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A Longitudinal Assessment of Diabetes Autoantibodies in the SEARCH for Diabetes in Youth study

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Abstract

Objective: To assess changes in diabetes autoantibodies (DAs) over time in children and young adults with diabetes and determine whether observed changes were associated with demographic characteristics, clinical parameters and diabetes complications.

Research design and methods: Participants had DAs measured at baseline (10.3 ± 7.1 months after diabetes diagnosis) and at 12, 24 months and ≥ 5 years after the baseline measurement. At the ≥ 5-year follow-up, presence of diabetes complications was assessed. We

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Author contributions:

L.M. and C.P. conceptualized the study. C.S. analyzed the data. C.P. and R. D. provided oversight for study analyses. L.M. prepared the manuscript. All authors reviewed and edited the manuscript and contributed to discussion. All authors have read and approved the final manuscript.

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The authors have no conflicts of interest to disclose.

Ethics approval statement and patient consent:

The study was conducted in accordance with principles of Good Clinical Practice. The study was approved by the appropriate regulatory agencies and institutional review boards/ethics committees. The parents, youth or young adult, or both provided consent or assent.

examined the associations between change in number of positive DAs and changes in individual DA status with the participants' characteristics and clinical parameters over time.

Results: Out of 4179 participants, 62% had longitudinal DA data and 51% had complications and longitudinal DA data. In participants with ≥ 1 baseline positive DA (n=1699), 83.4% remained positive after 7.3±2.3 years duration of diabetes. Decrease in number of positive DAs was associated with longer diabetes duration (p=0.003 for 1 baseline positive DA; p<0.001 for 2 baseline positive DAs) and younger age at diagnosis (p<0.001 for 2 baseline positive DAs). No associations were found between change in number of positive DAs in participants with ≥ 1 baseline positive DA (n=1391) and HbA1c, insulin dose, acute or chronic complications after 7.7±1.9 years duration of diabetes.

Conclusions: DA status likely remains stable in the first 7 years after diabetes diagnosis. Younger age at diabetes diagnosis and longer duration were associated with less persistence of DAs. Measuring DAs after initial presentation may aid in diabetes classification but not likely in predicting the clinical course.

Introduction:

The presence of one or more diabetes autoantibodies (DAs) (Glutamic Acid Decarboxylase antibodies (GAD), insulinoma-antigen 2 antibodies (IA-2), zinc transporter protein autoantibodies (ZnT8Ab), islet cell autoantibodies (ICA) and Insulin Autoantibodies (IAA)) has been used to classify individuals with diabetes as having autoimmune type 1 diabetes [1, 2]. However, few studies have assessed how DAs change over the course of diabetes and whether this change is associated with the clinical course including the development and progression of complications. Thus, whether DAs are informative in the classification of diabetes type beyond initial diagnosis and in predicting clinical course is not known.

There is a paucity of longitudinal DA data in youth with diabetes. The Type 1 Diabetes Genetic Consortium study documented that 80% of individuals with a clinical diagnosis of type 1 diabetes had DA positivity within 5 years of diagnosis. Positivity declined to 68% at 6–13 years diabetes duration [3]. The Belgian Diabetes Registry reported DA data obtained longitudinally within 4 years of diagnosis in individuals with type 1 diabetes and found that 93% had positive DA at diagnosis, and 94% of those with baseline positive DA remained DA positive at 4 years duration. An additional finding in the Belgian Diabetes Registry is that 24% of those with type 1 diabetes who were GAD negative at baseline became GAD positive within 4 years of diagnosis [4]. In individuals with type 2 diabetes, the TODAY study reported that 9.8% of youth with obesity and clinical diagnosis of type 2 diabetes had DA positivity [5].

A few small studies have evaluated whether DAs were related to the rate of decline in endogenous insulin production, insulin requirement, and rate of diabetes complications. In some studies, DA positivity was associated with rapid decline in beta cell function and higher insulin requirements [6–8], but in a separate study DA positivity was not associated with the clinical course [9]. Two studies found no association between positive DAs and the complications of diabetes in individuals with type 1 diabetes [10, 11].

