Serology and endoscopy in coeliac disease: applications and limitations
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Citation for published version (APA):

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Chapter 4

Immunoglobulin A deficiency as a cause of false negative coeliac serology

ASSOCIATION BETWEEN SERUM LEVELS OF TOTAL IgA AND IgA ENDOMYSIAL AND ANTIGLIADIN ANTIBODIES: IMPLICATIONS FOR COELIAC DISEASE SCREENING

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Eur J Gastroenterol Hepatol 1997; 9: 559-62
Abstract

Background: Patients with selective immunoglobulin A (IgA) deficiency and coeliac disease, and established association, lack serum IgA class antigliadin and endomysial antibodies (AGA, EmA). Diagnostic protocols relying on AGA and EmA to select patients for small bowel biopsy will not identify these patients.

Objective: To determine whether total IgA should be routinely measured in patients suspected of having coeliac disease as a supplementary screening test before biopsy.

Design: Prospective measurement of IgA, AGA and EmA in patients undergoing small bowel biopsy for suspected coeliac disease.

Patients: We studied 318 patients suspected of having coeliac disease. Sera from 1959 controls in a random population sample were assayed as controls.

Results: Thirty-one (10%) patients had villous atrophy, of whom 27 (87%) had EmA. Five (2%) of the 318 patients had undetectable total IgA (<0.07 g/l): two (40%) of these five had villous atrophy with negative EmA. Use of undetectable IgA as a selection criterion for small bowel biopsy as well as positive EmA would have improved sensitivity from 87% (27/31) for EmA alone to 94% (29/31), with a fall in positive predictive value from 100% (27/27) to 91% (29/32), but would have maintained high specificity and negative predictive value. Serum IgA was undetectable in 5 (4%) of 117 patients with AGA in the range 0-10 ELISA units (EU) compared with none of 201 with higher AGA (p=0.007, Fisher’s exact test). Compared with controls who had AGA 0-10 EU, patients were more likely to have undetectable IgA (5/117(4%) v. 3/706(0.4%): p=0.005). Overall, median IgA in patients with AGA 0-10 EU was lower than for those with AGA > 10 EU (1.89g/l v. 2.34g/l, p<0.001).

Conclusion: There is an association between IgA deficiency and low/negative EmA/AGA. Routine measurement of total serum IgA in patients suspected of having coeliac disease, either with EmA or where AGA is low, improves selection of patients for small bowel biopsy.
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**Introduction**

Serum immunoglobulin A (IgA) class endomysial antibody (EmA) has useful sensitivity and high specificity for coeliac disease, not only in symptomatic populations [1] but also, probably, in screening of the general population [2]. However, neither EmA nor IgA class antigliadin antibody (AGA) is detectable in patients with selective IgA deficiency, which is known to be associated with coeliac disease [3]. It is possible that negative or ‘low’ EmA and AGA might be markers for IgA deficiency and thus allow identification of patients with that condition, who might also have antibody-negative coeliac disease. In this study we wished to determine the prevalence of IgA deficiency among patients undergoing investigation for coeliac disease. The level of ‘normal’ EmA as measured by indirect immunofluorescence is difficult to quantify but measurement of AGA by enzyme-linked immunosorbent assay (ELISA) allows quantification in ELISA units (EU) with a normal range reported by our laboratory of 0-100EU [4]. As AGA is still widely used as a screening test, we also assessed whether patients with AGA at the lower end of the range are more likely to have IgA deficiency.

**Materials and Methods**

Subjects and techniques

Patients attending gastroenterology clinics for suspected coeliac disease had sera tested for total IgA, IgG and IgM levels and for AGA and EmA. Total IgA < 0.8 g/l was taken as low, and < 0.07 g/l as undetectable. AGA was measured by ELISA using a commercial kit (Labmaster, Turku, Finland) with results expressed in ELISA units (EU). EmA was detected by indirect immunofluorescence with commercially available monkey (*Maccaca fascicularis*) oesophagus (Biodiagnostics, Worcestershire, UK) as antigen, and a titre of 1:5 or greater taken as positive. Irrespective of AGA or EmA result, patients underwent small bowel biopsy by standard forceps from the distal duodenum during upper gastrointestinal endoscopy. At least three biopsies were taken and carefully orientated on filter paper before submission in formalin for assessment by experienced histopathologists.
Controls

Sera obtained from a random population as part of a study of coronary artery risk factors (MONICA: multinational MONItoring of trends and determinants in Cardiovascular disease) had previously been tested for total IgA, AGA and EmA as part of another study [5], and were used as controls.

Statistics

Results were expressed as medians with 95% confidence intervals (CI) where appropriate and analysed by Mann-Whitney U test for continuous and Fisher’s exact test for categorical variables, with p<0.05 considered as statistically significant.

