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Diagnostic methods beyond conventional histology in coeliac disease diagnosis

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ABSTRACT

Background: Coeliac disease diagnostic criteria currently require the detection of small bowel mucosal villous atrophy and crypt hyperplasia.
Aims: To compare conventional histological examination to the determination of small bowel mucosal intraepithelial lymphocytes (IELs) and to serum and intestinal coeliac autoantibodies in untreated coeliac disease with villous atrophy and in mild enteropathy coeliac disease.
Patients and methods: Study comprised consecutive adult patients with coeliac disease suspicion; villous height–crypt depth ratio (Vh/CrD), the densities of CD3+, γδ+ and villous tip IELs and serum and intestinal transglutaminase 2 (TG2)-targeted autoantibodies were studied. Coeliac disease was diagnosed in 223 and excluded in 608 patients. Further, 66 patients were considered to suffer from mild enteropathy coeliac disease. Control group consisted of 138 patients.
Results: Vh/CrD determination detected 77% of untreated coeliac disease patients. Serum coeliac autoantibodies had 84% sensitivity for untreated coeliac disease with villous atrophy and 70% sensitivity for mild enteropathy coeliac disease; the specificity was 100%. Intestinal TG2-targeted autoantibodies had sensitivities of 100% and 93%, and 100% specificity, respectively. γδ+ and villous tip IELs proved more reliable than CD3+ IELs.
Conclusions: Conventional histological examination as the golden standard in coeliac disease diagnosis is questionable. Serum and especially intestinal TG2-targeted autoantibodies seem promising in future coeliac disease diagnostics.

1. Introduction

Small bowel biopsy has remained the confirmatory cornerstone test for coeliac disease for the last 30 years; the diagnosis should only be made when during a normal gluten-containing diet villous atrophy and crypt hyperplasia can be detected in the small bowel mucosal specimens [1]. However, patchy villous atrophy [2] or poor quality of the biopsy specimens [3] can complicate or even hinder the correct diagnosis. Further, small bowel mucosal villous atrophy can also be associated with other diseases [4,5], and it is nowadays widely recognized that symptoms and even complications of coeliac disease may occur even before the development of villous atrophy [6–8]. Therefore, coeliac disease diagnostic criteria need revision. Besides conventional histology, serology and investigation of small bowel mucosal inflammation, especially determination of γδ+ intraepithelial lymphocytes (IELs), are considered helpful in borderline cases [9,10]. Furthermore, recently discovered intestinal transglutaminase 2 (TG2)-targeted coeliac disease autoantibodies seem particularly promising in coeliac disease diagnostics [11,12]. Coeliac disease autoantibodies are produced in the small bowel mucosa, and it has been recognized for decades that untreated coeliac disease patients have deposited IgA along basement membrane in their small bowel mucosa [13,14]. It was shown a few years ago that this deposited IgA is directed against TG2 and thus represents local autoantibody production [11]. However, the role of the intestinal autoantibodies in coeliac disease diagnostics compared to conventional histology still requires further elucidation, as do in fact, all of the methods above.

This study aimed at investigating the diagnostic significance of the determination of CD3+, γδ+ and villous tip IELs and serum and intestinal TG2-targeted coeliac autoantibodies compared to conventional histology in untreated coeliac disease with villous atrophy, and in mild enteropathy coeliac disease where the villous atrophy has not yet developed. In order to achieve reliable and statistically relevant results large patient material was required, and hence we pooled the data collected for our previous clinical studies and performed a meta-analysis. By these means we succeeded in

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increasing the number of mild enteropathy coeliac disease to 66 cases, and in addition to include a large untreated coeliac ($n = 223$) and non-coeliac ($n = 138$) group.

2. Methods

2.1. Patients and study design

This study was carried out at the Department of Gastroenterology and Alimentary Tract Surgery in Tampere University Hospital. In our gastroenterology department we have collected comprehensive clinical, serological and histological data of all adult patients undergoing endoscopy for coeliac disease suspicion since 1995. These subjects have participated in our previous studies [9,15–17], and we now aimed at summarizing all the data from these large patient series. All adult patients undergoing endoscopy for coeliac disease suspicion during the years 1995–2002 were included in the present study.

Altogether 223 patients were diagnosed with untreated coeliac disease based on typical small bowel mucosal histological findings [1] (Table 1). Furthermore, 66 patients were considered to suffer from coeliac disease without villous atrophy and will be referred to in this study as mild enteropathy coeliac disease patients. In these 66 patients coeliac disease diagnostic criteria were not fulfilled at the time of the baseline investigations, but they were still considered to suffer from coeliac disease. Firstly, 34 of these patients were subsequently shown to develop small bowel mucosal villous atrophy and crypt hyperplasia when gluten consumption continued, and thus suffered from latent coeliac disease [18]. Secondly, 12 had gluten-dependent skin lesions diagnostic for dermatitis herpetiformis, but apparently normal villous architecture in their small bowel mucosa. Finally, in 20 symptomatic patients the diagnosis of mild enteropathy coeliac disease was based on the clinical response to a gluten-free diet, and six patients also underwent later gluten challenge to confirm the diagnosis of coeliac disease [15]. All patients with available data were HLA DQ2 or DQ8 positive, and all patients with positive IgA-class endomysial antibodies (EmA) (18 out of 20) showed serological response to a gluten-free diet. Furthermore, histological response to a gluten-free diet (decrease in inflammation) was demonstrated in all 17 patients with available control biopsy result.