The SEARCH for Diabetes in Youth study (SEARCH) provides a unique opportunity to add novel cross-sectional and longitudinal data regarding DAs in youth with type 1 and type 2 diabetes. The objectives of this study are to assess longitudinal changes in DA status and in number of positive DAs over time and whether these changes are associated with demographics, clinical factors, Human Leucocyte Antigen (HLA) type, and clinical course including rates of diabetes complications. Our hypothesis is that DAs are useful clinical markers of autoimmunity in the first few years after diagnosis but their utility wanes over time. The change in DAs could be helpful in predicting the clinical course and complications of diabetes.

Methods:

SEARCH is a multicenter study that conducted population-based ascertainment of newly-diagnosed diabetes in youth younger than 20 years in 5 US sites (South Carolina; Cincinnati, Ohio and surrounding counties; Colorado with southwestern Native American sites; Seattle, Washington, and surrounding counties; and Kaiser Permanente, Southern California members). A detailed description of the SEARCH study methods has been published previously [12].

Participants in SEARCH with new onset type 1 and type 2 diabetes in 2002–2006 or 2008 (diagnosed by their medical provider and identified by medical record review) were recruited for a baseline research visit. Those who completed a baseline visit were invited to return at 12, 24, and 60 months for follow up visits. Clinical measures were collected, including height, weight, body mass index (BMI), hemoglobin a1c (HbA1c), insulin dose, fasting c-peptide levels (easier to obtain and follow over time than random or stimulated c-peptide), self-reported frequency of diabetic ketoacidosis (DKA) admissions and severe hypoglycemia in the past 6 months and insulin sensitivity (IS) score [13, 14]. The insulin sensitivity score (IS) was calculated as follows: $\log_e IS = 4.64725 – 0.02032$ (waist, cm) – 0.09779 (HbA_{1c}, %) – 0.00235 (Triglycerides, mg/dl) [13]. A subset of baseline participants who were older than 10 years at follow up and had diabetes duration ≥ 5 years were invited for an additional follow-up visit between 2011 and 2015 during which diabetes complications were assessed including presence of microalbuminuria, hypertension, arterial stiffness, retinopathy, cardiac autonomic neuropathy and peripheral neuropathy [15]. DAs were measured at baseline and at each of the above follow up visits. Participants were included in the study if they had longitudinal DA data (at baseline and at least one follow-up visit).

Complications of diabetes were not assessed in all participants. As a result, we analyzed 2 groups of participants (Figure 1). Follow up group 1 includes all participants with longitudinal DA data where DA measurement was available at baseline and at least one of the follow up visits (12, 24 or ≥ 5 years); If DA data was available for more than one follow-up visit, we used the last available visit data. Follow up group 2 describes those who had diabetes complications measures at ≥ 5 years after the baseline visit as well as longitudinal DA data. The study was approved by institutional review boards with jurisdiction for all participants. The parents, youth or young adult, or both provided consent or assent.

Laboratory analyses

Samples were collected at baseline and follow-up visits and analyzed for GAD and IA-2 for all visits (Northwest Lipid Metabolism and Diabetes Research Laboratories, Seattle, WA) and for ZnT8 at baseline and ≥5 year from baseline visits only due to funding limitation (Barbara Davis Center, Denver, CO) using a standardized protocol [16]. The cutoff values for positivity were 33 National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Units/mL for GAD, 5 NIDDK Units/mL for IA-2, and 0.02 NIDDK Units/mL for ZnT8. The specificity and sensitivity were 97 and 76% for GAD, 99 and 64% for IA-2, and 100 and 74% for ZnT8. We chose these 3 antibodies because they are used more frequently in the clinical setting. Insulin autoantibodies were not obtained because the baseline visit occurred after treatment initiation.

HbA_{1c} was measured by a dedicated ion exchange high–performance liquid chromatography instrument (TOSOH Bioscience, San Francisco, CA). Fasting C-peptide (FCP) was measured by a two-site immunoenzymometric assay (TOSOH Bioscience, San Francisco, CA) with level ≥0.23 ng/ml considered clinically significant [17]. HLA class II genotyping (HLA *DR-DQ*) was performed with a PCR-based sequence–specific oligonucleotide probe system in the laboratories of Drs. L. Gaur (University of Washington, Seattle, WA) and H. Erlich (Roche Molecular Systems, Indianapolis, IN).

