LETTER TO THE EDITOR

A novel quantitative ELISA as accurate and reproducible tool to detect epidermal transglutaminase antibodies in patients with Dermatitis Herpetiformis

Editor

Serology for anti-epidermal transglutaminase antibodies (Anti-TG3 Abs) has attracted interest, being inexpensive and non-invasive and could ideally replace skin biopsy for the diagnosis of Dermatitis Herpetiformis (DH).[1] The current commercially available ELISA (Elisa kit) for serum Anti-TG3 Abs overall has a good specificity (84–100%)[2-4]; however, its clinical use is limited due to variable sensitivity (45–100%) and low specificity in Coeliac Disease (CD) patients (47–85%) in whom Anti-TG3 Abs may be present in the absence of DH.[2,3,5-7] Here, we developed and evaluated the diagnostic performance of a novel ELISA to quantitatively measure Anti-TG3 Abs.

The novel ELISA was set-up using a human activated recombinant TG3[8] (Zedira, Darmstadt, Germany). To quantify serum Anti-TG3 Abs, we produced a human gut-derived monoclonal IgA Anti-TG3 Ab (hrm-IgA anti-TG3 Ab) and built a dose–response curve of the absorbance vs concentration correlating the optical density values of different known amounts of the hrm-IgA anti-TG3 Ab. The curve had high affinity and was highly specific and not cross-reactive with tissue transglutaminase (TG2). The intra- and inter-assay CV values for each concentration of the hrm-IgA anti-TG3 Ab and for each sample of our novel ELISA were good being <10% and 15%, respectively.

Stored serum samples of 46 DH patients, 212 CD patients, 120 patients with non-gluten related gastrointestinal diseases (CTRL) and 168 healthy controls (HC), collected at four University Hospitals, in Italy and Finland, between 2013 and 2017 were analysed at IRCCS Burlo Garofolo, Trieste, Italy, using the novel ELISA and the ELISA kit (Immundiagnostik, Bensheim, Germany). Both assays were run in duplicate for all sera.

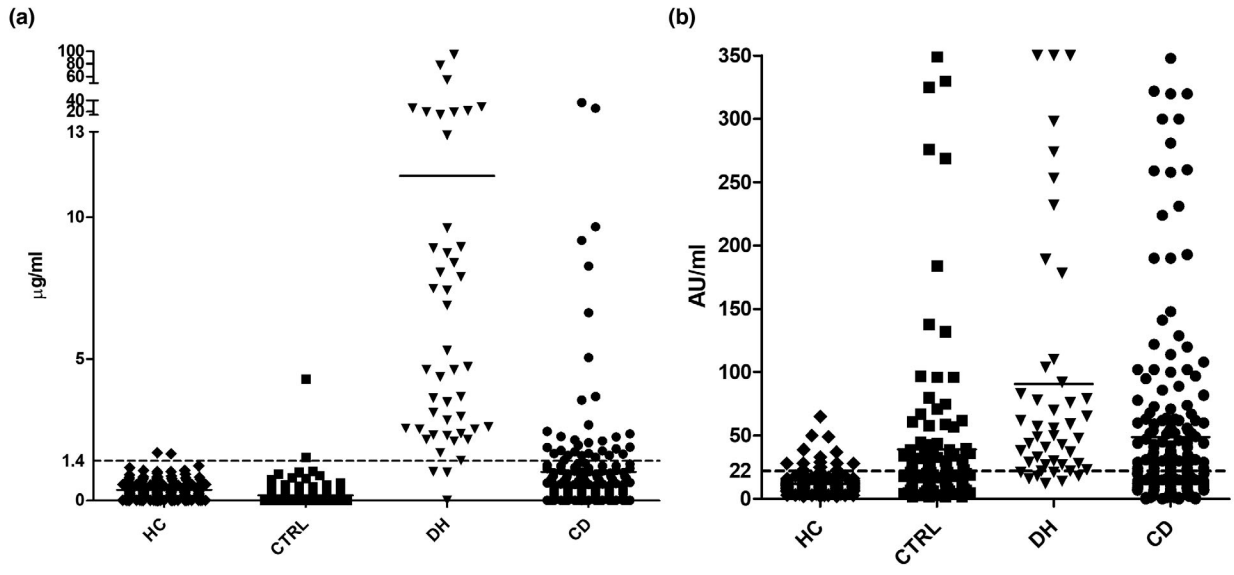

Figure 1 Serum IgA Anti-TG3 antibody concentrations in the four study groups were measured by the novel ELISA (a) and the commercial ELISA kit (b). Dashed lines mark the cut-off value for test positivity. Solid line in each study group is the mean value. DH, Dermatitis Herpetiformis (closed inverted triangles); CD, Coeliac Disease (closed circles); CTRL, non-gluten gastrointestinal disease (closed squares); HC, Healthy Controls (open circles).