Results

Patients

We studied 318 patients (mean age 42, range 11-88 years), of whom 192 (60%) were female. Primary indications prompting investigation for coeliac disease were diarrhoea (163 patients, 51%), anaemia (51, 16%), abdominal pain (39, 12%), chronic fatigue (23, 7%), weight loss (20, 6%), abnormal liver biochemistry (15, 5%), dermatitis herpetiformis (4, 1%), and family history of coeliac disease (3, 1%).

Small bowel biopsy and antibody results

A total of 31 patients (10%) had villous atrophy (VA). Of these patients, 14 (45%) reported diarrhoea. Primary indications for investigation in the remainder were anaemia (10, 32%), chronic fatigue (3, 10%), abnormal liver biochemistry (2, 6%) and dermatitis herpetiformis (2, 6%). Of these 31, 27 (87%) had EmA. All patients with EmA had VA, with AGA > 100 EU in 20 (75%) patients and 11-100 EU in 7 (26%). Of the four patients with negative EmA and VA, two had undetectable (<0.07g/l) IgA and AGA of 0 EU, one had AGA of 0 EU but normal IgA (0.93g/l), and one had AGA of 92 EU and normal IgA. Thus no patient with VA
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and negative EmA had AGA > 100 EU. *Giardia lamblia* organisms, in the absence of VA, were detected in three patients, two with low IgA and AGA (0.57 g/l, 6EU and 0.67 g/l, 2EU, respectively) and one with normal IgA and AGA of 10 EU. One patient with IgA of 0.75 g/l had lymphangiectasia. No patient with AGA >10 EU had a duodenal abnormality other than VA.

Impact of an IgA-related protocol on detection rates for small bowel biopsy

Table 1 shows sensitivity, specificity and positive/negative predictive values of two protocols for selecting patients for small bowel biopsy. By including patients with IgA < 0.07g/l as well as those with EmA, sensitivity rose from 87% to 94% and the positive predictive value fell from 100% to 91%. As no patient with VA and negative EmA had AGA > 100EU, addition of AGA to selection protocols added nothing to sensitivity or positive predictive value.

Table 1. Predictive values of two biopsy selection criteria (EmA; EmA or IgA < 0.07 g/l) for villous atrophy.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EmA</td>
<td>27/31 (87%)</td>
<td>287/287 (100%)</td>
<td>27/27 (100%)</td>
<td>287/291 (99%)</td>
</tr>
<tr>
<td>EmA or IgA &lt;0.07g/l</td>
<td>29/31 (94%)</td>
<td>284/287 (99%)</td>
<td>29/32 (91%)</td>
<td>284/286 (99%)</td>
</tr>
</tbody>
</table>

IgA and AGA levels

Low serum IgA (< 0.8g/l) was identified in 12 (10%) of 117 patients with AGA 0-120 EU. Five of these had undetectable (< 0.07g/l) IgA levels and AGA of 0 (4 patients) or 3 EU (one patient). One patient with low IgA (0.22 g/l) had an IgG paraproteinaemia. Another with undetectable IgA had reduced IgM: no significant abnormality in IgG or IgM was detected
in any other patient. Only one patient (0.5%) of the 201 with AGA > 10EU had low IgA (0.71 g/l, AGA 71 EU). There was thus a significant association between AGA 0-10 EU and both low IgA (<0.8 g/l) and undetectable IgA (<0.07 g/l): p < 0.001 and p = 0.007, respectively, Fisher’s exact test. The median total serum IgA was 1.89 (95% CI 1.72-2.00) g/l for patients with AGA 0-10 EU compared with 2.34 (1.99-2.36) g/l for patients with AGA > 10 EU (p < 0.001, Mann-Whitney U test).

