# Islet Autoantibody Type-Specific Titer Thresholds Improve Stratification of Risk of Progression to Type 1 Diabetes in Children

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## OBJECTIVE

**To use islet autoantibody titers to improve the estimation of future type 1 diabetes risk in children.**

## RESEARCH DESIGN AND METHODS

**Prospective cohort studies in Finland, Germany, Sweden, and the U.S. followed 24,662 children at increased genetic or familial risk to develop islet autoimmunity and diabetes. For 1,604 children with confirmed positivity, titers of autoantibodies against insulin (IAA), GAD antibodies (GADA), and insulinoma-associated antigen 2 (IA-2A) were harmonized for diabetes risk analyses.**

## RESULTS

**Survival analysis from time of confirmed positivity revealed markedly different 5-year diabetes risks associated with IAA ($n = 909$), GADA ($n = 1076$), and IA-2A ($n = 714$), when stratified by quartiles of titer, ranging from 19% (GADA 1st quartile) to 60% (IA-2A 4th quartile). The minimum titer associated with a maximum difference in 5-year risk differed for each autoantibody, corresponding to the 58.6th, 52.4th, and 10.2nd percentile of children specifically positive for each of IAA, GADA, and IA-2A, respectively. Using these autoantibody type-specific titer thresholds in the 1,481 children with all autoantibodies tested, the 5-year risk conferred by single ($n = 954$) and multiple ($n = 527$) autoantibodies could be stratified from 6 to 75% ($P < 0.0001$). The thresholds effectively identified children with a ≥50% 5-year risk when considering age-specific autoantibody screening (57–65% positive predictive value and 56–74% sensitivity for ages 1–5 years). Multivariable analysis confirmed the significance of associations between the three autoantibody titers and diabetes risk, informing a childhood risk surveillance strategy.**

## CONCLUSIONS

**This study defined islet autoantibody type-specific titer thresholds that significantly improved type 1 diabetes risk stratification in children.**


The development of islet autoantibodies is known to precede onset of clinical type 1 diabetes (diabetes). However, the time interval from initial seroconversion to the

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onset of clinical symptoms varies among individuals from weeks to many years, creating uncertainty in how to monitor for metabolic instability as well as when to potentially intervene with immunotherapy. The age at seroconversion and the number and combination of specific islet autoantibodies present are known to be associated with the duration of progression from seroconversion to onset of diabetes (1). However, the association between islet autoantibody titers and progression to diabetes is less well understood, and the results of prior studies are partly inconsistent. One of the earliest such studies showed an association between higher peak titer of islet cell antibodies (ICA) and higher risk of developing diabetes (2). In more recent studies, associations between higher titers of autoantibodies to insulin (IAA) and insulinoma-associated antigen 2 (IA-2A) have been observed to be associated with faster progression to diabetes (3–8). However, one study found these associations for IAA but not for IA-2A (9), and another reported that lower initial IAA titers predicted slower progression to diabetes (10). Yet another recent study estimated that the association between autoantibody titers and progression to diabetes was time-constant for IA-2A but decreased over time for IAA (11). For autoantibody titers of GAD antibodies (GADA), the results have also been mixed. Some studies have found no significant association between the risk of developing diabetes and GADA titers (3,8,9). Notably, one study reported that higher initial GADA titers were associated with more rapid progression to diabetes (6); another found an association between GADA titers and progression to diabetes that decreased over time (11). Despite the varying results, all evidence seems to suggest that antibody titers may be informative for stratification of diabetes risk in islet autoantibody-positive individuals. Although other autoantibody characteristics, such as IgG subclass, epitope specificity, and binding affinity may also be useful in stratifying diabetes risk, they require additional testing (12). Autoantibody titer is a simple marker and only needs one quantitative assay. However, titer measurements are not standardized between most of the currently used islet autoantibody assays; harmonization of quantitative results is thus required.

In this study, we assessed and quantified the association between the titers of different islet autoantibodies (IAA, GADA, IA-2A) and the risk of progression to diabetes by using harmonized titer values from our large prospective T1DI (Type 1 Diabetes Intelligence) study cohort (13). We focused our analysis on the time point of seroconversion; more specifically, the time at which newly detected islet autoantibody positivity was confirmed in a second consecutive sample. Our goal was to leverage islet autoantibody titers to refine diabetes risk stratification for children who developed confirmed-positive islet autoantibodies.

## RESEARCH DESIGN AND METHODS

### Study Population

Prospective cohort studies in Finland (Diabetes Prediction and Prevention [DIPP] [14]), Germany (BABYDIAB [15]), Sweden (Swedish Diabetes Prediction in Skåne [DiPiS] [16]), and the U.S. (Diabetes Autoimmunity Study in the Young [DAISY] [17] and Diabetes Evaluation in Washington [DEW-IT] [18]) have followed 24,662 children at increased genetic and familial risk for development of islet autoantibodies and diabetes from close to birth for a period of 15 years or until their diagnosis. Data from these studies were combined and harmonized in the T1DI study cohort (13). Only those children with confirmed positivity to IAA, GADA, or IA-2A and with autoantibody titer measurements before diagnosis of diabetes or the end of study follow-up period were selected for analysis (Supplementary Fig. 1). This cohort (study cohort 1) had 1,604 children, of whom 600 (37.4%) developed diabetes (Supplementary Table 1). There were a total of 32,660 visits, with a mean time interval between successive visits of 0.53 (SD 0.71) years. A more constrained second cohort, consisting of children with complete autoantibody titer measurements for all three autoantibodies, in the first overall islet autoantibody-positive and in the second consecutive positive serum sample, were selected for additional analysis (Supplementary Fig. 1). This second cohort (study cohort 2) had 1,481 children, of whom 570 (38.5%) developed diabetes (Supplementary Table 2). All T1DI constituent studies were approved by their respective ethics review boards.