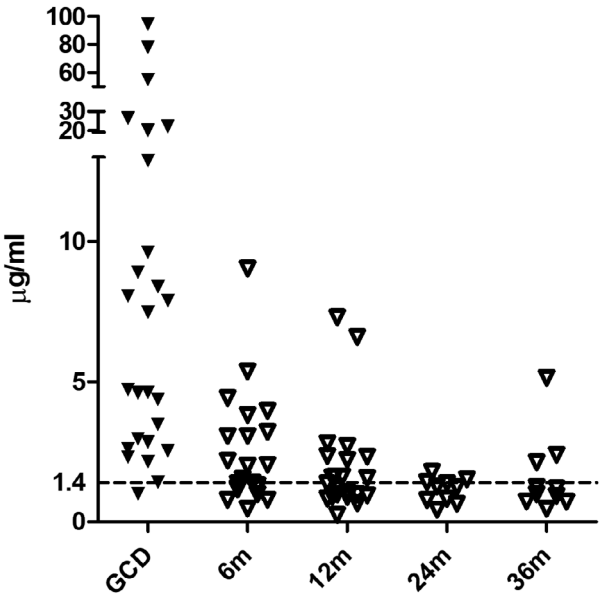

Figure 2 Serum IgA Anti-TG3 antibody concentrations were measured in 26 patients with Dermatitis Herpetiformis on gluten-containing diet (GCD) (closed inverted triangles) and after 6, 12, 24 and 36 months on gluten-free diet (open inverted triangles) using the novel ELISA. The dashed line marks the cut-off value for positivity (1.4 µg/mL).

Using the novel ELISA, we found that DH patients had significantly higher concentrations of Anti-TG3 Abs compared to all the other groups ($P < 0.0001$; Fig. 1a) and that Anti-TG3 Abs concentrations significantly decreased with gluten-free diet ($P < 0.0001$; Fig. 2). In DH patients, there was a direct correlation between Anti-TG3 and Anti-TG2 Abs concentrations ($R^2 = 0.3$) whereas no statistically significant relationship was found in CD patients ($R^2 = 0.01$).

For the novel ELISA, the ROC curve analysis identified a cut-off value of IgA Anti-TG3 Abs concentration of 1.4 µg/mL able to produce a sensitivity of 93% and a specificity of 99% (AUC 0.98) to distinguish DH patients from HC. This cut-off was used to define a positive test across all groups. Applying the manufacturer cut-off value (positive value >22 AU/mL), the ELISA kit gave a sensitivity of 83% and a specificity of 90.5% in the same cohort (Fig. 1b). In CD patients, the novel ELISA gave a specificity of 85% whereas the ELISA kit gave a specificity of 48%. Interestingly, the specificity of the novel ELISA varied when considering CD patients by age and was higher in patients ≤25 years (specificity 97%) versus patients >25 years (specificity 55%). This difference was not observed using the ELISA kit.

To conclude, in this multicentre study, we developed an accurate and reproducible quantitative novel ELISA assay for the measurement of Anti-TG3 Abs. One reason to explain the good reproducibility of the test could be the fact that to create the dose–response curve, we used a monoclonal antibody isolated from the intestinal mucosa, which is likely the primary site of Anti-TG3 Abs synthesis in DH patients.[9] The availability of a specific standard curve will improve the comparison of the results obtained from different laboratories and allow a shared diagnostic cut-off value for the diagnosis of DH. Given its high diagnostic performance, this novel ELISA is suitable as a screening tool in patients in whom DH is clinically suspected and may serve as the sole confirmatory test in younger patients. Further studies in larger cohorts are needed to confirm these results.

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[Correction added on 26 August 2020, after first online publication: the 'Funding Sources' has been corrected in this version.]

Conflicts of Interest

None.

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