Results of control sera

Sera were tested from 1959 controls (49% male), aged 12-64 years, of whom 706 (36%) had AGA 0-10 EU and 1253 (64%) had AGA > 10 EU. Eleven (1%) controls with AGA > 10 EU had EmA. Total IgA was low (<0.8g/l) in 27 (4%) patients with AGA 0-10 EU compared with 12 (1%) with AGA > 10 EU (p < 0.001, Fisher’s exact test). Total IgA was undetectable (<0.07 g/l) in 3 (0.4%) patients with AGA 0-10 EU compared with 1 (0.1%) patient with AGA > 10 EU (p = 0.14). Median (95% CI) total IgA was 1.93 (0.64-4.08) g/l for controls with AGA 0-10 EU compared with 2.34 (0.97-5.15) g/l for those with AGA > 10EU: as for patients, these were significantly different (p < 0.001). However, compared with controls with AGA 0-10 EU, patients with AGA in that range were significantly more likely to have IgA < 0.8 g/l (12 (10%) of 117 v. 27 (4%) of 706: p = 0.002) and IgA < 0.07 g/l (5 (4%) of 117 v. 3 (0.4%) of 706: p = 0.005). Prevalence of low IgA by AGA level among patients and controls is shown in Table 2.
Table 2. Comparison of IgA, AGA and EmA values in patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
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</thead>
<tbody>
<tr>
<td><strong>AGA 0-10EU</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>117 (37%)</td>
<td>706 (36%)</td>
</tr>
<tr>
<td></td>
<td>No. with IgA &lt;0.8g/l</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 (10%)</td>
<td>27 (4%)</td>
</tr>
<tr>
<td></td>
<td>No. with IgA &lt;0.07g/l</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 (4%)</td>
<td>3 (0.4%)</td>
</tr>
<tr>
<td></td>
<td>No. with EmA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td></td>
<td>No. with villous atrophy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (3%)</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>AGA &gt;10EU</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>201 (63%)</td>
<td>1253 (64%)</td>
</tr>
<tr>
<td></td>
<td>No. with IgA &lt;0.8g/l</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (0.5%)</td>
<td>12 (1%)</td>
</tr>
<tr>
<td></td>
<td>No. with IgA &lt;0.07g/l</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td></td>
<td>No. with EmA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27 (13%)</td>
<td>11 (1%)</td>
</tr>
<tr>
<td></td>
<td>No. with villous atrophy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28 (14%)</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

**Discussion**

The prevalence of 1:490 of very low IgA in our control population is similar to that described in population studies elsewhere in Western countries [6] and is much lower than among our patients. Since the initial description by Crabbe and Heremans [7] of steatorrhoea associated with selective IgA deficiency, several studies have established a link with coeliac disease [8,9]. Recent reports suggest a very high prevalence of IgA deficiency among patients with VA of 1:5-1:10 [10,11], which is similar to our figure of 1:16 (2 of 31). Our biopsies were not assessed for increased intraepithelial lymphocyte counts in the presence of normal villi, so the possibility of milder enteropathy in some of our patients (including the three patients with IgA < 0.07 g/l and no VA) was not considered. Screening with IgG class antigliadin and endomysial antibodies would not be influenced by IgA level, but we found them to have low sensitivity in a symptomatic population (57% and 39%, respectively) [4]. Collin
et al [8] reported a sensitivity of only 43% for IgG class antigliadin antibodies among IgA-deficient coeliac patients. We were thus prompted to assess total IgA as a supplementary screening test.

The comparison between results for our patients and those of a control group is important as investigation protocols shown to be effective in symptomatic patient groups are not necessarily so for the screening of population where the prevalence is relatively low. We previously reported a 100% specificity of AGA > 100 EU for VA among patients with high suspicion of coeliac disease [4]. In contrast, when Uibo et al [12] screened a healthy population using the same ELISA kit, none of 48 patients with AGA > 100 EU undergoing biopsy had VA. Similarly, our control group had a very low prevalence of EmA where AGA was > 100 EU, and even in our symptomatic patients the prevalence of EmA and VA was only 43% among those with AGA > 100 EU. This disparity with our initial study may stem from the inclusion of patients with relatively non-specific symptoms as possible coeliac disease cases.

A protocol for biopsy disregarding AGA levels and based on total IgA level as well as EmA would have increased the sensitivity of coeliac screening at the expense of a fall in the predictive value of a positive result but with no appreciable reduction in specificity or in the predictive value of a negative result. Two patients with VA had neither EmA nor undetectable IgA and would not have been identified by either screening protocol. Routine biopsy of all patients with IgA < 0.8 g/l would not have increased the detection of VA but would have identified three other cases of pathology (two giardiasis, one lymphangiectasia). Both conditions are associated with low IgA [13,14].

Although AGA performed poorly as a screening test, we have included the results as many units still use these antibodies for screening. Our results confirm that low serum IgA is associated with low AGA and is worth testing for if AGA is in the 0-10EU range.

Although a majority of patients with IgA < 0.8 g/l had normal small bowel biopsy, significantly more patients than AGA-matched controls had low IgA, raising the possibility of an aetiological association in the absence of VA or other demonstrated pathology. Patients
with IgA deficiency and no VA may have increased intraepithelial lymphocytes [15], and an immunohistochemical study has shown increased density of activated CD25+ cells in jejunal epithelium and lamina propria and raised mitotic activity in crypts [10]. Increased gut permeability associated with IgA deficiency is suggested by circulating milk precipitins and other dietary antigens [16,17].

In conclusion, testing for IgA deficiency improves selection of patients for small bowel biopsy. The significant association between symptoms and IgA deficiency is further evidence of an aetiological link.
References

15. Klemola T. Immunohistochemical findings in the jejunum of IgA-deficient persons: