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Selective measurement of anti-tTG antibodies in coeliac disease and IgA deficiency – an alternative pathway

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All authors contributed equally.

ABSTRACT

Objective

To determine the ability of selective antibody testing to screen for coeliac disease in the presence of IgA deficiency and to define the sensitivity of a pathway using this method (Figure1).

Method

All IgA and IgG anti-tTG tests performed at our centre between January 2008 and December 2009, using the Immunocap 250 analyser, were retrospectively reviewed. Positive results were correlated with histology. Results were used to validate our diagnostic pathway.

Results

12,289 consecutive serological tests were reviewed. IgA deficient patients gave either an "error" reading or very low response on the Immunocap 250 analyser. Subsequent testing of this sub-group demonstrated raised IgG anti-tTG antibodies in those with histologically proven coeliac disease.

Conclusions

Using our antibody screening pathway, which involves the selective use of IgG anti-tTG, sensitivity increased from 87% to 92% in those with IgA deficiency. Adoption of this pathway for coeliac screening would negate the routine screening of immunoglobulin levels, with resultant cost saving.

INTRODUCTION

Coeliac disease is a common disease that affects around 1% of the general population in the UK, and makes up a large proportion of the workload seen in general gastroenterology clinics [1]. The pathogenesis of coeliac disease is related to hypersensitivity to gluten in genetically susceptible individuals. As a result of ingestion of gluten containing products (wheat, rye and barley), damage is caused to the mucosa of the small bowel leading to villous atrophy. This can result in a wide spectrum of signs and symptoms which include lethargy, diarrhoea, bloating, weight loss and anaemia but, in some cases, patients can remain asymptomatic. However, as such symptoms and signs are non-specific, and are common to many other gastrointestinal diseases, the diagnosis of coeliac disease may be challenging. Therefore, a high index of clinical suspicion is needed to exclude coeliac disease and to prevent delays in diagnosis.

Duodenal biopsies remain the gold standard in diagnosis. However, identification of those patients to biopsy usually involves serology testing against specific antibodies. The type of antibodies being used has evolved over the years with more sensitive and specific assays replacing older ones. Currently, IgA anti-tissue transglutaminase (IgA anti-tTG) antibodies are recommended as a first choice test for the diagnosis of coeliac disease [2]. However, variation in the sensitivity and specificity of serology tests has been noted from previous studies [3]. Patients with IgA deficiency are prone to false negative antibody results [4]. To add to the problem, IgA deficiency is more prevalent in patients with coeliac disease than in those without coeliac disease [5,6]. The measurement of IgA immunoglobulin level is traditionally carried out at the same time as the IgA anti-tTG antibodies, in an attempt to identify patients with IgA deficiency [6]. However, such an approach involves carrying out two assays (anti-tTG

antibodies & IgA immunoglobulin) for each patient being tested for coeliac disease. In addition, some laboratories perform Endomysial Antibody (EMA) testing which increases the yield slightly but at an additional cost. Moreover EMA testing is subject to the same limitation as it is IgA dependent, and is not as reproducible as an ELISA method, as it relies on immuno-fluorescence for which there is inter-assay variation [7].

The use of IgG anti-tTG antibodies in screening for coeliac disease in patients with selective IgA deficiency has previously been demonstrated [8,9]. However, these previous studies did not clearly define a role for the use of IgG anti-tTG antibodies in the diagnosis of coeliac disease. It has been suggested that it may be used as a further screening test in those patients with IgA deficiency, as shown by very low IgA anti-tTG antibodies [10].

We believe that the most cost effective and practical approach, to aid in the diagnosis of coeliac disease, is by initially testing with IgA anti-tTG antibody and then selecting a subset to undergo IgG anti-tTG antibody testing, by an enzyme-linked immunosorbent assay (ELISA). Based on this we devised an ELISA-based serological pathway to simplify screening for coeliac disease and co-existent IgA deficiency (figure 1). The primary objective of this study is to validate our coeliac disease screening pathway, by determining its sensitivity and specificity.

