures the injury continuum for architecture and inflammation, but these are used as separate outcomes. The original Marsh and M-O classifications are rather contrived approaches to assess a biologic continuum, forcing the injury in categorical groups of unclear clinical relevance and where clinically significant changes may occur within 1 single category. Furthermore, the quantitation of the inflammation relies on binary assessment of normal or increased, which results in histology that is unscorable by M-O if villous atrophy persists without increased IELs.

In this article we propose a new metric for small intestinal enteropathy that we have named VCIEL. This was developed to test the hypothesis that a composite measure of architectural changes (Vh:Cd) and inflammation (IEL count) may be a more appropriate measure of mucosal health than either of the components alone.

Methods

Composite Histology Scale

The conversion for an individual's Vh:Cd and IEL readings to the VCIEL scale is given by

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What You Need To Know

Background

Though villus height to crypt depth ratio (Vh:Cd) and intraepithelial lymphocytes (IEL) are key measures of histology of the small intestine in celiac disease (CeD) there is no commonly accepted quantitative measure of mucosal injury.

Findings

A composite scale VCIEL comprising individual subject values for Vh:Cd and IEL with equal weighting appears to offer better accuracy and statistical precision, particularly for population analysis in clinical trials, as well as potentially offering a broader measure of mucosal health.

Implications for patient care

The VCIEL scale enables accurate measurement of mucosal health in CeD patients and may improve the powering of clinical trial designs. Use of VCIEL may lead to better outcome measures for potential new therapeutic treatments benefitting patients.

$$VCIEL = \left[\frac{Vh{:}Cd - <Vh{:}Cd>}{\sigma_{Vh{:}Cd}} - \frac{IEL - <IEL>}{\sigma_{IEL}} \right] \quad (1)$$

where $<Vh{:}Cd>$ and $<IEL>$ are the mean values and $\sigma_{Vh{:}Cd}$ and σ_{IEL} are standard deviations for the population. In many study designs, all treatment groups can be pooled to calculate standard deviation at baseline before any intervention (eg, active treatment or placebo). This formulation creates equally weighed distributions for Vh:Cd and IEL centered at zero. We chose to maintain the same direction of change as the Vh:Cd scale by adding IEL values after a sign reversal accounting for the negative sign in Equation (1). The difference between 2 measurements can be defined as

$$\Delta VCIEL = VCIEL_{final} - VCIEL_{baseline} \quad (2)$$

If individual patient values are not known, then one can compute $\Delta VCIEL$ from the mean population change $\Delta Vh{:}Cd$ and ΔIEL from baseline to final measurement as

$$\Delta VCIEL = \left[\frac{\Delta Vh{:}Cd}{\sigma_{Vh{:}Cd}} - \frac{\Delta IEL}{\sigma_{IEL}} \right] \quad (3)$$

where $\sigma_{Vh{:}Cd}$ and σ_{IEL} are defined as before. Equation (3) is also useful when evaluating a dose-dependent trend analysis where there are multiple dose groups in

addition to placebo, and the individual patient histology values are less important or unavailable. Another use for Equation (3) is when evaluating histology based on median values for which there is just 1 value in any population group.

A graphical representation of how VCIEL is calculated by Equation (1) is given in Supplementary Figure 1. This also makes clear that the VCIEL scale naturally has a peak in its distribution of patients at zero, with positive and negative values representing, respectively, better and worse mucosal health relative to the average for that population. The mean and standard deviation are calculated normally for the set of patient values in a population group.

It is also possible to scale the VCIEL values to "look" more like Vh:Cd values by fitting a linear function to a plot of VCIEL versus Vh:Cd and applying the equation

$$\text{VCIEL}_{\text{scaled}} = \text{VCIEL} \times m + b \qquad (4)$$

where the constants m and b are the slope and intercept, respectively. It is shown in the Discussion section that $\text{VCIEL}_{\text{scaled}}$ and VCIEL are statistically equivalent, and the conversion is simply to retain a Vh:Cd-like scale modified by IEL values.

The VCIEL algorithm was developed at ImmunogenX, Inc and is for unrestricted use.

Histologic Sample Preparation

We provide details of the biopsy sample preparations for the 4 studies evaluated in this manuscript. Further details can be found in the literature cited.[17,18,22,32] In general, upper gastrointestinal and duodenal biopsies were collected. The standard procedure is for biopsy samples to be individually formalin fixed, paraffin embedded, oriented, cut, and then stained with hematoxylin and eosin for Vh and Cd measurements and with $CD3^+$ for measurements of IEL densities. Because histologic lesions can be patchy, typically at least 4 biopsy samples are taken, with 3–5 cuts per sample. Readings are made by light microscopy by a gastrointestinal pathologist.

