Determination of anti-endomysium IgA antibodies in the diagnosis of celiac disease: Comparison of a novel ELISA-based assay with conventional immunofluorescence

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Abstract

AIM: To evaluate the novel anti-endomysium (anti-EMA) detection based on ELISA.

METHODS: Anti-EMA IgA was measured by a novel ELISA in 196 patients with gastrointestinal symptoms and suspected mal-absorption. Data were compared with those obtained by the conventional IF test.

RESULTS: A good concordance of 98% was found between these two assays. In sera of 161 patients (82%) both assays tested negative whereas in sera of 31 patients (16%) both assays tested positive for the presence of anti-EMA antibodies. Discrepancies between EMA-ELISA and EMA-immunofluorescence (IF) were found in only 4 patients (2%).

CONCLUSION: This ELISA can replace IF for the detection of anti-EMA antibodies and provide clinicians with an excellent tool to screen for celiac disease in patients with gastrointestinal complaints.

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Key words: Celiac disease; Endomysium; ELISA; Immunofluorescence; Histology

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INTRODUCTION

Celiac disease (CD) is the most common cause of intestinal malabsorption in the Western world with a recently described incidence of 1:100 to 1:300. Gastrointestinal damage is the result of gluten-dependent change in villous morphology and/or signs of immunological activation in the lamina propria, which is reversible on a strict gluten-free diet

Material and Methods

The study population consisted of 196 patients (male 35%, female 65%) with gastrointestinal symptoms and suspected mal-absorption, visiting the Department of Internal Medicine or the Department of Pediatrics of the Diaconessenhuis in Leiden or the Albert Schweitzer Hospital in Dordrecht, respectively, between November 2002 and May 2005. Total IgA was determined by immunoturbidimetric assays on the Hitachi-917 (Roche Diagnostics Corporation, Indianapolis, IN, USA) or the Beckmann-immage autoanalyzer (Beckman-Coulter, Mijdrecht, the Netherlands).

Detection of anti-EMA antibodies by IF was performed by using cryostat sections of monkey esophagus as an antigen substrate (The Binding Site, Indianapolis, IN, USA).
The detection of anti-EMA antibodies by an ELISA has been described before. To the best of our knowledge, this is the first anti-EMA assay based on endomysium antigens purified by affinity chromatography from primate liver and is not commercially available anymore.

We compared the EMA-ELISA and the EMA-IF by assaying 196 patients with gastrointestinal symptoms and suspected mal-absorption. A good concordance of 98% was found between both these assays. In sera of 161 patients (82%) both assays tested negative whereas in sera of 31 patients (16%) both assays tested positive for the presence of anti-EMA antibodies and the diagnosis of CD was confirmed by histological results. Discrepancies between EMA-ELISA and EMA-IF were found in only 4 patients (2%). In sera of 3 of these patients the presence of anti-EMA antibodies was detected by IF but not by EMA-ELISA, however in these sera anti-tTGA antibodies were absent (results not shown), suggesting the absence of CD. Since no histological data were available the diagnosis of CD could not be excluded or confirmed. In serum of 1 patient the presence of anti-EMA antibodies detected by ELISA could not be confirmed by IF and anti-tTGA antibodies were absent. The slightly increased anti-EMA antibody concentration (e.g. 22 U/mL) appears to be false positive since the biopsy results were not indicative for CD.

Quantitative results obtained by EMA ELISA are easy to interpret in contrast to qualitative results obtained after IF, providing the clinicians with an excellent tool to screen for CD in patients with gastrointestinal complaints in the absence of IgA deficiency.

In conclusion, this ELISA can replace IF for the detection of anti-EMA antibodies. In daily practice, testing for anti-EMA antibodies is frequently performed in conjunction with testing for anti-tTGA antibodies since this combination accounts for almost 100% sensitivity and specificity.[3, 4]

### RESULTS

None of the patients included in this study showed an IgA deficiency. Sera of 161 patients tested negative for anti-EMA antibodies with both IF and ELISA (Table 1). In sera of 31 patients anti-EMA antibodies were detected with both IF and ELISA and the diagnosis of CD could be confirmed by histological results. In sera of 4 patients discrepancies were found in anti-EMA results between IF and ELISA. In sera of 3 patients the presence of anti-EMA antibodies was only detected with IF. No histological data were available for these 3 patients. In serum of 1 patient the slightly increased anti-EMA concentration (22 U/mL) was only detected with ELISA, and CD could not be confirmed by histological results.

### DISCUSSION

To the best of our knowledge, this is the first anti-EMA assay based on endomysium antigens from human origin. The detection of anti-EMA antibodies by an ELISA has been described before[5]. This EMA-ELISA however is based on endomysium antigens purified by affinity chromatography from primate liver and is not commercially available anymore.

#### Table 1 Comparison of the results of anti-EMA antibody detection by IF and ELISA in 196 patients

| EMA-IF | EMA-ELISA | n (%) |
|--------|-----------|------|
| -      | -         | 161 (82) no biopsy results |
| +      | +         | 31 (16) biopsy: indicative for CD |
| +      | -         | 3 (1.5) tTGA -, no biopsy results |
| -      | +         | 1 (0.5) anti-EMA 22 U/mL, tTGA -, biopsy: not indicative for CD |

- = negative results; + = positive results; n = the number of patients.

Birmingham, UK) according to the manufacturer’s instructions. All slides were scored by two independent observers and a definite result was obtained by consensus.

Anti-EMA antibodies were also detected with an ELISA (Medipan Diagnostica, Selchow, Germany) according to the manufacturer’s instructions (www.genericassays.com). Microplate strips coated with purified human endomysium autoantigens (90-300 kDa) were incubated with 100 µL 1:50 diluted serum, a positive control and a 4-point calibrator curve were included in each run. The strips were incubated for 1h at 37 °C and washed six times with 300 µL washing buffer and 100 µL horseradish peroxidase-conjugated anti-human IgA was added. After 30 min incubation at 37 °C the plates were washed 6 times, 100 µL of substrate solution containing 3, 3', 5, 5' tetramethylbenzidine was added and the reaction was stopped after 10 min at ambient temperature in the dark with 100 µL 0.25 mol/L H2SO4. The absorbance was measured at 450 nm. A standard curve was established by plotting the optical density of each calibrator with respect to the corresponding concentration value in U/mL. From the OD of each sample the corresponding antibody concentration expressed in U/mL could be determined. The cut-off value for positivity was 20 U/mL. Patients who tested positive for serologic tests, underwent a small intestinal biopsy.

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