Serology and endoscopy in coeliac disease: applications and limitations
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Link to publication

Citation for published version (APA):

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Chapter 1

General introduction and outline of the thesis
Serological tests for coeliac disease

The gold standard for diagnosis of coeliac disease remains the small bowel biopsy, made easier by the endoscopic forceps technique which has largely replaced the suction capsule in routine use [1]. Biopsy allows the identification not only of villous atrophy but also of crypt hyperplasia and raised intraepithelial lymphocyte counts which are features of milder gluten sensitive enteropathy [2]. However the broad spectrum and the non-specific nature of many of the clinical manifestations of coeliac disease [3] makes biopsy as the initial investigation impossible, and much effort has been put into the identification of serological screening tests with adequate sensitivity and specificity.

Although IgA class antigliadin antibody (AGA) had very high sensitivity and specificity in our laboratory when assessed in a selected series of patients undergoing biopsy for suspected coeliac disease [4], this was not confirmed in general population screening elsewhere [5] or in our own coeliac population with a range of less specific symptoms [6]. Although it has been used for population screening, both in isolation and as part of a two step screening protocol with AGA positive patients then undergoing IgA class endomysial antibody (EmA) testing before selection for biopsy, most workers accept that EmA is a much more useful test [7]. Some EmA assays use monkey oesophagus as the substrate, which raises concerns of cost and ethics, but more recently human umbilical cord assays have been shown to have equivalent sensitivity and specificity [8,9].

The search for a high sensitivity/specificity screening test has gained fresh impetus after the proposal by Dieterich et al [10] that the autoantigen of coeliac disease recognised by EmA is tissue transglutaminase (tTG), a calcium dependent enzyme involved in mucosal repair and cell growth and differentiation. They suggested that complexes between tTG and dietary gliadin initiate the immune response of coeliac disease. Studies to date of anti-tTG as a test for coeliac disease suggest that it has similar, or slightly lower, sensitivity compared with EmA, and high specificity [11,12]. As anti-TTG is assayed by ELISA, unlike EmA (indirect immunofluorescence), it is more convenient and less subjective and labour intensive.

High specificity for both EmA and anti-tTG is not in question. Seronegativity is well recognised in the context of IgA deficiency, which is associated with coeliac disease [6,13]. However, Mulder’s group in the Netherlands has raised concerns about high sensitivities reported in the experimental setting with IgA deficiency excluded which do not apply in
clinical practice. Selecting patients for biopsy on the basis of positive EmA, then incorporating those patients in studies will lead to a “self-fulfilling prophecy” [14]. This has additional importance in the evaluation of anti-tTG, which appears to correlate with EmA. Secondly, many studies have evaluated serology in severe (subtotal or total) villous atrophy and not in milder (partial villous atrophy) lesions. Consequently, Rostami et al [15] reported an EmA sensitivity for TVA of 100%, STVA of 70%, and PVA of only 31%. Studies show that EmA disappears soon after starting a gluten-free diet, long before histological recovery, raising the possibility that seronegative coeliac disease reflects low gluten intake [16].

The prevalence and clinical presentation of coeliac disease: changing perceptions

One of the most significant recent developments in the clinical study of coeliac disease is the realisation that it is much commoner than reported prevalences based on recognised cases suggested. Typical figures included 1: 2200 in Denmark [17], 1:1050 in Sweden [18] and 1:1600 in Scotland [19]. A prevalence of 1:300 reported from Ireland [20] was perceived as unusual. Although most people in the United States are of European ancestry, the prevailing perception until very recently had been that coeliac disease there was even less common, supported by a community-based study which estimated a prevalence of 1:4860 from review of patients having intestinal biopsies over a 30-year period [21].
It has become clear in the last decade that most coeliacs do not fit the stereotype of an underweight marasmic patient presenting with diarrhoea and that many go undiagnosed because of mild or “atypical” presentations. A study of body mass index in our coeliac patients at first presentation [22] found that 22% were underweight, while 35% were overweight: two-thirds of male patients were overweight. First presentation in the elderly is common, frequently with delay in diagnosis [23,24].
Fewer than half of our patients report diarrhoea as a symptom [22]. Dyspeptic rather than bowel symptoms may predominate [25,26] raising the possibility of diagnosis during routine endoscopy.
Presentations involving organ systems other than the gut, in which gastrointestinal symptoms may be absence or trivial, are increasingly recognised. The skin rash of dermatitis
herpetiformis was the first example, and has been well researched. In 1990 Depla et al [27] described anaemia as the sole manifestation of coeliac disease. McIntyre and Long [28] identified coeliac disease in 3% of 114 patients undergoing GI investigation for anaemia, while Corrazza et al [29] reported a 5% prevalence among 200 anaemic patients. Four of 41 patients with iron deficiency anaemia and no gastrointestinal source of blood loss had villous atrophy [30]. Coeliac disease presenting with neurological symptoms (neuropathy, ataxia, epilepsy), osteoporosis and arthralgia, infertility and miscarriage, and abnormalities in liver biochemistry is well documented [3].