### Islet Autoantibody Measurements

The methods used by each study to measure IAA, GADA, and IA-2A have been previously described and are summarized in the supplement of Anand et al. (13). Each of the studies and their laboratories have participated—with satisfactory results—in both the Diabetes Autoantibody Standardization program (DASP) (19–21) and the Islet Autoantibody Standardization Program (22) proficiency workshops. Because in the T1DI study cohort titer values for the same autoantibody may originate from different assays with different units, they are not directly comparable. Therefore, as described in Supplementary Section S1 and Supplementary Fig. 2, all autoantibody titer measurements for IAA, GADA, and IA-2A were converted to multiples of the upper limit of normal (mULN) to facilitate comparisons and combined for analysis.

Autoantibodies to zinc transporter 8 (ZnT8A) were not consistently measured across all constituent T1DI studies. Several of the studies only measured ZnT8A if the child tested positive for one or more of the other three autoantibodies or had developed diabetes; as a result, ZnT8A was not included in our analyses.

Confirmed autoantibody positivity was defined as a positive test result (for the same autoantibody type) in at least two consecutive samples, regardless of the time interval between the visits. The first and second of these two consecutive visits will be hereby referred to as the initial visit and confirmatory visit, respectively (Supplementary Fig. 3). The mean (SD) time intervals, in years, between the initial and confirmatory visits were 0.4 (0.5), 0.5 (0.5), and 0.4 (0.7) for IAA, GADA, and IA-2A, respectively. The mean (SD) age, in years, at the confirmatory visit were 5.4 (4.1), 6.1 (4.1), and 5.7 (3.9) for positivity to IAA, GADA, and IA-2A, respectively (Supplementary Table 1).

In our analyses, we focused on the autoantibody data from the confirmatory visit (when autoantibody positivity was first confirmed) rather than the initial visit (when autoantibody positivity was first detected) because we assumed that the autoantibody response would be more robust and mature in confirmatory testing and would better reflect

the situation in practice in a screening scenario.

### Outcome Definition

Type 1 diabetes diagnosis was based on the World Health Organization and American Diabetes Association criteria (23).The main outcome of interest was the time to diabetes from the time of the confirmatory visit for positivity to IAA, GADA, or IA-2A. Specifically, the outcome was defined as the time, in years, from the confirmatory visit for one of the three specific autoantibodies, to the time of diagnosis of diabetes for events, or the time of last follow-up (censoring time) for nonevents.

### Statistical Analyses

Five different analyses were performed, each focused on addressing a different question.

1. How well can islet autoantibody titers stratify diabetes risk? Children in study cohort 1 were stratified based on autoantibody-specific titer quartiles from their confirmatory visit for autoantibody positivity. Time-to-event analysis was then used to examine whether progression from the confirmatory visit to clinical diabetes was associated with the autoantibody titer. Kaplan-Meier (KM) estimates with 95% CIs were used to estimate diabetes risk from the confirmatory visit. Log-rank tests were used to establish statistical differences between the strata in the KM analysis.
2. What islet autoantibody type-specific titer threshold maximizes 5-year diabetes risk stratification? Study cohort 1 was used to identify the lowest autoantibody type-specific titer threshold at the confirmatory visit that maximized the difference in 5-year diabetes risk between two groups (i.e., those with titers at or above the threshold vs. those with titers below the threshold). To do this, all possible threshold values between 1.0 and T (where T is the titer value corresponding to the 75th percentile of the respective autoantibody-positive cohort) were considered separately for IAA, GADA, and IA-2A. For each threshold value, the cohort was partitioned into two groups, as described above, and KM analysis was performed to estimate diabetes risk for both groups from the confirmatory visit. Next, the 5-year diabetes risk for each group was extracted, and the difference between them computed. Finally, the lowest titer threshold value resulting in the maximum risk difference was selected.
3. How well can the autoantibody type-specific titer thresholds stratify diabetes risk for single and multiple islet autoantibody-positive children? The autoantibody type-specific titer thresholds were then used in a KM survival analysis using study cohort 2 to stratify diabetes risk for children with single and multiple islet autoantibody-positive status. Information from the earliest confirmatory visit in the child's history was used to determine the single and multiple islet autoantibody status. Single autoantibody-positive children were stratified into two groups: those with titers at or above the autoantibody type-specific threshold and those with titers below the threshold. Multiple autoantibody-positive children were stratified into four groups: those with zero, one, two, or three titers at or above the autoantibody type-specific thresholds. Log-rank tests were used to establish statistical differences between the strata.
4. How significant are the associations between autoantibody titers and diabetes? Next, a series of multivariable analyses were performed using cohort 2 to quantify the significance of autoantibody titers from the earliest confirmed autoantibody positivity. Hazard ratios (HRs) and corresponding 95% CIs of association between titers and diabetes were estimated, using Cox proportional hazards regression. An initial model analyzed the association between autoantibody positivity (present or absent) at the earliest confirmatory visit and diabetes risk. The model was adjusted for HLA risk group, sex, and age at the earliest confirmatory visit and stratified by study site. As described in the supplement of Anand et al. (13), genotypes from individual studies were harmonized into four HLA risk groups (A, B, C, and D), ordered by decreasing risk. Autoantibody positivity indicators for IAA, GADA, and IA-2A, at the earliest confirmatory visit were the primary predictors. A second model analyzed the association between autoantibody titers and diabetes risk. In this model, the log-transformed autoantibody titers (log mULN) for IAA, GADA, and IA-2A, at the earliest confirmatory visit, were added to the initial model as the primary predictors. The proportional hazards assumption was tested using the Schoenfeld test (24). For covariates that did not satisfy the proportional hazards assumption at the 0.05 significance level, time-varying coefficients were used with time modeled linearly (25,26). *P* values corrected for multiple comparisons using the Benjamini-Hochberg method were reported (27).
5. How well can the autoantibody type-specific titer thresholds identify children with high diabetes risk based on autoantibody screening at different ages? Finally, an application of the autoantibody type-specific titer thresholds to identify children at high risk of developing diabetes in the next 5 years was explored for potential clinical trial recruitment. Specifically, the ability to use these titer thresholds to stratify diabetes risk (based on results from autoantibody screening at different age ranges) was assessed. The following age ranges were explored: 1–2.0, 2–3.0, 3–4.0, 4–5.0, 5–10.0, and $\geq$10 years. For each age range, children in study cohort 1 with at least one autoantibody titer measurement, and without already being diagnosed with diabetes, were included for analysis. These children were stratified based only on the observed autoantibody titers in that age range (when multiple measurements of the same autoantibody were available for a child within the age range, the earliest one was used). Each child was placed into 1 of 12 possible strata defined by single or multiple autoantibody positivity and the combination of IAA, GADA, and IA-2A titers at or above the autoantibody type-specific threshold. KM survival analysis was then performed to estimate the risk of diabetes from the time of the autoantibody measurement, for each age range and each stratum. Children belonging to strata that have an estimated 5-year diabetes risk $\geq$50% were labeled as