The 4 studies that served as test cases for evaluating the VCIEL composite measure included the following: the gluten challenge ALV003-1021 (NCT00959114) was conducted and biopsies read at the University of Tampere, Finland.[15,17] The nongluten challenge IMGX003-NCCIH-1721 (NCT03585478) study was multicentered, but all biopsies were read at the Mayo Clinic.[18] The gluten challenge ALV003-1221 (NCT01917630) study was conducted and biopsies read at the Mayo Clinic.[22] The noninterventional gluten challenge (NCT03409796) study was conducted at Massachusetts General Hospital and Beth Israel Deaconess Medical Center, and biopsies read at the Harvard Medical School.[32]

Statistical Analysis

The analyses of the histology data used 2-sided paired t test for within-group changes, 2-sided unpaired t test for between-group comparisons, and unpaired t test and analysis of covariance (ANCOVA) for the between-group differences. For the dose-dependent trend analysis, linear regression analysis was conducted returning R^2 and P values (slope). These analyses were performed using either GraphPad Prism 9.1.1 or XLSTAT 2021.1.1.

Results

The following sections illustrate the performance of VCIEL based on reanalysis of data from 4 studies.

ALV003-1021 (NCT00959114)

In this trial, subjects randomized to either latiglutenase 900 mg or placebo consumed 2 g/day of gluten for 6 weeks.[17] In Figure 1, the ΔVCIEL scale (Equations 1 and 2) visually seems to benefit from a narrower distribution for individual patients in each group, providing greater statistical significance compared with either the ΔVh:Cd or ΔIEL scales. The results for mean reduction of histologic damage for latiglutenase (IMGX003) versus placebo were 83%
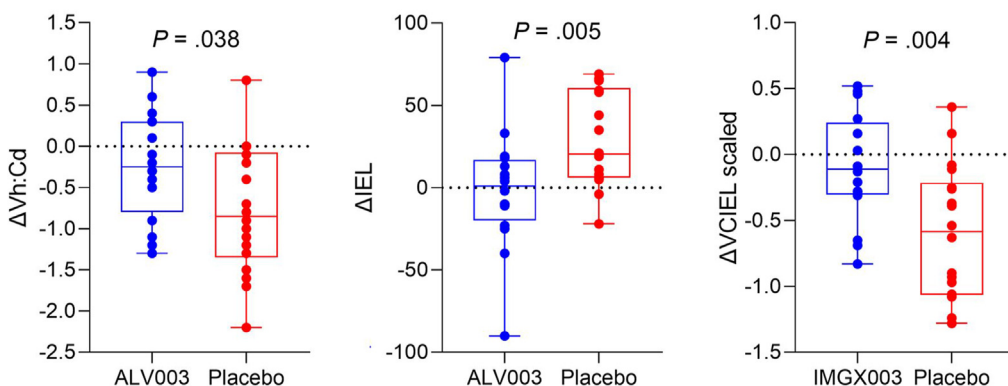
Figure 1. Histologic change to a gluten challenge in study ALV003-1021 for latiglutenase (ALV003, n = 16) versus placebo (n = 18) for different scoring scales. The *box* and *whisker plots* show min and max, 1st and 3rd quartile, and median values. *P* values are ANCOVA.

Table 1. Tabulation of *P* Values for Study ALV003-1021 Histologic Parameters

	Vh:Cd	IEL	VCIEL	Type
P values				
A: B to F	.179	.832	.342	PTT
P: B to F	.001	< .001	< .001	PTT
B: A to P	1.000	.948	.963	UPTT
F: A to P	.055	.010	.015	UPTT
Delta	.035	.008	.004	UPTT
	.038	.005	.004	ANCOVA
Mean Attn, %	70	107	83	
Median Attn, %	71	95	81	
Effect size	1.37	1.17	1.86	

NOTE. N = 16 and 18 for IMGX003 and placebo, respectively.
A, IMGX003; ANCOVA, analysis of covariance; Attn, attenuation; B, baseline; Delta, difference between B and F; F, final after GC period; IEL, intraepithelial lymphocyte; M-O, Marsh-Oberhuber; P, placebo; PTT, paired *t* test; UPTT = unpaired *t* test; VCIEL, Vh:Cd and IEL scale; Vh:Cd, villus height to crypt depth ratio.

