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Short Communications

Comparison of an EIA for GAD and IA2 autoantibodies with existing radioimmunoassays

B.E.P.B. BALLIEUX[1], Z. MENGI[1], M. BATSTRA[2,4] and B. ROEP[3]

Autoantibodies to glutamic acid decarboxylase-65 (GAD) are associated with the diagnosis of Diabetes Mellitus type 1 (DM1) and in combination with antibodies to the tyrosine phosphatase IA2 predictive for development of DM1 in 1st degree relatives of patients with DM1 (1). Recently, new enzyme immunoassays (EIA) for GAD- and IA2-antibodies have been developed, that showed enhanced clinical performance in the 3rd survey of the Diabetes Antibody Standardization Program (DASP) of the IDS (Immunology of Diabetes Society), http://www.idsoc.org/committees/antibody/antibody_committee.html. We compared the performance of these Enzyme-Immunoassays with conventional in house Radio Immunoprecipitation Assays (RIA) (2).

Methods

Sera from 55 patients with DM1 within five days of diagnosis and sera from 55 healthy siblings, not developing DM1 within at least 5 years after inclusion, were included in this study. These patients and relatives were participants in the KOLIBRIE study (3). RIA's were then performed as described in (2) using an in-house radioimmunoassay. 35S methionine labelled human recombinant GAD65 and the intracellular domain of IA2 (AA603-980) were used as tracer. These RIA's showed representative performance for immunoprecipitation assays in the 3rd survey of the IDS and at that time the Reinier de Graaf Group had been assigned as reference laboratory by the IDS.

In the present study both GAD-antibodies and IA2-antibodies were assayed in the same samples from the KOLIBRIE study using EIA's of Medipan, Dahlewitz/Berlin, Germany. The reagents for these EIA's were manufactured by RSR Limited, Cardiff, UK. The assays have been standardized against the WHO reference standard 97/550 for GAD autoantibodies and IA2 autoantibodies. In short, GAD or IA2 coated wells were incubated consecutively for 1 hour with 25 µl of serum, biotin-labelled recombinant GAD or IA2, streptavidine peroxidase and finally substrate (TMB). ROC curves were constructed for the EIA's and the RIA's to document clinical performance using the statistical software Analyse-it®.

Results

The clinical performance of the EIA for GAD-antibodies was better than the RIA. The area under the curve (AUC) was 0.94 for the EIA versus 0.87 for the RIA (Fig. 1A and 1B). The clinical performance of the EIA for IA2-antibodies was comparable to the RIA (AUC 0.82 vs. 0.83 , Fig. 1C and 1D). Based on these data optimal cut-off value for the EIA's could be selected for this study group. Using these cut-offs a sensitivity of 93% and specificity of 95% was calculated for the GAD antibody EIA and a sensitivity of 70% and a specificity of 90% was found for the IA2 antibody EIA. Total variation over 5 runs was 5% for the anti-GAD EIA and 4% for the anti IA2 EIA.

Discussion

The RIA's used in this study were assigned as reference method, based on previous surveys, that showed consistent performance and optimal sensitivity and specificity in relation to consensus values of the distributed sera. In the latest surveys results were related to clinical status resulting in maximal sensitivities of approximately 80%. Despite initial scepticism, the clinical performance of this new EIA for antibodies against GAD in the latest survey of the IDS was found to be superior to all RIA's. This enhanced performance is confirmed in our study. There was no significant difference in the performance of the IA2 antibody EIA compared to the RIA tested. We must bear in mind that the antibody prevalence in our reference population of non diabetic siblings is higher than in the general population. The constructed ROC curves therefore are solely applicable when performing prediction studies in first degree relatives. They are however not applicable to the general population and for diagnostic purposes. Similarly, clinically useful cut-off values should be defined based on the results in a large representative population of non-diabetic subjects. However, the differences in AUC between the EIA and RIA for GAD antibodies in this study population, which are essentially independent of the cut-off value chosen, seem to confirm the data of the IDS survey.