"high-risk." Prediction performance was measured using inverse probability of censoring weighted (28,29) positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity.

Analyses were performed using Python (scikit-learn, scikit-survival) and R (survival, survminer) software (30,31).

## RESULTS

### Stratification of Diabetes Risk by Islet Autoantibody Titer Quartiles

Survival analysis from time of confirmed positivity revealed markedly different 5-year diabetes risks associated with IAA ($n = 909$), GADA ($n = 1076$), or IA-2A ($n = 714$) when stratified by quartiles of titer, ranging from 19% (GADA 1st quartile) to 60% (IA-2A 4th quartile) (Supplementary Fig. 4). Histogram distributions and quartile thresholds of autoantibody titers at the confirmatory visit for positivity to IAA, GADA, and IA-2A are shown in Supplementary Fig. 5.

### Determination of Autoantibody Type-Specific Titer Thresholds That Maximize 5-Year Diabetes Risk Discrimination

The results of this analysis for IAA, GADA, and IA-2A are shown in Fig. 1 *A*, *B*, and *C*, respectively. The minimum titer value associated with a maximum difference in 5-year diabetes risk differed for each autoantibody type: $T_{IAA} = 3.6$ mULN, $T_{GADA} = 5.4$ mULN, and $T_{IA\text{-}2A} = 2.5$ mULN. These titer threshold levels corresponded to 58.6th, 52.4th, and 10.2nd percentile of children positive for IAA, GADA, and IA-2A, respectively.

These thresholds were used to stratify children positive to each autoantibody type into two groups: those with titers at or above threshold and those with titers below threshold. This stratification resulted in significantly different 5-year diabetes risks for all three autoantibody types (all $P < 0.0001$) (Supplementary Fig. 6).

### Improved Stratification of Diabetes Risk by Autoantibody Type-Specific Titer Thresholds

Stratifying single and multiple islet autoantibody-positive children (determined at time of the earliest confirmatory visit) using the autoantibody type-specific thresholds resulted in significantly different 5-year diabetes risks. For single autoantibody-positive children ($n = 954$), those with antibody titers at or above the autoantibody type-specific threshold ($n = 364$) had a 5-year diabetes risk of 21.9% (95% CI 17.0–26.4) compared with 6.1% (3.9–8.3) for those with titers below the threshold ($n = 590$) (Fig. 2*A*). For multiple autoantibody-positive children ($n = 527$), those with zero ($n = 49$), one ($n = 202$), two ($n = 216$), and three ($n = 60$) antibody titers at or above the autoantibody type-specific thresholds had a 5-year diabetes risk of 24.7% (95% CI, 10.1–36.9), 41.2% (33.7–47.9), 55.7% (48.2–62.1), and 75.1% (61.0–84.1), respectively (Fig. 2*B*). The corresponding follow-up times from the earliest confirmatory visit to 50% cumulative progression to diabetes were 8.5 years (95% CI, 7.1–15.0), 5.8 years (5.3–6.8), 4.0 years (3.3–5.1), and 2.3 years (1.6–3.3), respectively. A total of 276 children had a ≥50% risk of developing diabetes within 4 years of the first confirmed autoantibody positivity.