(median, 81%; ANCOVA $P = .004$) for ΔVCIEL compared with 70% (median, 71%; ANCOVA $P = .038$) for ΔVh:Cd and 107% (median, 95%; ANCOVA $P = .005$) for ΔIEL. Table 1 and Supplementary Figure 2 show the within-group and between-group *P* values along with effect size defined as the mean difference from baseline to Week 6 divided by the pooled baseline standard deviation. A key outcome is that effect size for ΔVCIEL increases because of the effective decrease in the pooled standard deviation resulting from merging the Vh:Cd and IEL data.

### IMGX003-NCCIH-1721 (CeliacShield, NCT03585478)

In this recently completed gluten challenge study, patients randomized to either a 1200-mg dose of latiglutenase or placebo consumed 2 g/day of gluten for 6 weeks.[22] The ΔVCIEL scale, similar to the ALV003-1021 example, enhanced the accuracy and statistical significance compared with either ΔVh:Cd or ΔIEL score (Figure 2 and Table 2). The results for mean reduction of

histologic damage for latiglutenase (IMGX003) versus placebo were 70% (median, 76%; ANCOVA $P = .007$) for ΔVCIEL compared with 88% (median, 83%; ANCOVA $P = .057$) for ΔVh:Cd and 60% (median, 72%; ANCOVA $P = .018$) for ΔIEL. Furthermore, because of the apparent increased precision of the composite VCIEL scale a statistical outlier (high 1-sided; $P = .05$) was evident. This was a patient randomized to placebo under a gluten challenge who oddly registered greater improvement in both Vh:Cd and IEL than any latiglutenase-treated patient. Removal of the outlier changed the ΔVCIEL reduction of histologic damage to 95% with greater statistical significance ($P = .001$). Table 2 and Supplementary Figure 3 show the within-group and between-group *P* values along with effect size, which increases for ΔVCIEL similar to the ALV003-1021 study, because of the effective decrease in the pooled standard deviation.

ALV003-1221 (CeliAction, NCT01917630)

This real-world study was used to demonstrate that the mean and median ΔVh:Cd and ΔIEL values (Equation 3) can be used for multidose studies to evaluate the improvement in the accuracy and statistical precision of the VCIEL scale relative to Vh:Cd and IEL individually.[18,33,34] In this study, the primary end point for Vh:Cd failed to show improvement with latiglutenase dose for the nominally 12-week long trial. However, 108 out of 430 subjects continued for an additional 12-week dose-dependent extension stage, and showed a positive trend for healing but did not reach statistical significance. Figure 3A-C for median values shows that the statistical significance (linear regression *P* value) of the trend improved for the composite VCIEL scale ($P = .119$) relative to Vh:Cd and IEL scales ($P = .283$ and 0.705, respectively). The mean values (Figure 3D) show improved statistical significance for VCIEL relative to Vh:Cd and IEL, but the overall results were not as statistically strong as for the median ($P = .983$, 0.426, and 0.295 for ΔVh:Cd, ΔIEL, and ΔVCIEL, respectively). Finally, we note that the

Figure 2. Histologic change to a gluten challenge in study IMGX003-NCCIH-1721 for latiglutenase (IMGX003, n = 21) versus placebo (n = 22) for different scoring scales. The *box* and *whisker* plots show min and max, 1st and 3rd quartile, and median values. *P* values are ANCOVA.
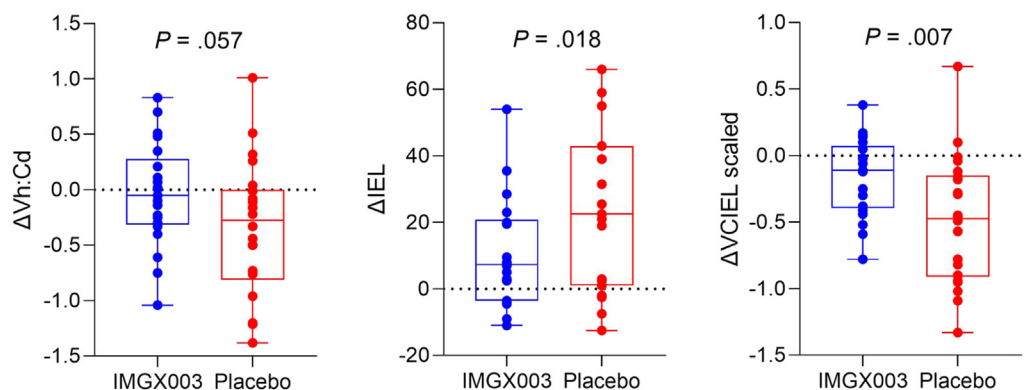
Table 2. Tabulation of *P* Values for Study IMGX003-NCCIH-1721 Histologic Parameters

