

IgA antiendomysium antibodies have a high positive predictive value for celiac disease in asymptomatic patients

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Many attempts have been made to find screening tests for celiac disease to reduce the need for biopsy, or to achieve better selection criteria before intestinal biopsy. We have recently analyzed apparently healthy blood donors for antigliadin antibodies (AGA) to select subjects for further gastrointestinal investigation. A prevalence of gluten enteropathy of at least 1/256 was found in this population. The positive predictive value (+ PV), however, was only 20%. In the present study we have analyzed IgA antiendomysium antibodies (IgA-EmA) to estimate the sensitivity and specificity of the test, and determine whether or not the + PV of the assay increases when screening for adult celiac disease in an asymptomatic population. We found that asymptomatic persons with celiac disease may have IgA-EmA. We found a 100% specificity of IgA-EmA in the tested population of blood donors, whereas the sensitivity was about the same as that of IgA-AGA. This result of a + PV of 100% indicates that a positive IgA-EmA could replace biopsy in diagnosing celiac disease. However, further extended studies are needed to determine whether this is applicable in other populations. To screen patients for celiac disease, we recommend the easy and cheap IgA-AGA assay as a preliminary test and the IgA-EmA to verify the diagnosis and avoid unnecessary biopsies.

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The diagnosis of celiac disease is based on the demonstration of a flat or almost flat jejunal mucosa in biopsy material from the small intestine and the regeneration of the mucosa after a gluten-free diet (24). Since celiac disease has been found in patients without typical gastrointestinal symptoms, the selection of patients for intestinal biopsy to verify the diagnosis is difficult (2, 4, 6, 7, 9, 12, 14, 17). Many attempts have been made to find screening tests for celiac disease to reduce the need for biopsy, or to achieve better selection criteria for intestinal biopsy.

Interest has been focused on various antibodies such as antireticulin, antigliadin, and antiendomysium antibodies (3, 5, 8, 10, 16, 18–21, 25, 26, 28–30). We have analyzed apparently healthy blood donors for antigliadin antibodies (AGA) with a view to further gastrointestinal investigation including jejunal biopsy (12, 13, 17). A fourfold higher prevalence of asymptomatic gluten enteropathy (1/256) than of symptomatic celiac disease (1/1000) was found in this population within the same geographic area (15).

This prevalence is comparable with the increasing incidence (1/300) of symptomatic celiac disease previously reported in Swedish children (1). The positive predictive value (+ PV) in our study, however, was only 20% when AGA was the only factor used for selection.

The aim of this study was to estimate the positive predictive value for celiac disease of IgA antiendomysium antibodies (IgA-EmA) as a single test or in combination with IgA-AGA in a population with no or few symptoms.

Material and methods

Blood donors

Sera from 42 donors previously selected because of high IgA-AGA (> the 96.8th percentile) but found not to have gut lesions (intestinal biopsy) and sera from 110 subjectively healthy donors (unbiopsied) were analyzed for IgA-EmA.

Patients with celiac disease

Sera from 16 untreated patients primarily selected because of various symptoms in the gastrointestinal tract and seven asymptomatic blood donors previously found to have high IgA-AGA (> the 96.8th percentile), as well as gut lesions suggestive of celiac disease, were further analyzed for IgA-EmA.

The intestinal biopsy was taken from the duodeno-jejunal junction with a Watson capsule and examined for changes characteristic of celiac disease, as described elsewhere (12).

Antigliadin antibodies (AGA)

A recently described micro-ELISA assay for detection of AGA in serum was used (12, 13). Microtiter plates were coated with 50 µg/ml gliadin dissolved in ethanol (Sigma, St Louis, MO, USA). The plates were incubated for 60 min with serum diluted 1/10 in phosphate-buffered saline (PBS) containing 0.5% human serum albumin, and then washed. Peroxidase-conjugated anti-IgA antibodies were used to detect AGA (Dakopatts, Glostrup, Denmark).