### Association Between Islet Autoantibody Titer and Diabetes Risk in Multivariable Analysis

The multivariable regression models that analyzed the association between autoantibodies at the earliest confirmatory visit and diabetes risk are shown in Supplementary Table 3. Model 1 used the autoantibody positivity indicators as predictors. Time-dependent covariates were used for both GADA and IA-2A

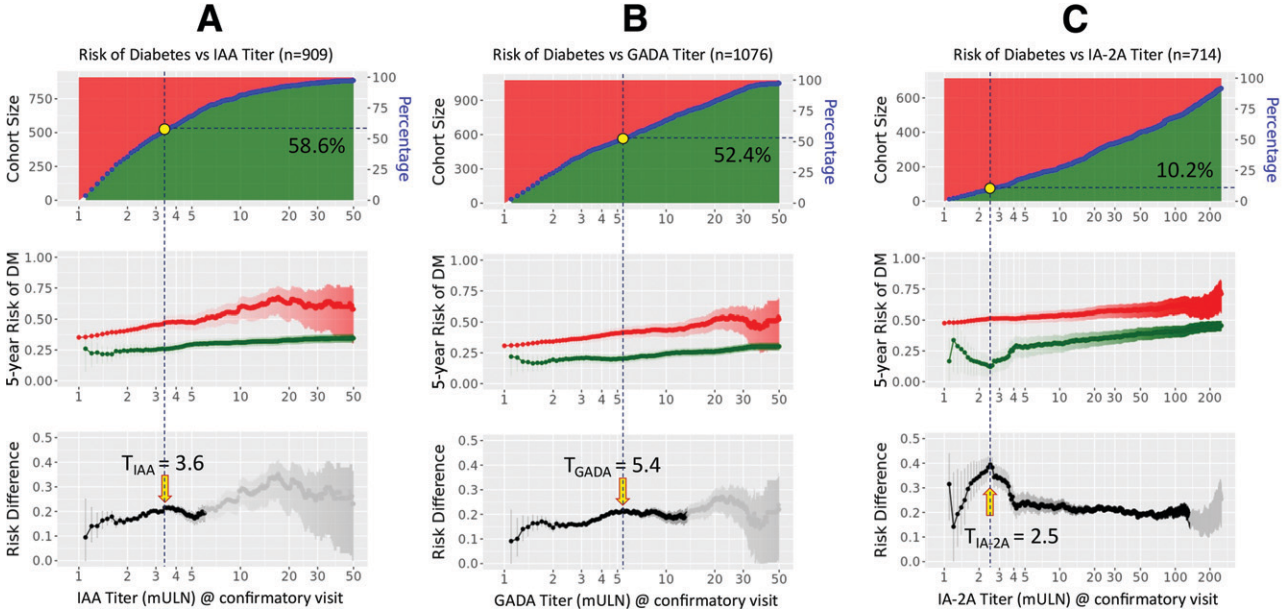


**Figure 1**—Identifying autoantibody type-specific titer thresholds for IAA (*A*), GADA (*B*), and IA-2A (*C*). Top panel: The size of the red cohort ($t \geq T$) and the green cohort ($t < T$) for each autoantibody titer threshold level. Middle panel: The 5-year risk of diabetes (diabetes mellitus [DM]) and 95% CIs from the time of the confirmatory visit for autoantibody positivity for the red and green cohorts for each titer threshold level. Bottom panel: The difference in the 5-year diabetes risk between the red and green cohorts for each titer threshold level. An arrow marks the lowest titer threshold level where there is a maximum risk difference between the cohorts and the threshold covers up to 75% of the cohort ($T_{IAA} = 3.6$ mULN, $T_{GADA} = 5.4$ mULN, and $T_{IA\text{-}2A} = 2.5$ mULN). The percentile of children who tested positive for the respective autoantibody corresponding to the final titer threshold is highlighted in the top panel ($T_{IAA} \rightarrow 58.6\%$, $T_{GADA} \rightarrow 52.4\%$, and $T_{IA\text{-}2A} \rightarrow 10.2\%$).

