Diagnosing coeliac disease by rectal gluten challenge: a prospective study based on immunopathology, computerized image analysis and logistic regression analysis

Arzu ENSARI†, Michael N. MARSH*, Shethah MORGAN†, Robert LOBLEY‡, D. J. UNSWORTH§, Daphne KOUNALI¶, Peter T. CROWE*, Jane PAISLEY‖, Kieran J. MORIARTY‖ and John LOWRY**

*University Department of Medicine, Hope Hospital, Salford, Greater Manchester M6 8HD, U.K., †Department of Pathology, University of Ankara, Sihhiye 06430 Ankara, Turkey, ‡Department of Chemical Pathology, Manchester Royal Infirmary NHS Trust, Manchester M13 9WL, U.K., §Department of Clinical Immunology, Southmead Hospital, Bristol BS10 5NB, U.K., ¶Medical Research Council Environmental Epidemiology Unit, Southampton General Hospital, Southampton SO1 6YD, U.K., ‖Department of Gastroenterology, Royal Bolton Hospital, Bolton BL4 0JR, Lancashire, U.K., and **Department of Oral Surgery, Royal Bolton Hospital, Bolton BL4 0JR, Lancashire, U.K.

ABSTRACT

The purpose of this study was to evaluate the use of rectal gluten challenge in the diagnosis of coeliac disease. A total of 103 patients with features suggestive of this diagnosis were prospectively enrolled into the study; a diagnosis of coeliac disease was based on strictly defined criteria used in judging the proximal jejunal biopsy. On that basis, 45 out of the 103 patients were deemed to have coeliac disease. A slurry of gluten powder in physiological saline was introduced into the rectum, and biopsies taken before and at 2 h or 4 h after the challenge were examined immunohistochemically by computerized image analysis. Cell counts were analysed by logistic regression, and the best equations were obtained for each challenge group. The 2 h challenge yielded diagnostic sensitivity and specificity of 69.6% and 78.6%, respectively. The 4 h challenge provided sensitivity and specificity of 100% and 100%, respectively. These results were compared with other clinical diagnostic predictors, including anti-endomysial antibodies, which yielded diagnostic sensitivity and specificity of 70% and 98%, respectively. It is concluded that a 4 h rectal challenge is a highly sensitive means of identifying gluten-sensitized individuals, and would be of particular value in cases showing negative antibody screening or equivocal biopsy appearances.

INTRODUCTION

Clinical observations from Rubin’s laboratory in Seattle over 30 years ago established the major morphological features of gluten-induced mucosal pathology in coeliac disease, before and after treatment [1,2]. Rubin’s group, however, also demonstrated that marked pathological changes could be induced both in the terminal ileal mucosa [3] and in the rectal mucosa in response to the local instillation of a wheat slurry, which caused acute

Key words: coeliac, compensated latent gluten sensitivity, diagnosis, immunopathology, gluten, logistic regression analysis, rectal challenge.

Abbreviations: df, degrees of freedom; HLA-DR, human leucocyte antigen D locus: DR sublocus; IEL, interepithelial space lymphocyte; IL2-R, interleukin-2 receptor.

Correspondence: Dr M. N. Marsh (e-mail aensari@ato.org.tr).
watery diarrhoea, abdominal pain and even bleeding [4]. Although the full significance of these phenomena was uninterpretable at the time, they clearly anticipated current precepts that coeliac disease is dependent on the presence of gluten-sensitized T lymphocytes circulating within the intestinal mucosa [5–7]. For several years, we have investigated the morphological and immunopathological features of the rectal mucosa (i) in untreated and treated coeliac patients [8–10], and (ii) during a rectal gluten challenge [11]. Importantly, the rectal mucosa in subjects with untreated coeliac disease reveals a high proportion of \( \gamma \delta \)− cells within an elevated population of T (CD3+) lymphocytes [10]; thus the detailed immunopathology is identical with that of the classical upper jejunal lesion. Our first morphometric study of the rectal response to gluten challenge prompted a prospective diagnostic trial, with resulting specificities and sensitivities of approx. 80 % [9]. Encouraged by these results, and the simplicity of this approach, we decided to initiate a larger, prospective challenge study in which we confined our immunohistochemical observations to shorter periods of 2 and 4 h.