The absorbance was read spectrophotometrically in a Dynatech minireader II (Dynatech, Alexandria, VA, USA), and a pool of positive sera was used as a standard. The optical density of the standard was transformed to units by a program for semilog fit. The cutoff point for positive outcome of the test was 40 units (the 96.8th percentile), and the end-point value was 300 units. All subjects were analyzed for serum IgA by a routine nephelometric assay (Kallestad, Austin, TX, USA).

Antiendomysium antibodies

IgA-EmA were analyzed by indirect immunofluorescence (IF) microscopy with fixed cryostat sections of monkey esophagus (Scimedx, Denville, NJ, USA) as antigen substrate.

Patient sera were frozen at -20°C for periods of up to 6 years before analysis and screened at a dilution of 1/10 in pH 7.6 PBS.

Polyclonal fluorescein isothiocyanate (FITC)-labeled rabbit antihuman IgA (Dakopatts, Glostrup, Denmark) was used as a secondary antibody (27). Positive sera were end-point titrated. The antibody titer was defined as the highest serum dilution yielding positive fluorescence.

Definitions of terms used in the study

Sensitivity: probability that a test will be positive when the disease is present

Specificity: probability that the test will be negative when the disease is not present

Positive predictive value (+PV): probability that the disease is present when a test is positive

Cutoff point: the arbitrary value used to separate positive from negative results for any given test.

Results*IgA-EmA in healthy adults and patients with celiac disease*

In the group of blood donors with high IgA-AGA ($n = 42$) but no gut lesions, no subject had detectable IgA-EmA (Table 1). In the unbiopsied donor group ($n = 110$), one subject had detectable IgA-EmA; the subject was biopsied and found to have a gut lesion suggestive of celiac disease. This donor was excluded from further calculations in this study. Five of the seven asymptomatic patients with celiac disease (blood donors) primarily selected because of high IgA-AGA also had IgA-EmA (titer 1/40–1/640).

In the group of 16 patients with celiac disease, selected by gastrointestinal symptoms, 13 (81%) had IgA-EmA (titer 1/40–1/1280). Eleven had IgA-AGA (69%) above the 96.8th percentile. Two of the three patients without IgA-EmA ($< 1/10$) were IgA-AGA positive. One patient had neither IgA-AGA nor IgA-EmA.

All subjects had normal or high serum-IgA concentration. Fig. 1 shows the typical immunofluorescence pattern produced by IgA-EmA.

Positive predictive value (+PV) of IgA-EmA

To calculate the +PV, we also estimated the specificity and sensitivity of the IgA-EmA assay, both as a single test and as a serial test together with IgA-AGA (the result is positive if both tests are positive), according to the Bayes theorem (11). Thirteen of 16 patients with symptoms and diagnosed as having celiac disease had IgA-EmA, and in the group of asymptomatic patients (blood donors) 5/7 had IgA-EmA. There was no obvious difference in titers between the two groups. This makes the estimated sensitivity of the assay 78% (18/23). The specificity

Table 1. Frequency of IgA-EmA in healthy subjects and in patients with celiac disease

	<i>n</i>	IgA-EmA
Blood donors without gut lesions and high IgA-AGA*	42	0
Subjectively healthy donors (unbiopsied)	109	0
Celiac disease patients with symptoms	16	13
Asymptomatic patients with celiac disease and high IgA-AGA*	7	5

* Selected for further gastrointestinal examination because of high IgA-AGA (> 96.8th percentile).