While lack of awareness of non-gastrointestinal manifestations is understandable though nowadays less excusable, there remains significant difficulty with “classical” coeliac disease symptoms among clinicians. Diarrhoea as a presentation led to a correct diagnosis in only 72% of initial hospital referrals, and anaemia in only 41% [24].

It is not surprising then that population screening, only possible in the last decade with the availability of relatively specific serological tests, reveals a much higher prevalence of coeliac disease than previously supposed.

Using EmA with histological confirmation where positive, Rostami et al in the Netherlands [31] identified two of 1000 blood donors with subtotal villous atrophy; a third had intact villi but raised intraepithelial lymphocytes and crypt hyperplasia, in keeping with milder gluten sensitive enteropathy [2]. In Sweden, AGA screening of blood donors with biopsy of positive cases revealed a prevalence of 1:260 [32], and 1:492 in a later study using AGA initially with EmA testing of positives before biopsy [33]. Subsequently, randomly selected Swedish adults yielded 1 in 190 from EmA testing followed by biopsy [34]. In the Italian peninsula, a prevalence of 1:300 was obtained among Italian schoolchildren (AGA followed by EmA before biopsy) [35] and 1:550 from San Marino adults using EmA [36].

Nott et al [37] screened sera from 2000 blood donors in the United States, initially with AGA by ELISA, with EmA testing of high AGA samples. Eight patients had EmA, suggesting a prevalence in the United States of at least 1:250, equivalent to the European experience. Biopsy confirmation was unfortunately not possible.

These prevalence figures, although high, may still be underestimates. Screening healthy blood donor populations is biased in favour of relatively asymptomatic coeliac patients. Although this is overcome by screening general populations, there remains the problem of seronegative coeliac disease. In this context the Northern Ireland population screening study
is of interest, in which a prevalence figure of 1:120 was obtained [38]. Rather than use a two step approach, Johnston et al tested for AGA and EmA in all patients, arranging biopsy if either was positive [39]. Of 10 patients identified as coeliac 6 had EmA and 7 had AGA. Two patients had EmA but not AGA, and four had AGA without EmA. AGA had poor specificity was poor, with only 4 of 34 (12%) with AGA alone having villous atrophy. However, reliance on EmA screening alone would have missed 40% of coeliacs. To date, there have been no studies of prevalence using anti-tTG, either alone or in parallel with EmA. If the figure obtained by Johnston et al is correct or even an underestimate, there is very significant underdiagnosis of coeliac disease in Northern Ireland. In January 2000 there were 1260 Coeliac Society members in Northern Ireland, against an expected figure in our population of 1.6 million of 13 000 (Coeliac Society of the UK, personal communication).

Case finding and screening for coeliac disease

Logan [40] discussed the implications of mass screening for coeliac disease in 1996. Coeliac disease meets the criteria for a condition likely to be worth screening for on two counts: it is common, and there are simple screening tests available of adequate sensitivity and specificity (EmA and now- probably- anti-tTG). However, the third criterion for effective screening- that treatment given at the stage identified by screening is more effective than treatment given later- is more contentious. While there is no doubt that coeliac disease is a risk factor for gastrointestinal malignancy and that a gluten-free diet reduces this risk [41], the likelihood in absolute terms is small, and cannot be quantified for relatively asymptomatic patients. Finally, it is by no means clear what proportion of coeliacs with minimal symptoms identified on screening could be persuaded to comply with gluten exclusion. His conclusion was that efficient case-finding might prove as effective and more acceptable.