METHOD

Subjects

Our centre is a university teaching hospital serving a population of over 450,000. Since 2007, serological screening for coeliac disease at our centre has consisted of performing ELISA testing for both IgA anti-tTG antibodies and IgG anti-tTG antibodies in all patients. All patients screened for coeliac disease by IgA and IgG anti-tTG antibodies during the 2 year study period (January 2008 and December 2009) were included. Patients diagnosed with coeliac disease prior to 2008 were excluded. Ethical approval was not required and all results in this retrospective study were anonymised.

Serology

Serology tests were performed using the commercial kits tTG-IgA Celikey and tTG-IgG Celikey (Phadia, Freiburg, Germany) and in accordance with the manufacturer's instructions the cut-off for negative results was set at <5 U/ml (the manufacturer's quote <10 U/ml). The Immunocap 250 analyser ® Phadia was used. In those patients with IgA deficiency, the Immunocap 250 will give either an 'error' reading or a very low response [11]. A very low response was defined by Response Unit (RU) of less than 50, as determined by the calibration curve on the Immunocap 250. Previous practice had been to refer members of this subgroup for duodenal biopsy, regardless of their IgG anti-tTG antibody result, to determine if they had coeliac disease.

Histology

The diagnosis of coeliac disease was defined as positive serology (IgA or IgG anti-tTG) in the presence of confirmatory histological findings (Marsh grade of 2 - 4). Latent coeliac disease was defined as positive serology in the absence of confirmatory

histology, while histology in the absence of serology was defined as possible serology-negative coeliac disease. Our electronic histology database was reviewed over the same 2 year period. Details were recorded of all patients diagnosed with coeliac disease through positive histology, on duodenal biopsy, regardless of serological result.

Pathway

A pathway for the selective use of IgG anti-tTG in serological screening for coeliac disease was designed (Figure 1).

Data-analysis

Results of all patients screened for coeliac disease were entered into an Excel spreadsheet. Serological and histological results were analysed to determine which patients met the criteria for a diagnosis of coeliac disease. Results were analysed in the context of the proposed pathway for serological screening for coeliac disease. Sensitivity and specificity for coeliac detection, if the pathway had been used, were calculated and used to determine its screening ability.

RESULTS

During the study period, 12,289 patients with no prior history of coeliac disease underwent serological screening for coeliac disease by IgA anti-tTG antibodies and IgG anti-tTG antibodies. Of these 12,289 patients, 87 were classified, through either confirmatory histology or elevated anti-tTG antibodies, as possibly having coeliac disease (Table 1).

IgA anti tTG and positive histology

Further analysis showed that 66 had both an elevated IgA anti-tTG antibody and confirmatory histology (Marsh grading 2 – 4). Therefore, in this group, the diagnosis of coeliac disease was suggested by using only the IgA anti-tTG antibodies serological test and then confirmed with histology. Thus, the sensitivity for diagnosing coeliac disease with IgA anti-tTG antibodies alone was 87% (specificity 99 %) (Table 2).

Negative IgA anti tTG and positive histology

Those patients identified as having either undetectable ("error" reading) or low IgA anti-tTG levels also had their IgG anti-tTG antibodies and duodenal histology checked. Of these patients, 4 had a positive IgG anti-tTG antibodies serological test and subsequently had positive histology on biopsy (table 3). Thus, these 4 patients had IgA deficiency (with an "error" reading on the Immunocap 250), but raised IgG anti-tTG antibodies and positive histology. They were diagnosed with coeliac disease. Therefore, the use of selective IgG anti-tTG improved the sensitivity for coeliac disease detection to 92% (specificity 99%).

Latent coeliac disease

In 11 patients with positive serology, histology was reported normal. In these patients the mean IgA anti-tTG level was 43 ± 35 and 8.1 ± 20 for IgG anti-tTG. Therefore, it

was assumed that these patients have latent disease and that they will manifest clinically at a later stage in their lives.