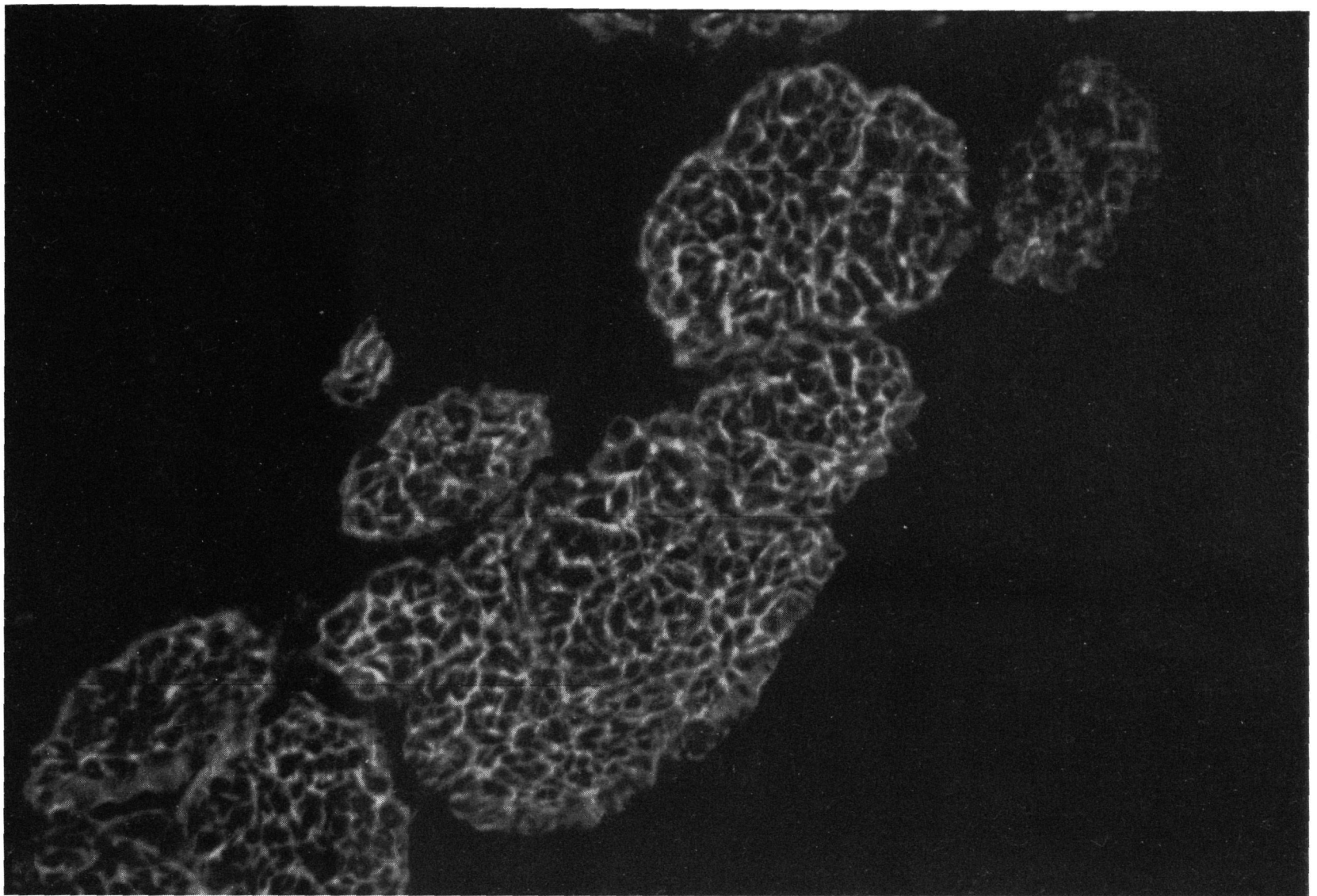


Fig. 1. Typical immunofluorescence pattern produced by IgA-EmA.

of the assay was estimated to be 100% (151/151). By application of the Bayes theorem in a population with no or few symptoms, the +PV for celiac disease was therefore 100% for IgA-EmA, both as a single test and in serial testing together with IgA-AGA.

Discussion

Celiac disease is often diagnosed in patients without classical gastrointestinal symptoms (2, 4, 6, 7, 9, 12, 14, 17). It was for this reason that the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) suggested new diagnostic criteria, and advocated laboratory tests providing better selection criteria for intestinal biopsy (31).

Various studies have raised important questions concerning the true prevalence of celiac disease. A study in Sweden using IgA- and IgG-gliadin antibodies as a screening test on a group of patients at a health center for farmers showed a high prevalence of celiac disease (1/114) in that population (2).