	Vh:Cd	IEL	VCIEL	Type
P values				
A: B to F	.691	.010	.058	PTT
P: B to F	.015	< .001	< .001	PTT
B: A to P	.769	.916	.859	UPTT
F: A to P	.061	.047	.025	UPTT
Delta	.074	.017	.008	UPTT
	.057	.018	.007	ANCOVA
Mean Attn, %	88	60	70	
Median Attn, %	83	72	76	
Effect size	0.76	0.98	1.14	

NOTE. N = 21 and 22 for IMGX003 and placebo, respectively.
A, IMGX003; ANCOVA, analysis of covariance; Attn, attenuation; B, baseline; Delta, difference between B and F; F, final after GC period; IEL, intraepithelial lymphocyte; M-O, Marsh-Oberhuber; P, placebo; PTT, paired *t* test; UPTT, unpaired *t* test; VCIEL, Vh:Cd and IEL scale; Vh:Cd, villus height to crypt depth ratio.

analysis of these data by Equation (3) did not require the individual histologic values for each patient, demonstrating flexibility of the VCIEL scale.

NCT03409796

This was a 2-dose nonintervention gluten-challenge trial[32] that enrolled 7 patients into each of 3-g and 10-g, 14-day gluten-challenge studies. This study showed strong dose dependence as measured by Vh:Cd and IEL. The measured change from baseline to Day 15 was relatively small and not statistically significant for the 3-g study and relatively large and statistically significant for the 10-g study. The computation of VCIEL values from these data showed an improved statistical significance relative to the component values of Vh:Cd and IEL as seen by the within-group paired 2-tailed *t* test *P* values from baseline to Day 15 (see also Supplementary Figure 4): 3-g Vh:Cd, IEL, VCIEL of 0.4554, 0.1341, 0.1301, respectively; 10-g Vh:Cd, IEL, VCIEL of 0.0050, 0.0031, 0.0014, respectively.
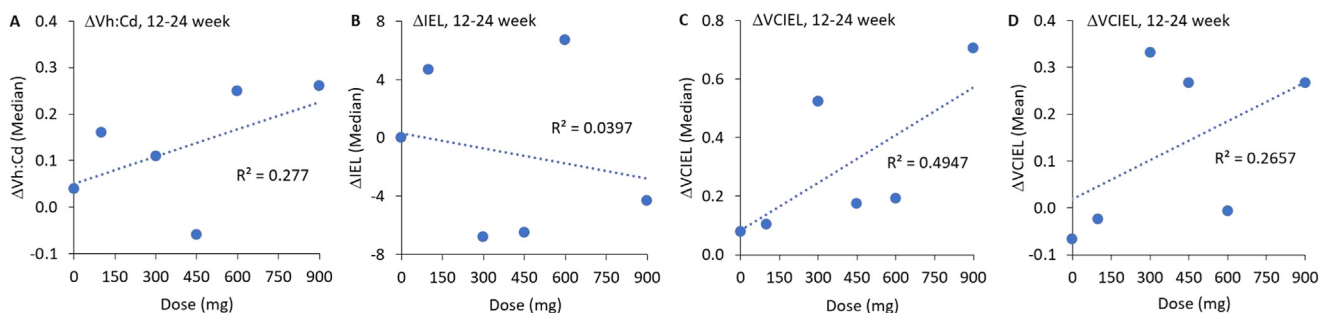
Other Considerations

In the Methods section it was described that the VCIEL results could be converted to a scale that resembles the Vh:Cd scale by Equation (3). Figure 4*A* gives the m and b values needed to complete the Equation (4) calculation. A similar plot for converting to an IEL scale is given in Figure 4*B*. The result for Vh:Cd versus VCIEL$_{scaled}$ is plotted in Figure 4*C* and shows excellent correlation. VCIEL$_{scaled}$ is a useful transformation because it allows for inclusion criteria and for clinical significance modeled after customary conditions for Vh:Cd. The VCIEL and VCIEL$_{scaled}$ results are statistically the same. The results of all histologic representations for the 3 featured gluten-challenge studies -1021, -1721, and the nonintervention study are given in Supplementary Figures 1–3, respectively.