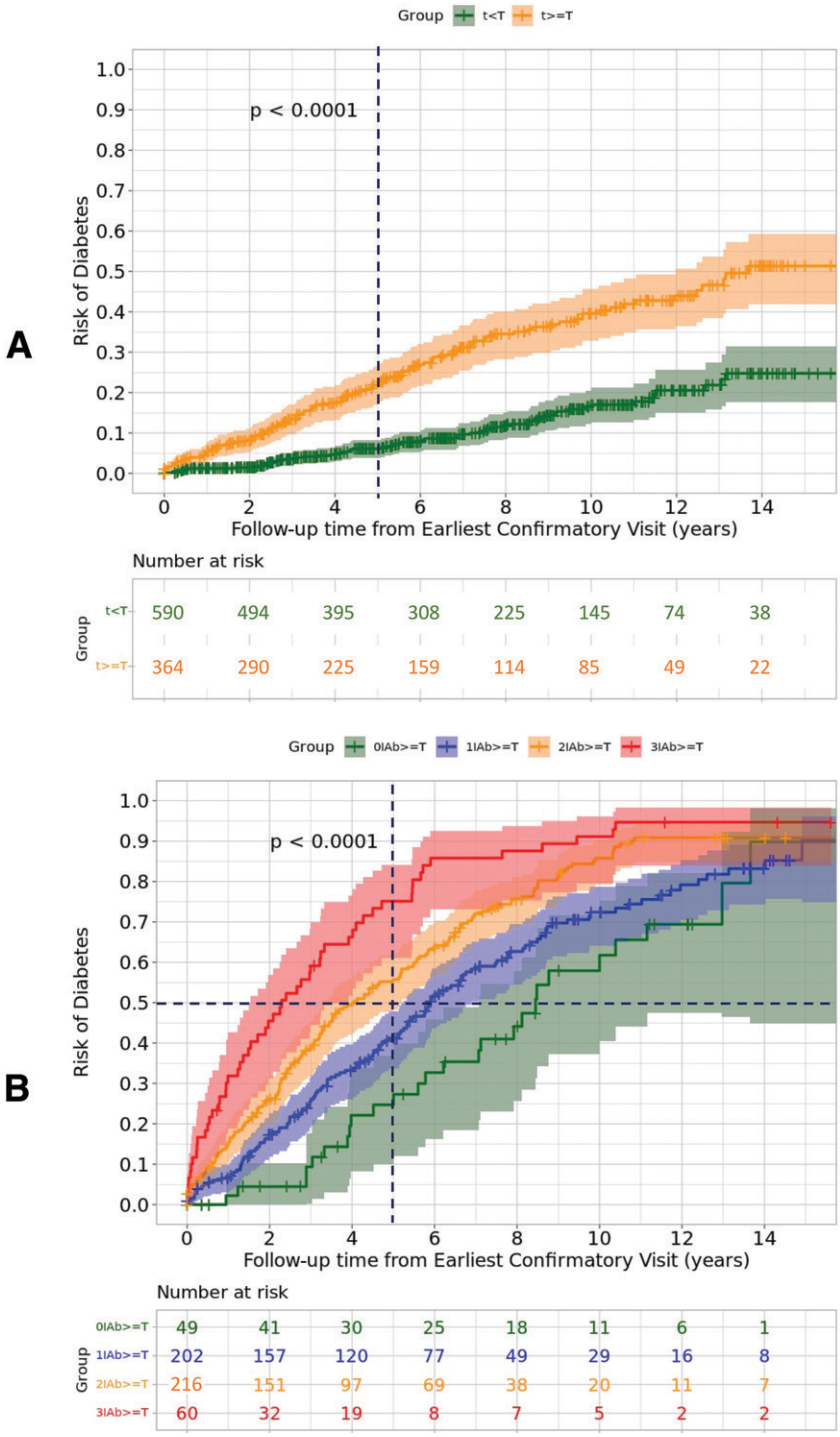


**Figure 2**—Progression to diabetes from the time of the earliest confirmatory visit in children with single and multiple autoantibody positivity. Stratification is based on the autoantibody titer measured at the earliest confirmatory visit and the identified autoantibody type-specific titer thresholds ($T_{IAA}$ = 3.6 mULN, $T_{GADA}$ = 5.4 mULN, $T_{IA\text{-}2A}$ = 2.5 mULN). *A*: Single autoantibody-positive children are partitioned into two groups: those with autoantibody titer below threshold (t < T) and those with titer at or above threshold (t ≥ T). *B*: Multiple autoantibody-positive children are partitioned into four mutually exclusive groups: those with no autoantibody titer at or above threshold (0IAb ≥ T), those with one autoantibody titer at or above threshold (1IAb ≥ T), those with two autoantibody titers at or above threshold (2IAb ≥ T), and those with all three autoantibody titers at or above threshold (3IAb ≥ T). The dashed vertical line marks the 5-year follow-up time point.

positivity since they did not satisfy the proportional hazards assumption. At the earliest confirmatory visit, the adjusted HR for GADA positivity was significant (HR 1.43; 95% CI 1.05–1.95; $P = 0.02$) and increased over time (HR 1.06 per year; 95% CI 1.01–1.12; $P = 0.02$). The adjusted HR for IA-2A positivity was significant (HR 3.93; 95% CI 2.95–5.23; $P < 0.0001$) but decreased over time (HR 0.95 per year; 95% CI 0.90–0.99; $P = 0.02$). The adjusted HR for IAA positivity was also significant (HR 2.10; 95% CI 1.74–2.55; $P < 0.0001$). Age at the earliest confirmatory visit and HLA risk group were also significant. Model 2 added the corresponding autoantibody titers as predictors. Note that all three of the autoantibody positivity indicators were no longer significant once the autoantibody titers were added. Time dependent covariates were used for IAA titer, since it was the only significant covariate that did not satisfy the proportional hazards assumption. At the earliest confirmatory visit, the adjusted HR for IAA titer was significant (HR 1.37; 95% CI 1.24–1.51; $P < 0.001$) and decreased over time (HR 0.98 per year; 95% CI 0.97–1.0; $P = 0.01$). The adjusted HR for GADA titer (HR 1.18; 95% CI 1.11–1.25; $P < 0.001$) and IA-2A titer (HR 1.17; 95% CI 1.10–1.24; $P < 0.001$) were also significant. Age at the earliest confirmatory visit and HLA risk group remained significant. Finally, model 2 showed higher concordance (SE) than model 1: 0.78 (0.01) vs. 0.75 (0.01).

### Effectiveness of Identifying Islet Autoantibody-Positive Children at High Risk for Diabetes at Different Ages by Using Autoantibody Type-Specific Titer Thresholds

The 12 strata, resulting from all possible groupings of single or multiple autoantibody positivity and the combinations of IAA, GADA, IA-2A titers above threshold, and their estimated 5-year diabetes risks are shown in Fig. 3 for each age range (the underlying KM analyses are shown in Supplementary Fig. 7). Strata that have a 5-year diabetes risk ≥50% are considered high-risk and are shaded in red. Supplementary Table 4 lists, for each age range, the individual high-risk strata, the "composite high-risk criteria" defined by forming a union of the individual high-risk strata, the total number

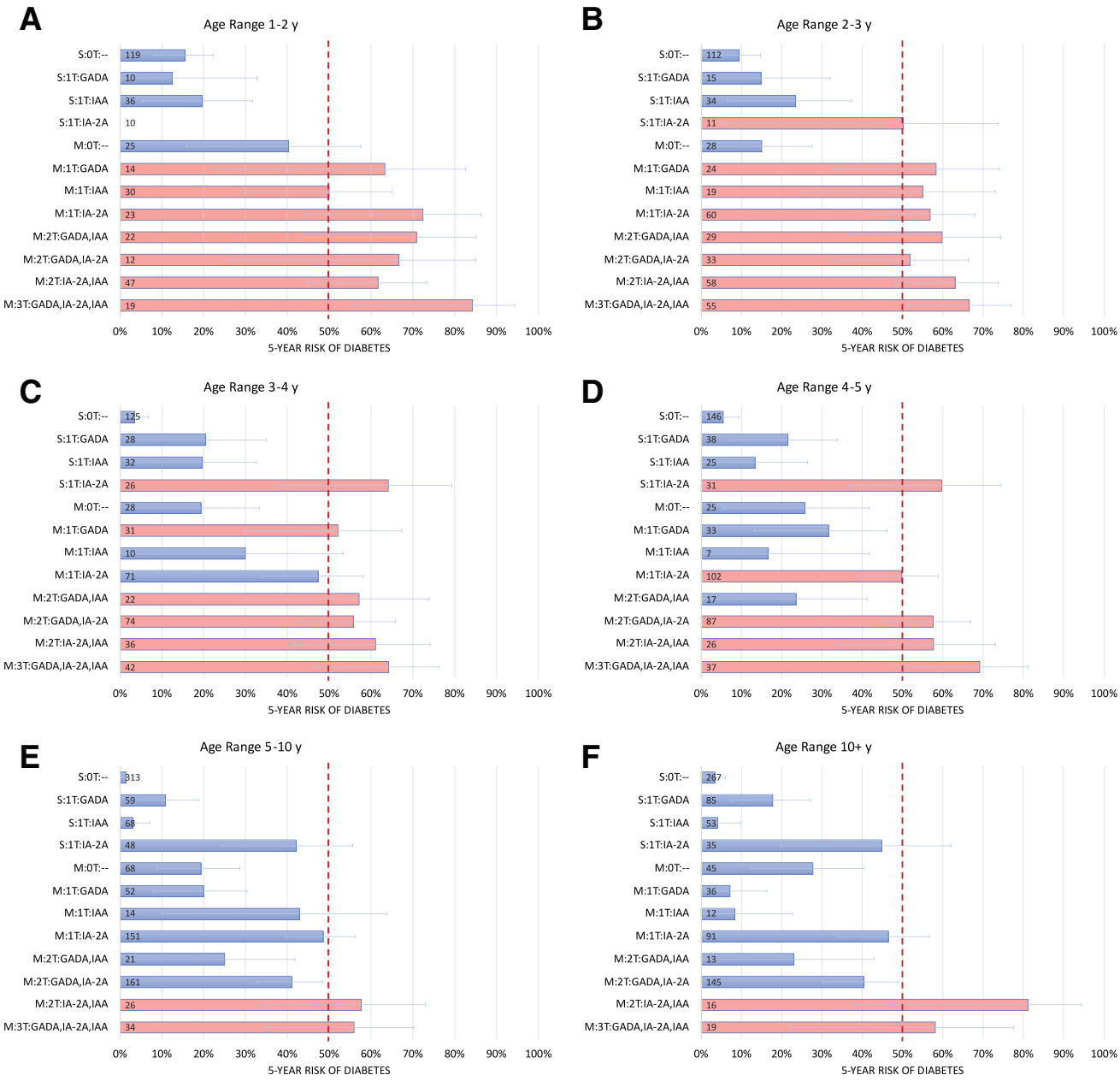


**Figure 3**—The 5-year risk of type 1 diabetes and 95% CIs in subjects who developed confirmed-positive islet autoantibodies, stratified by single or multiple autoantibody positivity and the combination of IAA, GADA, and IA-2A titers above thresholds ($T_{IAA}$ = 3.6 mULN, $T_{GADA}$ = 5.4, mULN, and $T_{IA\text{-}2A}$ = 2.5 mULN) for screening at different age ranges: 1–2.0 years (*A*), 2–3.0 years (*B*), 3–4.0 years (*C*), 4–5.0 years (*D*), 5–10.0 years (*E*), and 10+ y (*F*). The 12 strata are: S:0T:– = single positive, no autoantibodies above titer threshold; S:1T:GADA = single positive, one (GADA) above titer threshold; S:1T:IAA = single positive, one (IAA) above titer threshold; S:1T:IA-2A = single positive, one (IA-2A) above titer threshold; M:0T:– = multiple positive, no autoantibodies above titer threshold; M:1T:GADA = multiple positive, one (GADA) above titer threshold; M:1T:IAA = multiple positive, one (IAA) above titer threshold; M:1T:IA-2A = multiple positive, one (IA-2A) above titer threshold; M:2T:GADA,IAA = multiple positive, two (GADA, IAA) above titer threshold; M:2T:GADA,IA-2A = multiple positive, two (GADA, IA-2A) above titer threshold; M:2T:IA-2A,IAA = multiple positive, two (IA-2A, IAA) above titer threshold; M:3T:GADA,IA-2A,IAA = multiple positive, all three above titer threshold. The number of subjects in each stratum is shown at the base of each bar. The dashed vertical red lines mark the 50% 5-year risk of diabetes level. Strata that exceed that risk level are classified as "high-risk" and shaded red.