Altogether 608 patients were investigated due to coeliac disease suspicion, but small bowel mucosal samples showed normal villous architecture and coeliac disease with villous atrophy and mild enteropathy coeliac disease were excluded at the time.

In addition, 138 controls with no suspicion of coeliac disease were investigated. These patients suffered from dyspepsia ($n = 104$) or had intestinal diseases other than coeliac disease ($n = 34$) such as ulcerative colitis, Crohn's disease, collagen colitis, giardiasis lamblia or autoimmune enteropathy. Three patients with autoimmune enteropathy had villous atrophy and all of the remaining control patients had normal villous architecture.

2.2. Small bowel mucosal morphology and inflammation

Seven forceps biopsy specimens were taken from the distal part of the duodenum upon upper gastrointestinal endoscopy. Five of the small bowel specimens were processed and stained with haematoxylin-eosin (HE). Morphometrical analysis was carried out on 2-μm-thick formalin-fixed HE-stained small bowel sections and studied under light microscopy by specialist pathologists and experienced researchers of our study group. High-quality sections were required, and villous height–crypt depth ratio (Vh/CrD) was determined from several biopsy samples from multiple sites in order also to detect patchy forms of villous atrophy, as previously described [19]. Vh/CrD ≥ 2 was considered normal and coeliac disease was excluded, whereas Vh/CrD < 2 was considered compatible with untreated coeliac disease. The villous tip IELs were investigated using light microscopy in HE-stained small bowel samples; the villous tip IEL score was calculated as previously described and the reference value was set at 4.2 IELs/20 enterocytes [15]. In our laboratory the correlation coefficients for intraobserver variation for villous tip IELs were 0.89 and 0.87 for interobserver variation.

Two of the small bowel specimens were freshly embedded in optimal cutting temperature compound (Tissue-Tec, Miles Inc, Elkhart, IN, USA), snap-frozen in liquid nitrogen and stored at −70 °C until used. Immunohistochemical stainings for CD3+ and γδ+ IEL densities were determined as previously described [12,20]. The reference values were set at 37 cells/mm for CD3+ and at 4.3 for γδ+ IELs [9]. In our laboratory the correlation coefficients for intraobserver variation for CD3+ and γδ+ IELs were 0.95 and 0.98, and those for interobserver variation 0.92 and 0.98, respectively.

2.3. Small bowel mucosal TG2-targeted IgA deposits

It has already been shown that untreated coeliac disease patients have *in vivo* IgA deposits on TG2 in their small bowel mucosa. This IgA has been shown to target purified TG2 both in ELISA and in Western blot [11], and its ability to bind recombinant TG2 has been previously demonstrated [16]. Further, the disappearance of intestinal extracellular IgA deposits after disrupting the binding of TG2 to fibronectin has been proven [11]. These experiments have collectively demonstrated that intestinally deposited autoantibodies are specifically directed against TG2.

In this study the detection of intestinal TG2-targeted autoantibody deposits *in situ* in tissue sections was based on the co-localization of IgA with TG2 when double-labeled by direct immunofluorescence (IF) as previously described [11]. Small bowel

Table 1
Demographic data and primary reason for endoscopy and small bowel biopsy in study groups.

	Untreated CD with villous atrophy $n = 223$	Mild enteropathy CD $n = 66$	CD suspected but excluded $n = 608$	Non-CD controls $n = 138$
Age; median (range), years	42 (16–81)	45 (21–74)	42 (15–88)	49 (19–74)
Female; *n* (%)	147 (66)	46 (70)	432 (71)	82 (59)
Primary reason for endoscopy; %				
- Abdominal symptoms[a]	35	50	54	94
- Malabsorption or anaemia	9	6	20	3
- Skin symptoms suggesting DH	23	14	4	0
- Atypical symptoms[b]	14	18	16	3
- Silent, screening at-risk groups[c]	19	12	6	0
First-degree relatives with CD; *n* (%)	56/145 (39)	19/38 (50)	58/197 (29)	6/67 (9)

CD = coeliac disease, DH = dermatitis herpetiformis.

[a] Diarrhoea, flatulence, indigestion, abdominal distension, abdominal pain.

[b] Neurological symptoms, dental enamel defects, mouth ulcerations, osteoporosis, infertility, alopecia areata, arthritis, elevated liver enzymes.

[c] Insulin-dependent diabetes mellitus, autoimmune thyroid disease, Sjögren's syndrome, family history of coeliac disease.