The purpose of the present study was to determine the efficacy of 2 h and 4 h rectal gluten challenges in the diagnosis of coeliac disease, using immunohistochemistry, computerized image analysis and logistic regression analysis in order to establish numerical (diagnostic) scores. The diagnosis of each patient entered into the trial was determined by jejunal biopsy – being judged either normal (or within normal limits) or coeliac (demonstrating the classical flat lesion). The study indicates that a 4 h rectal gluten challenge accurately predicts the presence of gluten sensitivity. In prospectively evaluating the diagnostic power of other currently used tests for coeliac disease, it became clear that a positive (coeliac) logistic score assumes an even greater significance when it was demonstrated that gluten-sensitive individuals do not necessarily present with elevated anti-endomysial, or anti-\( \alpha \)-gliadin, antibody titres. They comprised referrals with one or other symptoms, or findings, of weight loss, diarrhoea, recurrent oral aphthous ulceration, iron-deficiency anaemia or the rash of dermatitis herpetiformis; this last diagnosis was confirmed by demonstrating the typical granular IgA deposits at the dermo–epidermal junction of uninvolved skin, in addition to the presence of severe changes in the upper jejunal mucosa.

The remaining 58 patients (32 female/26 male), of median age 50 ± 14.5 years, were considered as ‘disease controls’ for the purposes of this study. This work was approved by the local ethics committee, and all patients gave informed written consent. All investigations were carried out at Hope Hospital.

### Procedure for rectal challenge

Each patient had fasted from 22.00 hours on the previous evening. No attempt at ‘bowel preparation’ was permitted; this important procedural detail avoids structural alterations or unwanted cellular infiltration of the rectal mucosa before the actual challenge was carried out. Before proceeding further, the gluten challenge was prepared by slowly suspending either 6 g (2 h challenge) or 12 g (4 h challenge) of gluten powder (BDH, Poole, Dorset, U.K.) in 40 ml of physiological saline in a plastic beaker. The resulting even slurry was then decanted into the barrel of a 50 ml syringe, to which was attached a Luer-ended catheter of soft plastic tubing, 10 cm in length (Kwill; Hinders-Leslies Ltd, London, U.K.). With the patient lying in the left lateral position, a pre-challenge mucosal biopsy was obtained. Then, with a gloved finger inserted through the rectal sphincter, the Kwill catheter was guided into the rectum, and the contents of the syringe were discharged.

The patient was asked to retain the material for at least 2 h, or longer if a 4 h challenge was being undertaken. Once the challenge slurry per rectum had been given, a Crosby capsule was then passed orally and located to the duodenojejunal flexure under fluoroscopic control, and a mucosal biopsy of the upper jejunum was obtained.

### Clinical laboratory investigations

Blood was taken under fasted conditions, at the time of jejunal biopsy, and analysed in the hospital laboratory for haemoglobin level, white cell count and MCV (mean corpuscular volume); serum albumin concentration (normal reference range 29–38 g l\(^{-1}\)); immunoglobulins of the A (0.1–1.5 g l\(^{-1}\)), M (1.6–5 g l\(^{-1}\)) and G (5–18 g l\(^{-1}\)) isotype; serum (4–28 \( \mu \)g l\(^{-1}\)) and red cell (80–120 \( \mu \)g l\(^{-1}\)) folate, vitamin \( B_{12} \) (150–950 ng l\(^{-1}\)), ferritin (30–275 \( \mu \)g l\(^{-1}\)) and total iron-binding capacity (45–70 \( \mu \)mol l\(^{-1}\)).

Daily faecal fat output (as mmol of fatty acid; reference range 0–17 mM per day) was calculated using a pooled

### METHODS

#### Subjects

A total of 103 patients were prospectively enrolled into the study. These patients came from the Outpatient Clinic of the University Department of Medicine at Hope Hospital (M.N.M.), and The Gastroenterological Clinic (K.J.M.) and the Oral Surgical Clinic (J.L.) of Royal Bolton Hospital. Of these 103 consecutive patients, 45 (27 female/18 male), of median age 39 ± 14.7 years, were deemed to be gluten-sensitized, based on ‘gold-standard’ entry criteria to the trial of a severe, avillous (‘flat’) mucosal lesion of the upper jejunum.
5-day sample collected at home. The percentage urinary excretion of D-xylose in 6 h (25 g oral dose after a 12 h fast) was also calculated (reference value > 20% of ingested oral dose).