of children, the number that progressed to diabetes within 5 years, the number of high-risk children identified using the composite high-risk criteria, and the associated PPV, NPV, sensitivity, and specificity performance metrics. There were 167, 289, 231, 283, 60, and 35 high-risk children identified for the age ranges 1–2.0, 2–3.0, 3–4.0, 4–5.0, 5–10.0, and ≥10 years, respectively. The PPV was consistent across the age groups, ranging from 55 to 65%. Sensitivity ranged from 56% and 74% between ages 1 and 5 years but dropped significantly to 12% and 14% for ages 5–10 and ≥10 years, respectively. As the age of the child being screened increased, not only were more

stringent autoantibody criteria needed to identify those with high diabetes risk, but it also became more difficult to reliably identify them. A summary of the process to identify high diabetes risk children is illustrated in the decision flowchart in Fig. 4.

## CONCLUSIONS

This study showed that islet autoantibody titers can stratify risk of progression to diabetes in children beyond information about the number and type of islet autoantibodies present. Furthermore, these titers matter in different ways for different autoantibodies, and customized islet autoantibody type-specific titer thresholds could be defined that maximized discrimination of the 5-year diabetes risk. The combination of these titer thresholds effectively identified among islet autoantibody-positive children those with a 5-year risk of diabetes of ≥50% who could be potential candidates for participation in intervention trials.

The study used data from a large cohort of children prospectively followed in five different birth cohorts, harmonized autoantibody titers across these five studies, and combined them for analysis. Stratification of diabetes risk based on islet autoantibody titer quartiles showed for each of IAA, GADA, and IA-2A that higher titers were associated with higher diabetes risk, complementing findings from prior studies (1,3–8). Islet autoantibodies with high titers usually involve multiple IgG subclasses and are directed against multiple epitopes on the target antigen, likely reflecting a more intense and prolonged autoimmune response and associated with the progression of diabetes development (12). The current analysis also revealed that different autoantibody types exhibited different patterns (Supplementary Fig. 4). On the basis of the 5-year diabetes risk, there was no significant separation between neighboring quartiles for IAA, indicating a relatively smooth risk distribution as a function of titer. For GADA, the only significant separation was between the second and third quartiles, indicating a bimodal risk distribution with a gap around the median. For IA-2A, there was only separation between the first and second quartiles, indicating a bimodal risk distribution with a gap around the first quartile. Plots of the cohort percentile as a function of titer threshold at confirmed positivity (Fig. 1, top panel) also revealed different distributional behaviors. For IAA, the concave-shaped plot indicated that there were more IAA-positive children with lower IAA titers. The linear-shaped GADA plot indicated an even GADA titer distribution. For IA-2A, the convex-shaped plot indicated that IA-2A–positive children tended to have higher IA-2A titers.

To identify islet autoantibody type-specific titer thresholds, we used a novel analytical approach that has potential advantages. Specifically, our method was a data-driven approach to automatically identify thresholds that maximize a given outcome. It does this by scanning over the possible threshold values (e.g., increasing autoantibody titers), splitting the cohort based on each threshold value, performing survival analyses on the two resulting groups, and computing the outcome. Using the difference in the 5-year risk of diabetes as an illustrative outcome of interest, we identified the islet autoantibody type-specific titer thresholds. Furthermore, translation of the islet autoantibody type-specific titer thresholds into percentiles of autoantibody-positive children is important