DISCUSSION

Our objective on commencement of this study was to determine the role of IgG anti-tTG in screening for coeliac and to define the sensitivity of a pathway using such a method. Through this study we reviewed the results of non-selective IgA and IgG ant-tTG screening at our centre and applied these results to the proposed pathway. This enabled us to determine the sensitivity (92%) and specificity (99%) for coeliac detection that such a pathway would generate (Figure 1).

We show that this proposed Immunocap 250 pathway is able to identify coeliac disease in spite of IgA deficiency. These individuals produced a very low or "error" result on IgA anti-tTG testing, but were subsequently shown to have raised IgG anti-tTG antibodies. Without testing IgG anti-tTG in these patients, a false negative result in those with IgA deficiency would have been generated (4 patients out of 76 had IgA deficiency, but their IgG anti-tTG was elevated).

Furthermore, in those patients without coeliac disease, but with IgA deficiency, an "error" reading was also found. However, in this patient subset, the subsequent measurement of IgG anti-tTG antibodies showed a negative result. This would have allowed a decision to be made that a small bowel biopsy was not necessary. This would not only have benefited the patient by avoiding an unnecessary and invasive investigation, but would also have generated significant cost saving.

Additionally, the proposed approach is likely to be even more cost effective and less labour intensive. Both IgA and IgG anti-tTG antibodies can be carried out on the same ELISA plate. Furthermore, we believe that adhering to this pathway would

negate the routine screening of immunoglobulin levels, with resultant additional cost saving.

Additional saving, without clinical detriment, is of great importance, given the increasing awareness of coeliac disease leading to an increasing demand for testing. As with all serological testing, it does not purport 100% specificity or sensitivity. In our series we report a 92% sensitivity which must be borne in mind when requesting such tests.

A limitation of this study was that the researchers did not have input into which patients were selected for screening. This may have influenced sensitivities and specificities. Decision to screen was made purely by the referring clinicians. Duplicate screens may have been performed on patients, but as coeliac disease is a dynamic disease it is reasonable to repeat serology over time. Our low prevalence rate may also be explained by the mixed ethnic population in Coventry.

CONCLUSION

Previous studies have demonstrated that the addition of IgG anti-tTG antibodies is a useful tool in diagnosing coeliac disease in patients with IgA deficiency. We expand on this by demonstrating how it can be adopted into a specific pathway [8,9] The benefit of this is that it offers a selective and economical approach, which can easily be adopted in most laboratories.

MAIN MESSAGES

1. IgA anti-tTG identifies most people who may have coeliac disease
2. Selective use of IgG anti-tTG detects coeliac disease in those with IgA deficiency

3. Use of comprehensive clinical/biochemical pathway allows for an economical approach to detecting coeliac disease

CURRENT RESEARCH QUESTIONS

1. To validate the proposed pathway using EMA instead of IgG in the presence of IgA deficiency

2. To measure corresponding mucosal tTG, especially in those with IgA deficiency

3. To determine the validity of our proposed pathway in primary care setting

4. To determine the validity of our pathway using different manufactures' automated platforms

TABLES

Table 1: Summary of patients with coeliac disease

Screening modality which identified patient	Number of patients
Traditionally detected coeliac disease group (raised IgA anti-tTG, histology positive)	66
IgA deficient coeliac disease group (IgA anti-tTG antibodies showed "error" but raised IgG anti-tTG and positive histology)	4
Presumed latent coeliac disease group (raised IgA and IgG anti-tTG, but histology negative)	11
Histology suggestive of coeliac disease alone (both IgA and IgG anti-tTG negative)	6
Total number of patients with manifest or latent coeliac disease	87

Table 2: IgA anti-tTG antibody results correlated with histological confirmation

	Negative biopsy	Positive biopsy	Total
Positive anti-tTG IgA	11	66	77
Negative anti-tTG IgA	12202	10	12212
Total	12213	76	12289

Sensitivity of IgA anti-tTG for detecting coeliac screening = 0.8

Specificity of IgA anti-tTG for detecting coeliac screening = 0.9

Table 3: Detection with proposed subsequent IgG anti-tTG antibody results correlated with histological confirmation