Anti-reticulin antibodies were assessed by indirect immunofluorescence on a composite block of rat tissues (liver, kidney and stomach). Frozen sections (6 μm) were treated with patients’ sera diluted 1:10 (v/v) in PBS [12,13]. An R1 pattern [14], comprising discrete staining of fibrillar elements in all three tissues, especially rat liver parenchyma, was regarded as positive. Isotype staining showed that R1 anti-reticulin antibody positivity was confined to IgA antibodies. Anti-endomysial antibodies [12] were assayed on sections of Marmoset monkey oesophagus (The Binding Site, Birmingham, U.K.). Anti-gliadin antibody titres were determined for IgA and IgG with a commercial ELISA kit (Medical Innovations) using fasting blood samples taken at 0, 2 and 4 h post-challenge; similarly, soluble interleukin-2 receptor (IL2-R) was measured by ELISA (T Cell Sciences, Cambridge, MA, U.S.A.). Values were determined by computerized photometry (Dynatech, High Wycombe, Bucks, U.K.) of the peroxidase-coloured reaction product, according to manufacturer’s protocols.

At the beginning of the study, blood samples were taken pre-challenge and at 2 h and 4 h post-challenge; this practice was terminated once interim results failed to reveal any alterations in either anti-gliadin antibody (IgA and IgG) or IL2-R titres during the evolution of either challenge.

**Tissue handling**

**Biochemical assays**

Rectal tissue (4 h challenge only) was assayed for a panel of brush border oligosaccharidases and peptidases. Biopsies were homogenized in 2 ml of ice-cold 50 mM mannitol/2 mM Mops buffer at pH 7, with the homogenate being passed through a 23-gauge hypodermic needle immediately before assay.

All enzymes were assayed fluorimetrically. Maltase (EC 3.2.1.20), sucrase (EC 3.2.1.48), lactase (EC 3.2.1.23) and trehalase (EC 3.2.1.28) were determined by a micro method [15], and alkaline phosphatase (EC 3.1.3.1) [16], 7-glutamyltransferase (EC 2.3.2.2) [17] and dipeptidyl aminopeptidase IV (EC 3.4.14.1) [18] were assayed using methods described previously. Aminopeptidase N (EC 3.4.11.2) was measured with l-alanine-7-amido-4-methylcoumarin as substrate, and aminopeptidase A (EC 3.4.11.7) with 1 mM α-L-glutamyl-7-amido-4-methylcoumarin as substrate in 0.1 M potassium phosphate buffer, pH 6.9 [19]. All enzyme activities were expressed as units g protein, where 1 unit represents either 1 μmol of disaccharide hydrolysed, or 1 μmol of product liberated for non-disaccharidase enzymes. A fluorometric glycogen assay [20] was employed to measure total mucosal protein per specimen.

**Mucosal biopsies**

Upper-intestinal mucosal specimens were obtained with a Crosby capsule from the first loop of jejunum and retrieved rapidly. Rectal biopsies were taken from just within the internal anal sphincter (5–10 cm from the anal margin) by grasp forceps (Chevallier-Jackson 5 mm-cup instrument) operated through an illuminated disposable sigmoidoscope. On retrieval, jejunal and rectal specimens destined for routine histology were rapidly spread out, oriented with the mucosal face upwards on dental wax, and flooded in 2.5% ultrapure glutaraldehyde in 0.1 M cacodylate buffer for 4–6 h. Specimens were washed in fresh cacodylate solution and then dehydrated progressively in an ascending ethanol series, infiltrated with Araldite, sectioned at 1 μm in an OMM-3 Reichert ultramicrotome and stained with Toluidine Blue to await computerized morphometric image analysis.

