

LETTER

In response to comment on improved prediction of symptomatic type 1 diabetes using a luciferase-based assay to measure (pro) insulin autoantibodies

To the Editor,

We welcome the opportunity to respond to the correspondence from Desai *et al.* and share additional data. We thank them for their positive comments and suggestions, and are pleased that they agree that the central findings of our study stand. We discuss below why we believe the analyses they propose would not substantially alter the original conclusions drawn.

As stated in our published report, the participants were pre-selected for inclusion in the study, including oversampling of individuals who went on to develop diabetes, and the absolute risks presented should be interpreted in the light of this. The survival estimates and hazard ratios were reported for tests on the same samples and therefore the pre-selections would not bias the comparison between methods. The survival estimates and hazard ratios were reported to support interpretation of the comparison between methods. While re-weighting the hazard ratios would make them more generalisable: this was not the aim of the study.¹

Desai *et al.* raise an important point that thresholds used to identify positive levels of analytes should be appropriate to the population studied. Differences by age for zinc transporter 8 autoantibody (ZnT8A) ELISA thresholds have been identified² and therefore we have investigated this further. In a recent research letter, we found no substantial difference between the threshold for glutamate decarboxylase 65 autoantibodies (GADA) determined in 495 UK schoolchildren (median age 11 years, range 9–13 years) and 520 adult blood donors (median 43 years, range 17–69 years).³ In addition, there was no clear differences found when comparing two different constructs across two different assay systems, radiobinding assay (RBA) and luciferase immunoprecipitation system (LIPS). Subsequently, IAA were tested in these adult blood donors, where sera were available. Measured by RBA, 12 (2.3%) of 517 were above the 97.5th percentile threshold established

in schoolchildren with no evidence that a different threshold was required in adults (Fisher's exact, $p=0.809$), and given the GADA data the threshold is unlikely to differ for LIPS. The IAA threshold is therefore not expected to influence the proportion of 'discordant' older progressors. We note that recent analysis by Templeman *et al.* 2025 of the TrialNet cohort, which compared diabetes development by age, did not use age-specific positivity thresholds.⁴

We thank the authors for their commentary regarding continuous variable modelling. While both methodologies suggested are useful when evaluating continuous risk gradients, our study was designed as a method-comparison study to assess the utility and risk stratification performance of the two assays. The positivity thresholds in our study were chosen a priori, based on the 97.5th percentile of the healthy control group, avoiding post hoc cut-off optimisation or multiple testing concerns. The two variables highlighted, age and IAA titre, were derived from the distribution of the data, without reference to the outcome. Categorisation aided description and interpretation of the method comparison. Age (at 24 years) strongly mirrored the innate bi-model parent/sibling structure of the study but was modelled and presented as a continuous variable in table 3 and figure 3 of our original report. Similarly, categorisation of IAA titre at the median positive level was only included to estimate whether level could explain the apparent benefit of testing IAA by LIPS in addition to RBA. We agree establishing what level of IAA is clinically meaningful would require a more nuanced analysis, but this lay outside the scope of the method comparison.

IAA affinity data were not included in time-to-event analysis because it was missing (as stated in the original manuscript). However, the affinity analysis was intended to provide mechanistic insight into the reasons for the differences between methods. It was not evaluated as a primary outcome where missingness could alter the survival curves. The assumptions of proportional hazard models

were satisfied when Schoenfeld residuals were considered. With regards to multiplicity, the subgroups examined were pre-planned, biologically based stratifications grounded in established type 1 diabetes risk factors.⁵ All key *p*-values showed sufficiently strong evidence of differences between groups, such that Bonferroni or FDR corrections would not alter our conclusions; the exception is the subgroup with multiple islet autoantibodies excluding IAA (*p* = 0.0132). These are the highest 'risk' group where stratification by IAA would be expected to have least clinical impact, except in the very young (~under 5 years old). Recent analysis of differences between methods using samples from the TrialNet natural history study did not correct for multiple testing but presented 95% CI (as we have done).⁶

Additional adjustments may alter the exact figures reported and their generalisability, but the conclusions regarding the potential benefits of the LIPS methodology remain sound. The LIPS method remains a high-performing choice for islet autoantibody analysis,⁷ with particular value where low-volume, highly sensitive methods are critical.⁶

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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