Departments of Clinical Chemistry[1] and Immunohaematology[3] of the Leiden University Medical Centre, the Reinier de Graaf Group, Delft[2] and the Erasmus MC, department of Pediatrics, Rotterdam[4]

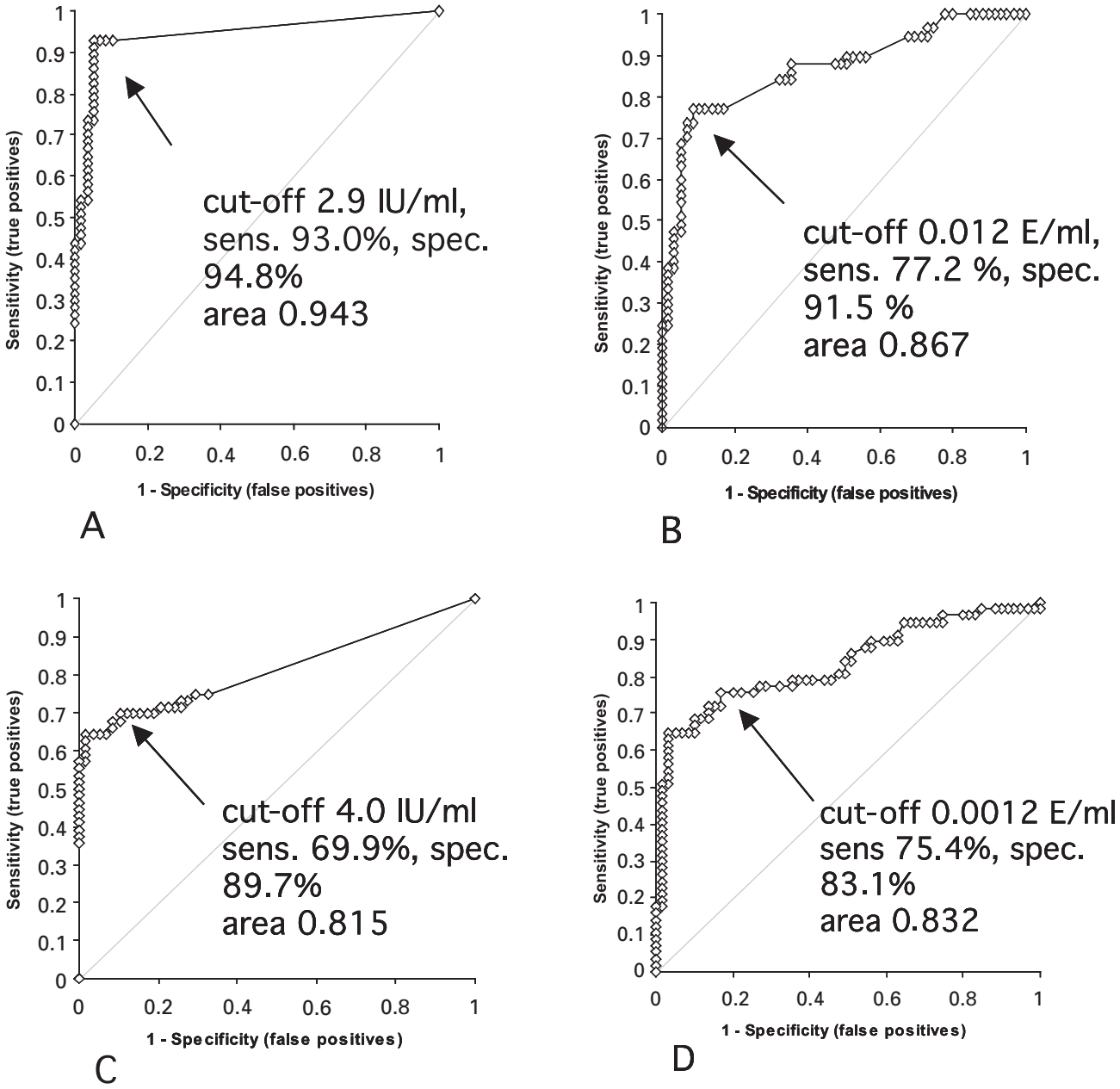

Figure 1. ROC analysis of diabetes associated autoantibody assays. A: anti-GAD EIA, B: anti-GAD RIA, C: anti-IA2 EIA, D: anti-IA2 RIA

Conclusions

The introduction of this EIA for GAD-antibodies significantly enhanced diagnostic performance compared to existing RIA's in this population of children, and may improve risk profiling in 1st degree relatives of patients with DM1. If this EIA performs equally well in adults, it may provide a useful and reliable tool to distinguish early DM type 2 and LADA in recently diagnosed young adults. Before this assay can be used in clinical studies cut off values have to be determined using a large population of healthy non-diabetic volunteers.

Literature

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