For immunocytochemical analysis, mucosae were embedded in an OCT (optimum cutting temperature) compound (BDH) after orientation on wax, snap-frozen in melting isopentane (−160 °C) cooled with liquid nitrogen, and stored in Dewar flasks. Blocks were subsequently sectioned at 5 μm thickness using a cryostat and stored at −20 °C. All specimens were carefully mounted on carrot in order to maintain strict orientation during the frozen-sectioning process (see Figure 2). A series of monoclonal antibodies (Dako, High Wycombe, Bucks., U.K.; T Cell Sciences) was employed to quantify CD3+ and γδ+ (TCSI) lymphocytes, neutrophils (CD15) and macrophages (CD68), and the expression of MHC class 2 alloantigen [HLA-DR (human leucocyte antigen D locus: DR sublocus)] and IL2-R (CD25) by double staining. Monoclonal antibodies were employed at various dilutions (1:15 to 1:50) in a two-step indirect immunoperoxidase method in which diaminobenzidine was used as substrate and nickel chloride as enhancing agent [21]. Nuclei were counterstained with Mayer’s haematoxylin, and sections were then mounted in Apathy’s medium (BDH). The APAAP (alkaline-phosphatase–anti-alkaline-phosphatase complex) technique was used for detection of CD25 and CD15 with Fast Red as substrate; sections were then counterstained and mounted as above.

**Computerized image analysis of mucosal biopsies**

Jejunal mucosae were analysed using previously published methodology from our laboratory. The categorization of coeliac (flat) mucose depended on the increased nuclear diameter and elevated mitotic index of interepithelial space lymphocytes (IELs) [22], and an
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Figure 1 Immunohistochemical procedure employed in the present study

(a) Four frozen sections (5 μm each) are applied to a slide and stained alternately with anti-CD3 and anti-γδ monoclonal antibodies. (b) The numbers of each cell type overlying successive lengths of muscularis mucosae are counted, and accumulated values are obtained. A length of muscularis of 200 μm is suggested, but other lengths may be used, so long as a total of 1000 μm is surveyed. (c) Cumulative means have been plotted, indicating that it is necessary for counting to extend to 1000 μm of muscularis mucosae in order to obtain a constant result for each cell type (see [21]). In order to convert values into absolute populations per 10^4 μm^2 muscularis mucosae, each raw count is multiplied by 10/11 (0.909).

elevated γδ+ IEL complement within our coeliac range (per 10^4 μm^2 muscularis mucosae) (A. Ensari and M. N. Marsh, unpublished work). These data are not considered further. The concurrent immunohistochemical analysis of all rectal tissues was performed without knowledge of their respective diagnostic categories.

After reacting rectal mucosae with individual monoclonal antibodies (Figure 1) specific for CD3, γδ, CD15, CD68 or CD25, and with anti-HLA-DR class antibodies, individual profile counts for each decorated cell type were accumulated with respect to the epithelium or lamina propria (as appropriate), relative to a constant length of muscularis mucosae, and expressed per 10^4 μm^2 muscularis mucosae [5,8,10,11,23–26]. Our statistical analysis (below) indicated that, of all the variables studied in this trial, information of diagnostic value was contained only in post-challenge CD3+, CD15+ and γδ+ cell counts.

Thus, in order to obtain the diagnostic score, the total number of cells overlying a total length of 1000 μm of muscularis mucosae for (i) epithelial CD3+ cells and (ii) epithelial and lamina propria γδ+ cells is determined. The cumulative total for each cell group is multiplied by 0.909 to obtain absolute counts for each of these three cell populations, per 10^4 μm^2 muscularis mucosae [21,23–26], and entered into the equation (see worked examples below).

Statistical analysis

Comparisons of differences in cell populations before and after challenge, for disease-control and gluten-sensitized groups, were determined by paired r tests, with P values of < 0.01 being accepted as significant.

Logistic regression analysis of all variables was employed to determine which single variable, or small group of variables, best predicted the gluten-sensitized patients, at either 2 h or 4 h after gluten challenge (SPSS package).

RESULTS

Of the 103 patients enrolled consecutively into the study, jejunal biopsies of 45 revealed the accepted criteria for untreated coeliac disease. None of the patients enrolled had taken a gluten-free diet.

2 h challenge

This study involved 23 disease-controls (12 female) and 14 patients with untreated coeliac disease (8 female). At 2 h post-challenge, the rectal mucosae from patients with coeliac disease revealed significant changes in the numbers of CD3+ or γδ+ lymphocytes within the epithelium and lamina propria (Figure 2). There were, however, no significant rises in numbers of neutrophils (scored as CD15+ or macrophages (scored as CD68+), and no evidence of cell ‘activation’ (IL2-R or HLA-DR expression) (Figure 3).