An increasing incidence (1/300) of celiac disease in Swedish children has previously been reported (1). This agrees with a study on Italian school-children in which a prevalence of 1/200 was found

(22). We have also found a prevalence of asymptomatic celiac disease of at least 1/256 in a population of blood donors (12, 13, 17). When high levels of IgA-AGA were used to select donors for further examination, the +PV varied between 18 and 25% depending on how the cutoff points were set (12, 13). Cutoff values were defined both as the mean plus 2 SD and as the 97.5th percentile, and they were also related to the age of the donor (12, 13).

Application of the Bayes theorem is important, especially in screening situations (11). The predictive value of any test is dependent on the quality of its sensitivity and specificity, as well as the prevalence of the disease in the population being investigated. In the present study, we measured the +PV for IgA-EmA in a healthy donor population. We assessed the assay both as a single test and as a serial test together with IgA-AGA to see whether +PV increases.

When one uses more than one test as a diagnostic tool, it is important that the tests be independent of each other. Our results show that patients may have a high concentration of IgA-AGA but not of IgA-EmA, a finding which indicates that there is no connection between the two antibodies per se. Our results also show that the presence of IgA-EmA does

not correlate with any symptoms of celiac disease because five of the seven asymptomatic donors with gut lesions had raised IgA-EmA titers. Possibly the other two donors with biopsies suggestive of celiac disease had not yet developed IgA-EmA, and it cannot be excluded that IgA-EmA has a prognostic value for the risk of developing clinical symptoms. On the other hand, it would be difficult to explain why all the patients (13/16) with gastrointestinal symptoms and diagnosed as having celiac disease did not exhibit raised IgA-EmA titers. It may be advisable to perform a control test for IgA-EmA at a later time if it was negative at the first sampling.

The + PV for the assay was 100%, analyzing IgA-EmA both as a single test and as a serial test together with IgA-AGA, since the specificity of IgA-EmA was 100%. This result agrees with a study on IgA-EmA in a group of patients with suspected celiac disease where a + PV of 100% was found (23). The sensitivity of IgA-EmA was found to be 89% in that study.

In another study on celiac disease, a + PV of 91% was found in patients without signs of malabsorption who here served as control group (8). Two of these patients had IgA-EmA; one of them had insulin-dependent diabetes mellitus, and the other thyroiditis, i.e., diseases known to be associated with increased incidence of celiac disease. In that study, IgA-EmA was found to have 100% sensitivity (8).

However, in the present study, we show that serial testing (IgA-AGA/IgA-EmA) decreased the sensitivity both in a symptomless population (5/7) and in untreated patients with more typical symptoms of celiac disease (9/16). If one instead uses both antibody tests (IgA-AGA/IgA-EmA), such that at least one of them must be positive, the sensitivity increases to 96% (22/23) in untreated patients with celiac disease.

If we compare both assays used in this study, the cost of analyzing the same number of samples is approximately fivefold greater for the IgA-EmA assay than the IgA-AGA assay. The time required for analysis is almost the same for both assays. For assessment of the results, however, the IgA-EmA assay requires more experience than the IgA-AGA assay.

When one screens large populations with no or few symptoms, it is desirable to have a high + PV to avoid unnecessary biopsies. In the decision of which test(s) to use, it is important to consider the Bayes theorem (11), the cost of the assay, and the field of application of the test(s). The last factor is dependent on the degree of selection of the subjects in the population studied, which in turn affects the prevalence and also the + PV.

We conclude therefore that when screening for celiac disease in large populations with no or few

symptoms, IgA-AGA should be used, mainly because of its low cost and high sensitivity. The suspicion of celiac disease indicated by a positive IgA-AGA assay should be confirmed by IgA-EmA assay. The results of the present study suggest that a positive IgA-EmA might obviate jejunal biopsy because of its high specificity (100%), but further studies are needed to confirm this. However, a negative IgA-EmA does not exclude celiac disease; therefore, such patients should be further examined by biopsy.

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