The following populations might benefit from case-finding through EmA or anti-tTG testing:

First degree relatives of coeliac patients. Up to 18% may have VA, although Rostami et al reported that reliance on AGA/EmA testing to select patients for biopsy will miss a high proportion, particularly of those with partial villous atrophy [42];

Insulin-dependent diabetics, in whom coeliac disease may aggravate weight loss and interfere with diabetic control, and in whom the risk of coeliac disease is 1:20 to 1:50 [43];
Patients with autoimmune thyroid disease, in whom coeliac disease might cause similar symptoms to thyrotoxicosis, and in whom the risk is 1:30 [44];
Patients with unexplained infertility or miscarriage [45];
Patients with osteoporosis [46];
Patients with abnormality of liver biochemistry, particularly primary biliary cirrhosis [47].
The availability of serological tests means that for the first time case-finding is possible by hospital practitioners other than gastroenterologists, and even in the setting of primary care [48]. However, these practitioners must be aware that biopsy confirmation before starting a gluten-free diet is required, and that a significant minority of coeliacs will be missed by reliance on serology.
Case-finding among patients who present with anaemia and with dyspepsia offers the opportunity for biopsy as part of routine investigation, overcoming the problem of false-negative serology. There seems no doubt that all patients with iron deficiency anaemia should have duodenal biopsy [28], which can be easily done during the routine upper gastrointestinal endoscopy. While routine biopsy in all patients with dyspepsia is not practical, endoscopic markers of villous atrophy are well described and may help select patients [49,50], although the sensitivity of these markers remains unclear.

The research presented in this thesis firstly assesses the role of EmA in case-finding in two distinct situations: in the primary care setting, and in primary biliary cirrhosis. We then explore the limitations of EmA testing: firstly in the generally recognised setting of IgA deficiency, and then in the more common though less well documented situation of normal serum IgA. We assess EmA titres in patients following dietary gluten exclusion and their correlation if any with histological recovery. Next, we study anti-tTG as a screening test for coeliac disease, in a population where EmA seronegativity is common. Finally, we study the value of endoscopic markers for villous atrophy in patients undergoing routine gastrointestinal endoscopy.
Outline and Aims of the Thesis

Chapter 1: Introduction

Chapter 2: The availability of EmA testing which has high specificity for villous atrophy allows for the first time an important role for primary care practitioners in diagnosis of coeliac disease. This study assessed the use of EmA testing by family doctors in our catchment area and the diagnostic yield.

Chapter 3: EmA testing also allows case finding among patients with conditions known to be associated with coeliac disease. In this study we assess the prevalence of EmA positive coeliac disease among patients with primary biliary cirrhosis, for which there is anecdotal evidence of a link.

Chapter 4: The next section of the thesis addresses limitations of EmA testing. An important cause of false negative EmA testing is IgA deficiency. We assess the significance of IgA deficiency among patients undergoing diagnostic tests for coeliac disease.

Chapter 5: False negative EmA may occur outside the setting of IgA deficiency. We assess its prevalence in a population of coeliac patients who were selected for biopsy on clinical rather than serological criteria.

Chapter 6: Formation of EmA is thought to require exposure to dietary gluten and may therefore correlate poorly with histology once a gluten-free diet is started. We compare EmA titres with histology in patients having follow-up biopsy after twelve months dietary modification.

Chapter 7: Antibodies to tissue transglutaminase (tTG), the recently identified autoantigen of coeliac disease, have been reported to correlate with EmA. We assess the sensitivity of anti-tTG in our population with a high prevalence of EmA negative disease, in order to determine its role in diagnosis.
General introduction and outline of the thesis

Chapter 8: Endoscopic markers of villous atrophy may allow selection for duodenal biopsy of patients undergoing routine endoscopy. We report their prevalence among 500 patients having open access endoscopy and their predictive value for coeliac disease.

Chapter 9: In a follow-up study, we compare endoscopic appearances with duodenal histology in patients undergoing routine endoscopy in order to determine sensitivity as well as specificity.

Chapter 10: shows examples of endoscopic markers of villous atrophy.

Chapter 11: summarises these studies.

Chapter 12: summarises these studies in Dutch.
References

General introduction and outline of the thesis

29. Corazza GR, Valentini RA, Andreani ML et al. Subclinical coeliac disease is a frequent
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