Although Vh:Cd and IEL both measure mucosal injury and generally move in the same direction, there is little correlation between baseline values and changes with intervention on an individual patient basis. This was observed in both the ALV003-1021 and IMGX003-NCCIH-1721 studies as shown in Supplementary Figure 5 for the latter study. R^2 values for the baseline and delta correlations were, respectively, 0.005 and 0.208 for the -1021 study and 0.023 and 0.055 for the -1721 study. It is evident in Figure 4 that the baseline correlation (active and placebo populations) for Vh:Cd and IEL versus VCIEL has a much higher correlation of $R^2 = 0.576$ (being the same in both cases because of the symmetry of the VCIEL computation).

Discussion

One of the weaknesses of the M-O scale is the emphasis on Vh:Cd and de-emphasis of the changes in IELs. As noted in morphometric inflammatory analyses in clinical practice and drug trials,[10,16–22] and as pointed out by Kuitunen et al,[14] most agree that greater consideration to changes in IELs are needed.[35] This supports the concept of a composite histology scale, such as VCIEL, which assesses overall mucosal health rather than individual measures of histologic change as assessed by Vh:Cd and IEL. In our opinion, VCIEL could be useful for

Figure 3. Dose response for histologic change (median) for patients continuing through weeks 12–24. n = 30, 14, 24, 12, 16, and 12 for the doses ranging from placebo to 900 mg. R^2 and *P* values are for the trend line. (*A–C*) Median values of ΔVh:Cd, ΔIEL, and ΔVCIEL, respectively. (*D*) Mean values of ΔVCIEL.

Figure 4. Plots for the IMGX003-NCCIH-1721 study for baseline pooled IMGX003 (n = 21) and placebo (n = 22) patients. (*A*) Vh:Cd versus VCIEL. (*B*) IEL versus VCIEL. (*C*) Vh:Cd versus VCIEL$_{scaled}$.
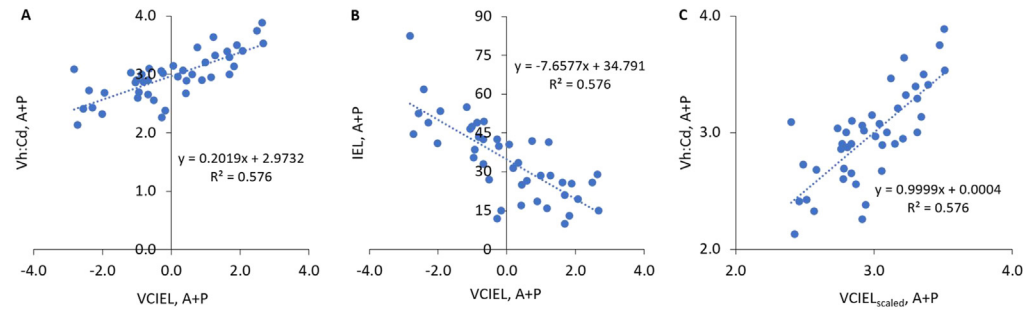
assessing the effect of therapeutic interventions and selection of patients for clinical trials. A prospective clinical trial will be required to set parameters for clinically meaningful change in VCIEL including sensitivity analyses based on Vh:Cd and IEL alone.

As noted in the Results section there is little correlation between baseline values and changes with intervention for Vh:Cd and IEL on an individual patient basis. Optimized sampling, processing of specimens cannot eliminate intrinsic sampling issues because of the patchiness of disease, which could contribute to this issue. Additionally, perhaps the improvement of these variables follows different time courses with treatment. Longer trials would be needed to establish the time courses of these 2 variables. The basis for the greater accuracy and statistical precision of the VCIEL scale is presumably caused by averaging over some of the measurement uncertainty in individual patient and timepoint Vh:Cd and IEL values and creating a composite of different histologic properties.