The best logistic regression model for this group was based on post-challenge numbers of CD3+ cells in coeliac rectal epithelium and lamina propria, γδ+ cells

Figure 2 Group results for coeliac patients after a 2 h challenge

The left-hand panels show pre- and post-challenge data for CD3+ lymphocytes (per 10^4 μm^2 muscularis mucosae) in epithelium (EP) and lamina propria (LP). The right-hand panels show corresponding results for γδ+ lymphocytes.

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Rectal gluten challenge in gluten sensitivity

Figure 3 Data for neutrophils (CD15<sup>+</sup>) and ‘activated’ cells (IL2-R<sup>+</sup>; HLA-DR<sup>+</sup>) during a 2 h challenge (left) and a 4 h challenge (right).

Note the increased responses, quantitatively, at 4 h post-challenge. No statistically significant changes were observed after the 2 h challenge.

Figure 4 Group results for coeliac patients after a 4 h challenge

The left-hand panels show pre- and post-challenge data for CD3<sup>+</sup> lymphocytes (per 10<sup>4</sup> lmm<sup>2</sup> muscularis mucosae) in epithelium (EP) and lamina propria (LP). The right-hand panels show corresponding results for γδ<sup>+</sup> lymphocytes.

in epithelium, and CD15<sup>+</sup> (neutrophils) in lamina propria (where ‘post’ denotes post-challenge count):

Score = -27.4 + 0.04 × (post lamina propria CD3<sup>+</sup>)
+ 0.12 × (post epithelial CD3<sup>+</sup>)
+ 0.52 × (post epithelial γδ<sup>+</sup>)
+ 0.16 × (post lamina propria CD15<sup>+</sup>)

With degrees of freedom (df) = 8, the Hosmer–Lemeshow Chi-square was 1.70 (P < 0.98). Jacknife assessment of the predictive diagnostic power of this model revealed a specificity of 100% and a sensitivity of 78.6%.

4 h challenge

This part of the study involved 35 disease-controls (20 female) and 31 patients with untreated coeliac disease (19 female). In comparison with the 2 h challenge, at 4 h there were, in addition to highly significant rises in the numbers of epithelial and lamina propria CD3<sup>+</sup> and γδ<sup>+</sup> cells (Figure 4), highly significant increases in CD15<sup>+</sup> cells, and in IL2-R and HLA class 2 expression (Figure 3).

Figure 5 Diagnostic scores (log<sub>10</sub>) for gluten-sensitive patients, and disease-controls, obtained by logistic regression analysis of the post-challenge data

The two groups are clearly delineated by this procedure.

The best logistic regression model for this group was based on post-challenge epithelial CD3<sup>+</sup> cells and post-challenge epithelial and lamina propria γδ<sup>+</sup> cells, with each population determined as the absolute number of cells overlying the 10<sup>4</sup> lmm<sup>2</sup> test area of muscularis mucosae:

Score = -193 + 11.4 × (post lamina propria γδ<sup>+</sup>)
+ 0.9 × (post epithelial CD3<sup>+</sup>)
+ 6.01 × (post epithelial γδ<sup>+</sup>)

With df = 6, the Hosmer–Lemeshow Chi-square was 0 (P = 1). Jacknife assessment of the predictive ability of this model revealed a specificity of 100% and a sensitivity of 100% (Figure 5).

Worked examples

These data pertain to actual cases in the 4 h challenge.

Gluten-sensitive (untreated)
Values are as follows. Post-challenge lamina propria γδ<sup>+</sup> population = 7; 7 × 11.4 = 80. Post-challenge epithelial CD3<sup>+</sup> population = 132; 132 × 0.9 = 119. Post-challenge epithelial γδ<sup>+</sup> population = 35; 35 × 6 = 210. Thus, using the model given above:

Diagnostic score = -193 + 80 + 119 + 210
= 216 [log<sub>10</sub> = (+)2.34]

Disease-control
Values are as follows. Post-challenge lamina propria γδ<sup>+</sup> population = 0. Post-challenge epithelial CD3<sup>+</sup> popu-
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Figure 6 Diagnostic sensitivities and specificities for conventional tests employed in the work-up for suspected coeliac patients