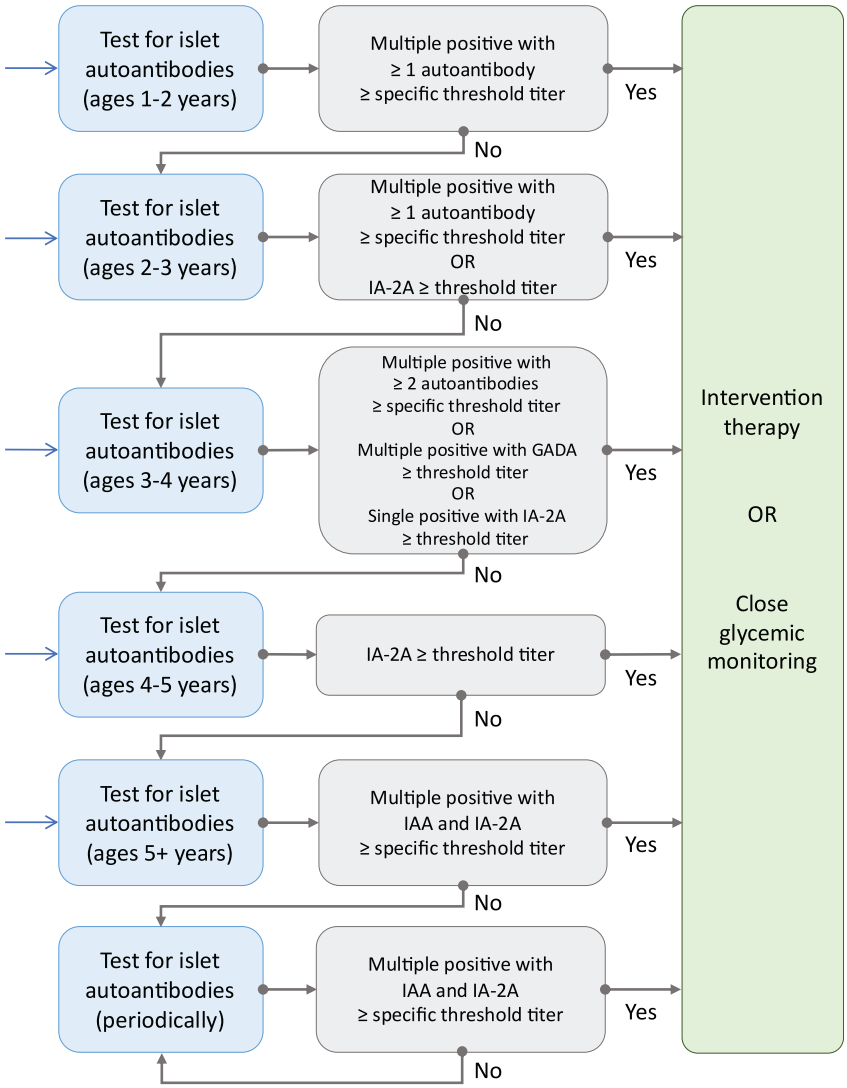


**Figure 4**—A proposed flowchart to discover islet autoantibody-positive children and then evaluate their antibody titer to identify those at high risk (≥50%) of developing type 1 diabetes within 5 years. A child can enter the flowchart by autoantibody testing at any age via a blue arrow and appropriate blue box. Those with antibodies fulfilling the titer criteria shown in the corresponding gray box are at high risk and could be considered for intervention therapy trials or close glycemic monitoring. Islet autoantibodies tested include IAA, GADA, and IA-2A.

because it allows the thresholds to be applied to external data sets that may have different assay characteristics and normalization methods. The appropriate threshold will depend on the application; notably, the method developed to identify the thresholds is flexible and generalizable. It can be easily reconfigured to accommodate different outcomes, and it may even be adapted for use with other quantified biomarkers such as plasma glucose or glycated hemoglobin. To demonstrate this, we selected a different outcome (e.g., difference in 3-year diabetes risk) and reran the analysis. A different set of autoantibody type-specific thresholds were identified that maximized the stratification of 3-year diabetes risk (Supplementary Fig. 8) and selected smaller groups of children with higher risk of fast progression to diabetes (Supplementary Figs. 9 and 10). It should be noted, however, that because the thresholds were determined based on our study cohort, there may be some uncertainty when extrapolating to other data sets, and they may not be as predictive when applied to other cohorts.

The multivariable regression analysis found that the autoantibody positivity indicators for IAA, GADA, and IA-2A at the earliest confirmatory visit were all significantly associated with diabetes risk. However, when the corresponding autoantibody titers were added, these indicator variables were no longer significant. Instead, all three of the titer variables became significant, indicating that the titers contain more information than the indicators. The HR was time-constant for GADA and IA-2A but decreased over time for IAA. Prior work has found associations between titers and progression to diabetes that were time-constant for IA-2A but decreased over time for GADA and IAA (11).

The age-based autoantibody screening simulation analysis was able to identify children with a high risk of developing diabetes by using autoantibody positivity and the islet autoantibody type-specific titer thresholds. Of note, the presence of IA-2A above titer threshold alone was sufficient to identify high diabetes risk in children aged 2–5 years, even in the absence of IAA or GADA. It is known that IA-2A usually occurs together with autoantibodies against other β-cell antigens and is therefore highly specific and predictive for progression to clinical diabetes (3,32).

Overall, the results of this study may contribute to improved risk counseling for families of affected children and improved screening for participants for intervention therapy trials aimed at preventing or delaying progression to clinical disease. Since titers add value beyond autoantibody type and number, islet autoantibody standardization programs (e.g., Islet Autoantibody Standardization Program) should continue to focus on improving titer standardization, to facilitate quantitative comparisons across assays and study sites.

This study has some limitations. First, the autoantibody titers were measured using different assays across the study sites. Although the titers were harmonized, some residual biases may remain. In addition, the current data are based on radio binding assay results. Islet autoantibody type-specific titer thresholds and respective percentiles of positives may need to be adjusted for other assay formats, such as those based on electrochemiluminescence (33), the luciferase immunoprecipitation system (34), or agglutination-PCR (35) technology.

Second, due to differences in the visit intervals of the study protocols, it is possible that the actual time of the earliest autoantibody positivity was missed, with the consequence that the measured time is biased. Off-schedule visits may also impact the timing of the confirmatory visit.

Third, only children with increased genetic and familial risk for development of islet autoimmunity and diabetes were enrolled into the studies, and the study populations were predominantly Caucasian, which may limit generalizability of the results.

Fourth, the analyses have not been validated on an independent cohort.

There are several possible directions for future work. First, the analyses should be replicated in higher time resolution datasets with more frequent prospective follow-up (e.g., The Environmental Determinants of Diabetes in the Young [TEDDY] [36]).

Second, validation in independent cohorts with broader population inclusion criteria (e.g., Fr1da (37) or Autoimmunity Screening for Kids [ASK] studies [38]) should be undertaken.

Third, the age-based risk stratification performance should be validated in a cross-sectional study.

Fourth, the utility of islet autoantibody titers as a continuous variable should be further explored in diabetes risk prediction (39) and disease progression modeling (40).

In summary, this study harmonized islet autoantibody titers across multiple birth cohorts, combined them for analysis, and defined autoantibody type-specific titer thresholds that significantly improved type 1 diabetes risk stratification in children.

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