This study has several limitations. This was a retrospective analysis of several short-term studies so the general applicability especially with longer trials remains to be demonstrated. We are confident that VCIEL will be able to detect longitudinal change in response to treatment and will be superior to the use of Vh:Cd or IELs separately. We believe it will be useful even in unusual circumstances where the differences between Vh:Cd and IELs are more pronounced, such as because of different kinetics of mucosal healing versus mucosal damage, and depending on time and amount of gluten ingestion/restriction. Increased IELs may persist despite normalization of villous architecture.[23,24] Conversely, newly diagnosed patients with CeD with a gluten-induced manifest mucosal lesion may have normal IEL densities;[24] the sensitivity of increased density of CD3$^+$ IELs to detect untreated CeD was reported to be only 82%–89%.[36] With additional use in randomized clinical trials of varying lengths, we will be able to further validate the VCIEL and demonstrate its clinical utility and responsiveness to change.

In conclusion, a composite VCIEL scale comprising Vh:Cd and IEL values seems to provide better accuracy and statistical precision relative to either Vh:Cd and IEL alone. Use of VCIEL seems to result in increased capability to detect significant changes, which may be a benefit for improving the power of histologic measure in clinical trials and offering the potential to measure small intestinal mucosal health more holistically.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://doi.org/10.1016/j.cgh.2023.10.031.

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Correspondence
Address correspondence to: Jack A. Syage, PhD, ImmunogenX, Inc, 1600 Dove Street, Suite 330, Newport Beach, California 92660. e-mail: jsyage@immuongenx.com.

Supplementary Material

We now present a graphical means to explain the VCIEL algorithm, which is rather simple when realized that it is just averaging the Vh:Cd and IEL values based on where they lie on their respective distributions divided by the standard deviation of the distributions. Supplementary Figure 1 shows a set of arbitrary, but realistic, Vh:Cd and IEL values for 4 hypothetical patients. It is known (see manuscript) that Vh:Cd and IEL values do not correlate strongly on an individual patient basis because of variability in each person's mucosal health and also because change in condition (eg, newly diagnosed patients with CeD first adhering to a strict GFD or in a clinical trial setting taking a gluten challenge)

will show different change dynamics for Vh:Cd and IEL. On a population basis these scores better correlate because of averaging, but the lack of individual correlation, particularly for changing situations, leads to a wide variance from patient to patient, which in turn contributes to a large standard deviation for a particular population.

In Supplementary Figure 1 it is evident that the VCIEL distribution is narrower in terms of standard deviation than the individual Vh:Cd and IEL distributions because of the averaging effect of these different patient mucosal profiles.

Supplementary Figures 2–5 show individual patient response to gluten under either latiglutenase (ALV003 or IMGX003) or placebo for the ALV003-1021 or IMGX-NCCIH-1721 gluten-challenge studies.
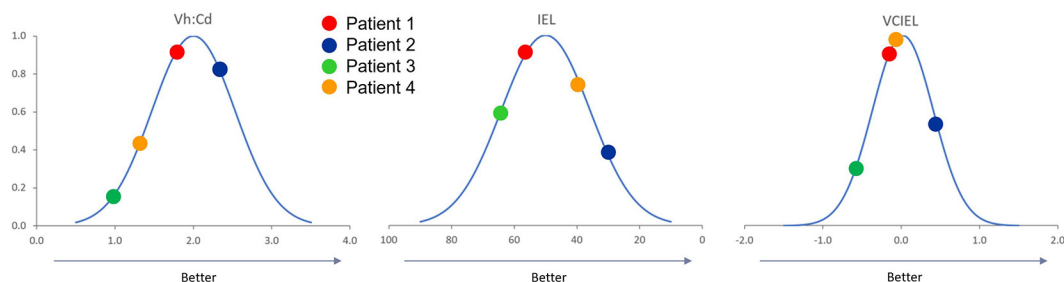