In this prospective study, serological tests (anti-gliadin antibodies; anti-endomysial antibodies) did not perform as well for this cohort of untreated patients as is suggested elsewhere in the literature. Abbreviations: ARA, anti-reticulin antibodies; AGA, anti-gliadin antibodies; EMA, anti-endomysial antibodies.

\[
\text{Diagnostic score} = -193 + 0 + 37 + 30 = -126 [\log_{10}(-2.10)]
\]

Other clinical aspects

It has already been stated that no discernible changes in the serological anti-gliadin or IL2-R antibody titres were detected in the blood during either challenge. This aspect of the study was therefore discontinued. The biochemical assays for brush border enzyme activity appeared to be of little value, as also found by others [27]. These data were likewise not considered further.

The comparative, prospective aspects of the clinical tests (specificity and sensitivity) (Figure 6) showed poor diagnostic value. More importantly, the serological findings, overall, yielded low sensitivities for coeliac diagnosis, in terms specifically of anti-gliadin antibodies (IgA/IgG or combined: 50–60%) or anti-endomysial antibodies (sensitivity ∼ 70%; specificity 98%). These sera were all analysed ‘blind’ and in a separate laboratory.

DISCUSSION

The results of this 5-year prospective study demonstrate the use of rectal challenge as an incisive means of identifying gluten-sensitive individuals. Furthermore, this study has permitted, in parallel, an evaluation of conventional laboratory procedures, as well as recent antibody tests that are used in screening for gluten sensitivity. In this setting, the 4 h rectal challenge was shown to achieve both a sensitivity of 100% and a specificity of 100% in detecting coeliac disease within an unselected, prospective cohort of 103 patients.

It was first demonstrated over 30 years ago that the rectum mounts an inflammatory response to the local instillation of gluten [1–4]. In the light of our knowledge of the physiology of the (mucosal) immune system, it is evident that the rapid response noted by Rubin and colleagues [4] is most likely explained by the local recirculation of sensitized immunocytes back to the mucosa, and their redeployment and accumulation at the site of subsequent antigen challenge [6,7]. Previous animal work has also emphasized the localized nature of a secondary mucosal immune response [28], which can be elicited at sites remote from the locus of primary intestinal immunization.

Our own immunohistological studies of the rectal mucosa in patients with untreated coeliac disease have revealed the presence of a lymphoplasmacytic infiltration that is responsive to gluten withdrawal [5,8–11]. It must therefore be concluded that antigenic fragments derived from partially digested, or undigested, gluten presumably enter the colon and hence the faecal stream, thereby evoking the antigen-specific lymphoid infiltrates that have been documented in the distal rectum, since such infiltrates are markedly reduced during dietary gluten restriction [10]. It should be noted, in this regard, that immunohistochemistry, performed according to a strict morphometric procedure, is considerably more sensitive than high-resolution (oil immersion) optical microscopy with 1 µm epon sections [10,11]. Despite such technical handicaps, Loft and co-workers [9] in their earlier prospective study achieved sensitivities and specificities of 80–90% with a 6 h challenge. Our aim was therefore...
(i) to utilize immunohistochemistry, as cell recognition and counting is far more accurate than the use of conventionally stained cells and (ii) to reduce the time of the challenge.

Our data show that a 2 h challenge gives insufficient time for the immune response to develop. From the derived logistic equation, the diagnostic scores for each patient were dependent solely on the 2 h CD3 lymphocyte count; interestingly, the sensitivity/specificity of this 2 h test was virtually equivalent to that achieved by morphological criteria in Loft et al’s [9] original prospective 6 h diagnostic challenge.

Conversely, the 4 h challenge achieved complete separation of the two groups. Furthermore, the 4 h score was now dependent not only on post-challenge CD3 infiltrating lymphocytes, but also on the specific contribution of post-challenge γδ receptor lymphocytes to gluten, which was relevant at 4 h post-challenge but not at 2 h post-challenge. The converse has also been noted: on treatment, the γδ receptor lymphocyte population remains long after the CD3 lymphocyte complement of lymphocytes has disappeared from the jejunal [29] or rectal [10] mucosa.