Statistical analysis

Descriptive analyses present means and standard deviations for continuous measures and counts and percentages for categorical variables.

Change in DA status was categorized as follows for each participant with complete DA data at both baseline and follow-up: 1. Negative DA at baseline, 2. Baseline 1 DA, follow-up negative, 3. Baseline 1 DA, follow-up >= 1 DA, 4. Baseline 2+ DA, follow-up negative, 5. Baseline 2+ DA, follow-up 1 DA, 6. Baseline 2+ DA, follow-up 2+ DA. We then ran simple bivariate tests on this variable vs. the demographics and clinical characteristics measured at a single timepoint. Demographics were mostly measured at baseline. Clinical characteristics were measured at follow-up. For example, for participants who had only one positive DA at baseline, we examined whether they had ≥ 1 positive DA at follow-up versus none. We then examined whether there was a relationship between this dichotomous variable (≥ 1 positive DA at follow-up or no positive DA at follow-up (yes/no)) and factors of interest at follow up visits. For participants who had ≥2 positive DAs at baseline, we examined whether they had 0, 1, or ≥2 positive DA at follow-up. We then examined the association of this 3-level variable with factors of interest measured at group 1 and group 2 visits. We also compared the longitudinal change in the number of DAs between those with baseline 1 positive DA and those with baseline ≥2 positive DAs in association with risk factors at group 1 and group 2 visits. Because only a small percentage of participants with baseline negative DA (4.1%) converted to positive, we did not analyze their longitudinal data.

We also evaluated the longitudinal change in each DA status separately (whether they remained positive, became positive, remained negative or became negative) in association with participants' characteristics. In addition, for those with only one positive DA at

baseline, we compared some of the clinical features (C-peptide level, insulin dose and Hba1c) at follow up by the type of the positive baseline DA. A log transformation was applied to C-peptide data due to the skewed distribution.

Bivariate relationships between the change in antibody status, demographic and limited clinical characteristics for group 1 and additional measures of diabetes complications for group 2 were investigated using Chi-squared tests, Fisher's Exact tests, and one-way ANOVAs, as appropriate based on sample sizes and whether variables were measured on a categorical or continuous scale. The relationship between diabetes duration and DA titer was modeled by performing a repeated measures one-way ANOVA over all visits. All analyses were performed with SAS v. 9.4 and used a 0.05 level of significance.

Results:

Figure 1 describes the number of participants with their percentage of DA positivity at the baseline visit (10.3 ± 7.1 months after diabetes diagnosis) and in the 2 follow-up groups described in the methods section. At follow up, 62% of the baseline participants (2589/4179) had longitudinal DA data available and 51% of the baseline participants (2125/4179) had complications and longitudinal DA data available. The participants in the follow up groups had a distribution of characteristics similar to the baseline cohort on demographic factors (age at diagnosis, sex and race), clinical variables (Hba1c, BMI z-scores, fasting c-peptide) and socioeconomic factors (insurance, household income and parental education)[15].

For follow-up group 1, 86.0% had a clinical diagnosis of type 1 diabetes (at baseline: 11.3% negative DA, 15.9% 1 DA and 72.8% ≥2 DA), 13.2% had a clinical diagnosis of type 2 diabetes (at baseline: 89% negative DA, 3.9% 1 DA and 7.1% ≥2 DA), and 0.9% had other/unknown type of diabetes (at baseline: 61.1% negative DA, 16.7% 1 DA and 22.2% ≥2 DA). The clinical characteristics of participants with a clinical diagnosis of type 1 diabetes and negative DA and of those with a clinical diagnosis of type 2 diabetes and positive DA are included in supplementary table 1. The change in DA over time by clinical diagnosis at baseline is shown later in table 1.

A small percentage (4.1%, n=14) of individuals with baseline negative DAs (all 3 DAs) had at least one positive DA at follow-up; 7 were GAD positive, 2 were IA-2A positive, 4 were ZnT8 positive and 1 was both GAD and ZnT8 positive. Because of the small number, we did not analyze their longitudinal data.