Supplementary Figure 1. Graphical representation of the VCIEL algorithm as exemplified by a set of arbitrary Vh:Cd and IEL values for 4 hypothetical patients.
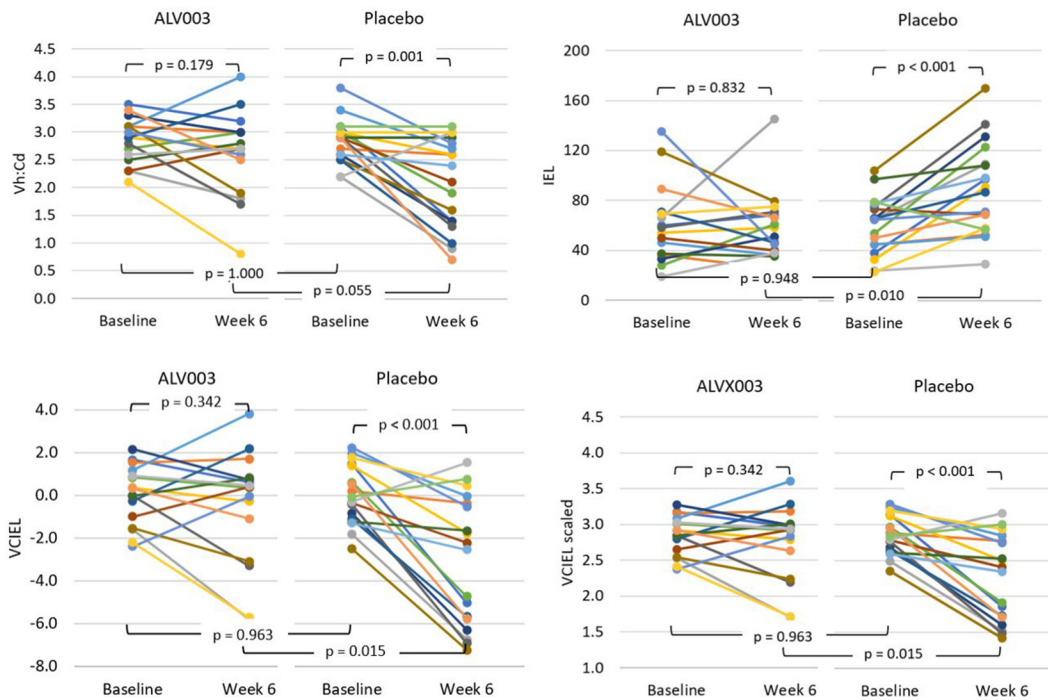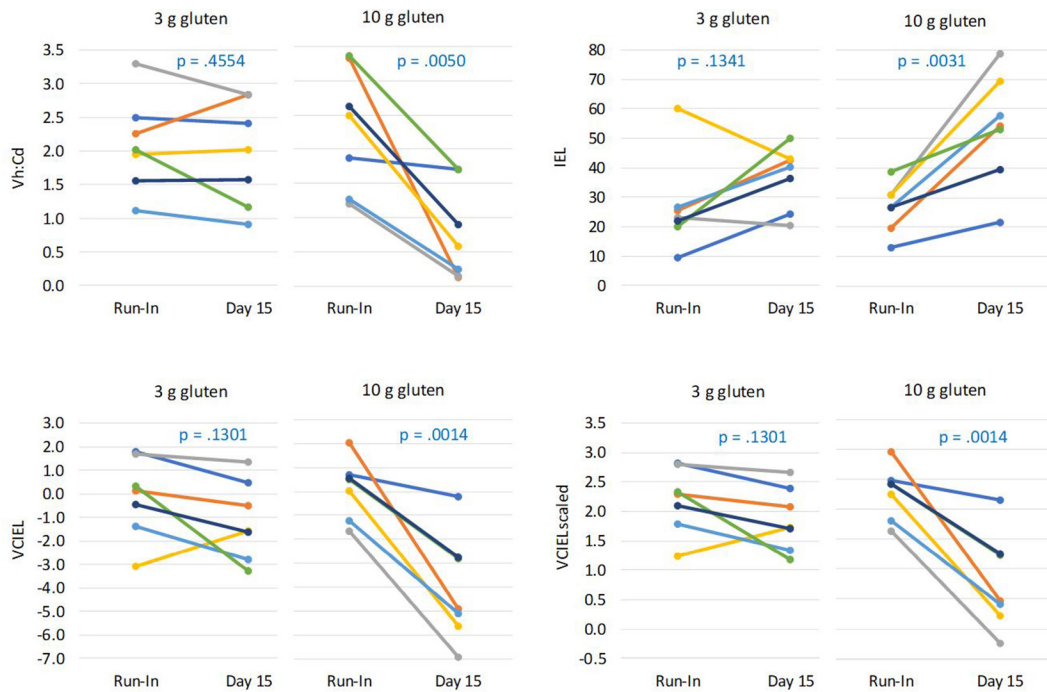
Supplementary Figure 2. Patient responses for study ALV003-1021 showing the change in Vh:Cd, IEL, VCIEL, and VCIEL$_{scaled}$ for baseline to Week 6 for ALV003 (now IMGX003) and placebo. The within-group and between-group *P* values are from Table 1.
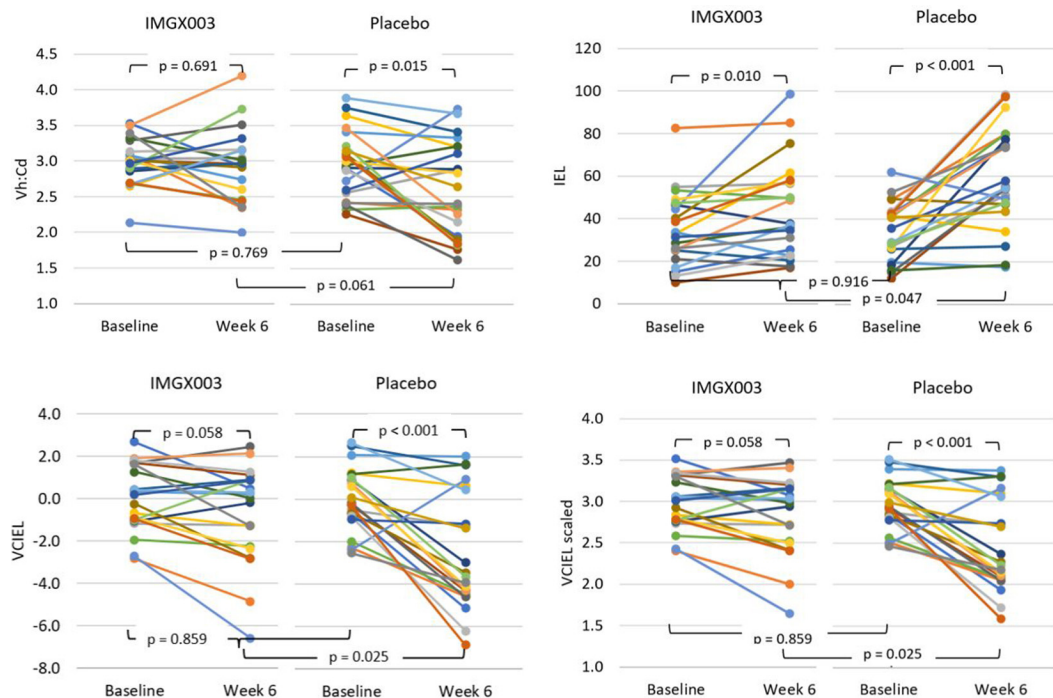
Supplementary Figure 3. Patient responses for study IMGX003-NCCIH-1721 showing the change in Vh:Cd, IEL, VCIEL, and VCIEL$_{scaled}$ for baseline to Week 6 for IMGX003 and placebo. The within-group and between-group *P* values are from Table 2.