Since the 4 h score is dependent solely on the absolute counts of CD3+ and γδ+ lymphocytes in the post-challenge biopsy, a pre-challenge biopsy is not required in the conduct of the test. Also, since it is necessary for the patient to have the challenge dose of gluten inserted into the rectum, without any previous preparation, the test can be performed as a simple office procedure that needs no specialized apparatus; the biopsy can be processed subsequently by routine methodology through any immunohistochemistry laboratory. For reproducible results, 1000 μm of muscularis mucosa must be surveyed, and the overlying CD3 γδ lymphocytes summed and expressed as absolute values per 10^6 μm^2 muscularis; once inserted into the equation, the diagnostic score can be calculated.

Rectal challenge is thus a rapid, convenient and easy test that provokes a dynamic T cell mucosal response that is presumed to be exclusive to gluten-sensitized subjects. Its performance is impressive when compared with that of other screening tests, particularly those involving the detection of antibody-specific tissue components, such as rat kidney (anti-reticulin) or monkey oesophagus (anti-endomysium). Furthermore, these latter tests seem to be dependent on extensive mucosal (jejunal) damage, thus performing badly in the presence of milder lesions. In this respect, it might be suggested that the admixture of dermatitis herpetiformis cases to the trial skewed the anti-endomysial antibody tests to such a low level. However, the 4 h gluten-sensitized group only contained four skin biopsy-positive patients with dermatitis herpetiformis, of which only two were negative for anti-endomysial antibodies, despite having the gold standard entry criterion of a typically ‘flat’ upper jejunal mucosal biopsy.

Paradoxically, it is people with mild lesions, and compensated latent gluten sensitivity, who need accurate methods of diagnosis, since in these individuals serology is invariably negative or unhelpful [30,31]. Thus the advantage of a dynamic test of the underlying T lymphocytic sensitization to gluten becomes evident.

There are cogent reasons for the deployment of a rapid and highly sensitive test for the identification of gluten-sensitized subjects. Firstly, continued exposure to gluten clearly predisposes the subject to cancer and lymphoma [32], even in the presence of minimal proximal lesions [33–35] where the conventional (histological) IEL count may not be raised [36,37], a phenomenon that can predate onset of lymphoma by several years. More importantly, there is a 10-fold increase in deaths from malignancy in the families of known gluten-sensitive subjects [38]; it is in these family members where the greatest likelihood of compensated latent disease will be present and in whom minimal-change jejunal histology, or negative antibody tests, may fail to provide the correct diagnosis.

Secondly, atypical symptomatology may either hinder early diagnosis or even exclude the possibility of gluten sensitivity, especially when clinical features, such as abdominal pain, obesity or disturbed liver function tests, do not immediately point to an origin in the small intestine [39–41]. It is in such cases that a rectal biopsy could provide a rapid means of excluding this possibility, among other possible differential diagnoses.

Finally, it should be appreciated that bone mass in asymptomatic, latent cases is invariably reduced; moreover, it has been shown that adult female gluten-sensitive patients, even if recognized and treated by diet, will remain osteopenic, thus predisposing them to more severe skeletal deterioration during the menopause and beyond. Here again there is a pressing need for an incisive diagnostic procedure for predicting occult gluten sensitivity.

In summary, the present study offers to the clinician a rapid and highly accurate predictive test of gluten sensitivity. Rectal challenge is probably less obtrusive than upper intestinal biopsy pursued either endoscopically or with a small-bowel capsule. It requires neither hospitalization nor patient preparation, obviates the need for endoscopic or roentgenological screening facilities, and is available to all patients, including those with an aversion to swallowing tubes or instruments, the young and the old, and pregnant women.

This test is a direct, provocative assay of underlying T cell sensitization to gluten protein, and its performance is extremely impressive compared with that of other common, conventional screening tests. It should also give a positive result when antibody tests are inapplicable, especially with the commonly associated IgA deficiency or other rare immunodeficiency syndromes [42], and, more importantly, when mucosal histology and/or antibody screening tests give equivocal, and hence
indeterminate, results [43,44]. The next logical step
requires a further controlled, prospective study com-
prising subjects in whom current diagnostic tests are
equivocal and in whom diagnosis is thus uncertain.

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