Figure 2 describes by 2-year intervals the percentage of participants with baseline positive DA who remained DA positive for each individual DA (series of cross-sectional data). Number of participants for each DA is included for the different diabetes durations evaluated. The percentage of participants who remained DA positive declined over time for the 3 DAs. IA2 appeared to be the most persistent and ZnT8 the least persistent among the 3 DAs studied.

Regarding longitudinal change in number of positive DA, for those with ≥ 1 positive DA at baseline (n=1699), at mean duration of diabetes of 7.3 years, 35.0% had 1 positive DA, 28.0% had 2 positive DAs, and 12.9% had 3 positive DAs. An additional 7.5% of

participants had at least 2 positive DAs (GADA and IA2) but did not have ZnT8 measured so we were not certain if they had more than 2 positive DAs. DA titer was negative for all DAs in 16.6% of these participants.

Table 1 describes the longitudinal change in the number of positive DAs by participants' characteristics using the follow-up group 1. Because ZnT8 data were not available for all visits, only 1900 participants could be classified into one of the six categories included in the methods. Among those with baseline 1 DA, 38% had negative DA at follow-up compared to 8% in those with baseline ≥ 2 DA. There was a significant difference in the change in number of positive DAs between those with baseline 1 DA and those with baseline ≥ 2 positive DAs that was associated with race/ethnicity, clinical diagnosis at baseline, diabetes duration, IS score, HbA1c and HLA category. In those with baseline 1 DA, we found a significant association with clinical diagnosis at baseline ($p=0.023$); 63% of those with clinical diagnosis of type 1 diabetes had ≥ 1 DA at follow up vs. 27.3% of those with clinical diagnosis of type 2 diabetes had ≥ 1 DA at follow up. There was also an association with diabetes duration ($p=0.003$). At <3 years duration of diabetes all participants had the same number of positive DA, with some converting to negative DA after 4 years duration. After 9 years diabetes duration, 54 % had negative DA. In those with baseline ≥2 positive DAs, there was a significant association with diabetes duration ($p<0.001$). After 9 years, 11% had negative DAs and 30% had only 1 DA. There was also an association with age at diagnosis ($p<0.001$); 14% of those diagnosed at <5 years of age and 11% of those diagnosed at 5–10 years of age vs. 5% of those diagnosed at 11–15 years of age had negative DA at follow up ($p<0.001$ for sub-group analyses).

Table 2 describes the longitudinal change in the number of positive DAs using follow-up group 2 by clinical parameters including complications. We also include in this table the participants with baseline negative DA. There was a significant difference in the change in number of positive DAs between those with baseline 1 DA and those with baseline ≥2 positive DAs that was associated with HbA1c ($p=0.019$) and with C-peptide ($p<0.001$). In those with baseline 1 DA, there was a significant association with arterial stiffness ($p=0.049$). In those with baseline ≥2 DAs, there was a significant association with HbA1c ($p=0.033$) and insulin dose ($p=0.014$) where those with persistent 2 DA at follow-up appeared to have slightly lower HbA1c and insulin dose compared to those with no DA or only 1 DA at follow up. There was no association between the change in the number of DAs (whether baseline 1 DA or baseline ≥2 positive DA) with frequency of DKA or hypoglycemic episodes in the past 6 months, presence of microalbuminuria, hypertension, retinopathy, cardiac autonomic neuropathy or peripheral neuropathy. The results did not change after adjusting the models for age, sex, ethnicity, BMI Z-score, HbA1C and HLA (data not shown).

Comparison of characteristics in those with baseline positive DA and longitudinal change in DA for individual DAs

We evaluated the association of the overall longitudinal DA status with the participants' characteristics for each of the DAs. For those with baseline positive GAD (supplementary table 2), we observed differences with sex, age at diagnosis and diabetes duration where

male sex, younger age at diagnosis and longer diabetes duration were associated with lower percentage of remaining GAD positive. For those with baseline negative GAD, C-peptide was lower (mean (SD) 0.43 (0.81) ng/mL) in those who became GAD positive compared to those who remained negative (mean (SD) 1.04 (1.75) ng/mL) (p=0.04) (data not shown).