Supplementary Figure 4. Patient responses for the nonintervention study NCT03409796 showing the change in Vh:Cd, IEL, VCIEL, and VCIEL$_{scaled}$ for baseline to Day 15 for 3-g and 10-g gluten challenge.
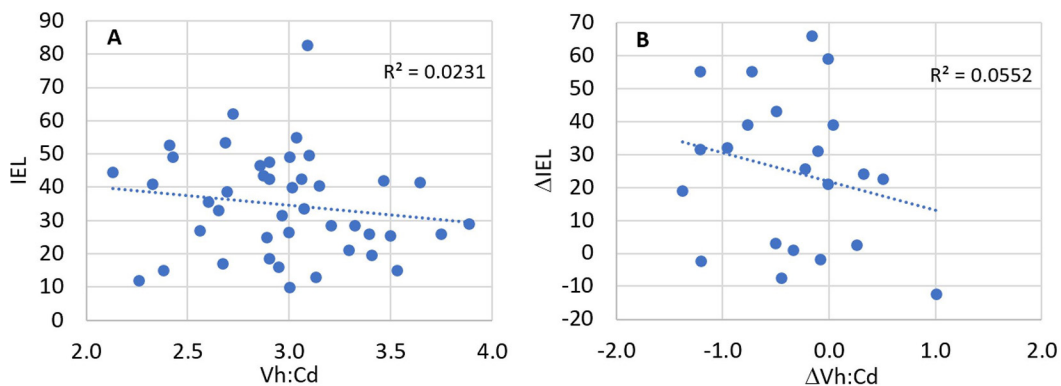
Supplementary Figure 5. Correlation plots for the IMGX003-NCCIH-1721 study. (*A*) Baseline Vh:Cd versus IEL for pooled IMGX003 (n = 21) and placebo (n = 22). (*B*) ΔVh:Cd versus ΔIEL for placebo (n = 22).