	Negative biopsy	Positive biopsy	Total
Positive IgA anti-tTG or IgG anti-tTG	11	70	81
Negative IgA with negative IgG anti-tTG	12202	6	12208
Total	12213	76	12289

Sensitivity of subsequent IgG anti-tTG for detecting coeliac screening = 0.9

Specificity of subsequent IgG anti-tTG for detecting coeliac screening = 0.9

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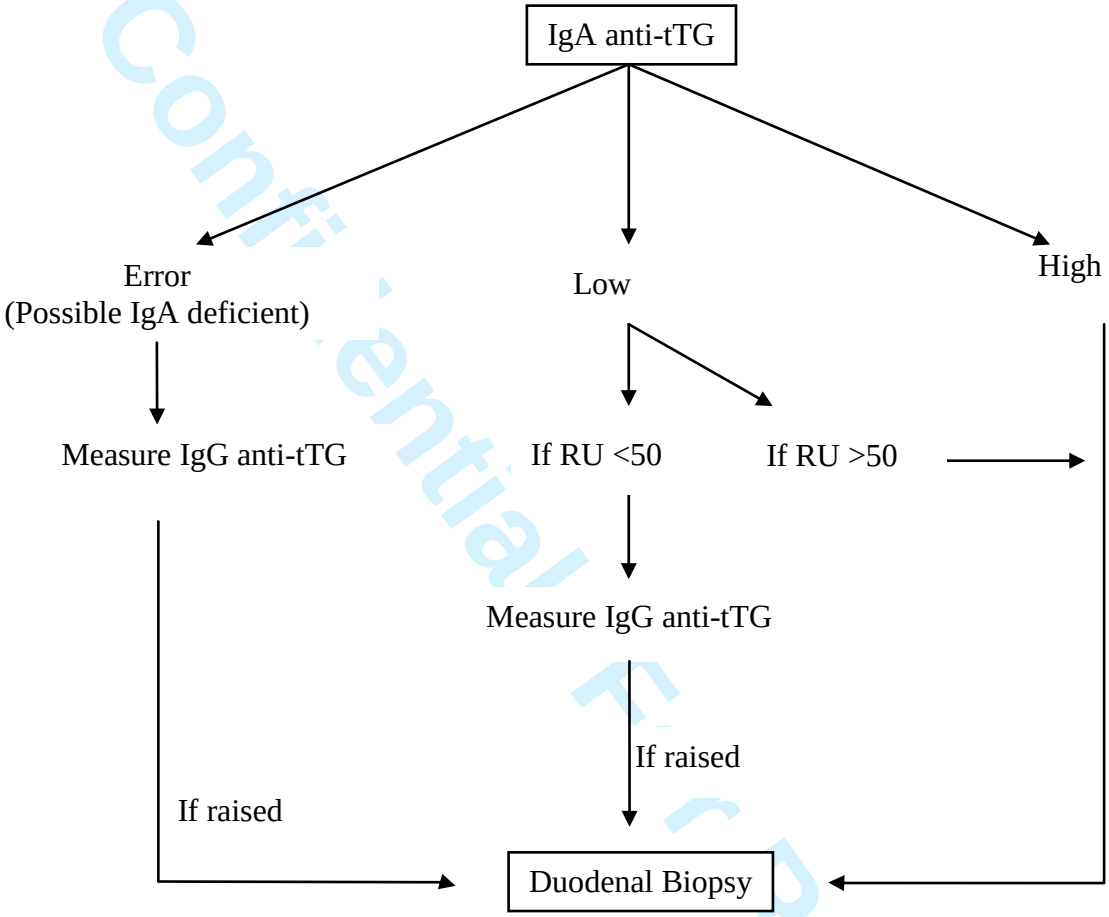
Figure 1: Pathway of selective testing with anti-tTG for coeliac disease



RU = Response unit determined by calibrated curve on the Immunocap 250