For those with baseline positive IA2 (supplementary table 3), there were differences with race/ethnicity, age at diagnosis, diabetes duration and IS score. Native American race, younger age at diagnosis, longer diabetes duration and higher IS score were associated with lower percentage of remaining IA2 positive. For those with baseline positive ZnT8 (supplementary table 4), there were significant differences by sex, age at diagnosis, diabetes duration and IS score. Male sex, younger age at diagnosis, longer diabetes duration and both lower and higher IS score (compared to intermediate score) were associated with lower percentage of remaining ZnT8 positive.

In those with only one positive DA at baseline, we compared clinical outcomes at follow-up with the type of positive DA at baseline (data not shown). We found difference in C-peptide levels at follow-up; those with baseline positive GAD had C-peptide level of 0.40 (0.99) ng/mL vs. those with baseline IA2 had C-peptide 0.10 (0.19) ng/mL vs. ZnT8 had C-peptide level 0.17 (0.52) ng/mL ($p<0.001$). There was no difference in HbA1c or insulin dosing at follow-up in association with the type of baseline positive DA.

All three DAs had a significant decline in their titers over time. Supplementary figure 1 shows the mean DA titer in participants with baseline positive DA by diabetes duration. There was no association between HLA risk category and the mean decline in DA titers for any of the DA (data not shown).

Discussion:

We found that DA positivity persisted at 7 years duration of diabetes for most participants with positive DAs at baseline. Only 4.1% of individuals with baseline negative DAs had one or more positive antibody at follow-up. Thus, DA is likely a useful measure to classify type of diabetes for an extended period after diagnosis. Having only one positive DA at baseline was associated with higher rate of becoming DA negative. Younger age at diagnosis and longer diabetes duration were associated with decrease in the number of positive DAs over time and less persistence of DA positivity in all 3 DAs. The change in the DA in those with baseline positive DA was not associated with the clinical course or the presence of diabetes complications.

Our study provided longitudinal DA data for both type 1 and type 2 diabetes. In the literature, studies mainly evaluated DAs in type 1 diabetes. The Type 1 Diabetes Genetics Consortium reported that the frequency of any DA positivity (GAD or IA2) at 6–13 years after diagnosis was 68% [3]. The Belgian Diabetes Registry reported longitudinal DA data in individuals with type 1 diabetes but only for 4 years after diagnosis and found that 93% remained positive[6]. Savola et al. measured 4 DAs in 90 Finnish children and adolescent at diagnosis, 2, 5, and 10 years later and found that two thirds still tested positive for at least one DA after the first 10 years of diagnosis and 42% had 2–3 DAs detectable [18].

In a cross-sectional study, Maraschin et al evaluated GAD in 92 Brazilian individuals with type 1 diabetes and found that about half had positive GAD. Even though the positivity rate declined over time, it can stay positive up to 15 years after diagnosis, and the best diagnostic performance of GAD was in patients with diabetes duration < 15 years, and with diabetes onset after 16 years of age [19]. In comparison, our study followed patients for a mean diabetes duration of 7.3 years and found that 83% of participants with baseline positive DA remained positive. This indicates that measuring the DAs can be useful in classifying diabetes for years after diagnosis. As in the TODAY study, in which 9.8% of youth with a clinical diagnosis of type 2 diabetes had a positive DA (GAD and/or IA2) [5], our study reported that 10% of youth with a clinical diagnosis of type 2 diabetes had positive DAs at baseline; of those 38.7% converted to negative DA at follow up. We also found only a small percentage of participants with baseline negative DA who converted to DA positivity which supports the importance of measuring DAs at baseline even if there is strong suspicion for type 2 diabetes as they can aid in accurately classifying diabetes at presentation.

The Belgian Diabetes Registry also found a higher percentage of GAD negative participants converting to GAD positive (24%) at 4 years compared to 3.5% who converted to GAD positive after 7.3 years in our study. This is likely due to the shorter duration of follow up in the Belgian study [4] in addition to the fact that their study population only included individuals with clinically diagnosed type 1 diabetes whereas our study included both type 1 and type 2 diabetes. Also, the methods for DA analysis have been shown to make a difference regarding specificity of each assay. The Northwest Lipid Research Laboratory and the Barbara Davis Laboratory where DA analyses were performed, have participated in harmonization efforts to ensure that DA assays are comparable between international laboratories [16].

The finding that younger age at diagnosis and longer diabetes duration were associated with less persistence of DA positivity has been reported previously, and could be explained by the rapid rate of beta cell loss post diagnosis in younger patients [20]. In the Type 1 Diabetes Genetic Consortium study, while GAD positivity was influenced by age at diagnosis as it was more common and persisted more in older subjects in the first 5 years after diagnosis, IA2 was not. Similarly, in that study both GAD and IA2 were less likely to remain positive with increased diabetes duration [3]. The Belgian registry data study showed that DAs disappeared more rapidly in patients younger than 7 years [4]. In our study, the change in all 3 DAs had an interaction with diabetes duration and age at diagnosis. Our finding suggests that age at diagnosis and diabetes duration should be considered when using the DAs outside of the initial presentation for diabetes classification and negative results should be interpreted with caution as they do not rule out type 1 diabetes. For example, current guidelines recommend genetic screening for maturity-onset diabetes of the young (MODY) when various criteria are met and one of these criteria is absence of DAs [21].

Furthermore, our study suggests that measuring the DAs can be still helpful years after diagnosis in accurately classifying diabetes type in youth with clinically diagnosed type 2 diabetes. Unrecognized islet autoimmunity in this population can result in metabolic decompensation with rapid onset of a requirement for insulin. Making the right diagnosis may help avoid DKA and its associated morbidity. Studies suggest that there are clinically

significant differences between individuals with clinical signs of type 2 diabetes and islet autoimmunity compared with those without autoimmunity. The Prospective Diabetes Study (UKPDS) showed that in participants who had positive GAD and physician-diagnosed type 2 diabetes, oral treatment failed significantly more rapidly than in those without autoimmunity (94 vs. 14% required insulin at 6 years) [22]. A smaller report of adolescents initially diagnosed with type 2 diabetes but later determined to have markers predictive of risk for type 1 diabetes indicated that they required insulin therapy within 4 years [23]. Additionally, having islet autoimmunity is associated with higher risk for other autoimmune diseases such as thyroid and celiac disease that warrants regular screening. Finally, youth with obesity and diabetes being considered for inclusion in research studies evaluating outcomes of type 2 diabetes should have their DAs measured for accurate classification, as the clinical course and outcomes for those with autoimmunity may be significantly different [15].

The change in the number of positive DAs over time was not associated with the clinical course such as frequency of DKA or severe hypoglycemia episodes. C-peptide was lower at follow up in those with baseline ≥2 positive DAs compared to those with baseline 1 positive DA. In those with baseline ≥2 positive DAs with persistent 2 DAs at follow-up, HbA1c and insulin dose were slightly lower compared to those with less persistent DAs but the difference did not seem clinically significant. Although C-peptide level was higher at follow up in those with baseline positive GAD compared to those with baseline positive IA2 and ZnT8, the insulin dose and HbA1c were not different. Previous studies had inconsistent findings about the association between the baseline DAs and the clinical course. Decochez et al reported that high titers of islet cell antibodies at diagnosis were associated with a rapid decline in C-peptide levels within the first 2 years [6]. Juusola et al found that positivity for ZnT8 at diagnosis was associated with rapid decline in beta cell function, higher insulin requirements, and more frequent ketosis [7]. Sabbah et al found that positivity for multiple DAs is associated with accelerated beta cell destruction and an increased requirement for insulin over the second year of disease. In contrast, Karjalainen et al found that insulin autoantibodies were a poor predictor of diabetes clinical course and response to insulin which is consistent with our findings [9].

There was also no interaction between the change in DA status and the rate of diabetes complications except for higher rate of arterial stiffness in those with baseline positive 1 DA who had negative DA at follow–up compared to those with persistent DA. Previous studies found no association between GAD and neuropathy, nephropathy or retinopathy [10] and no effect of the presence of DAs on the development time of diabetic microvascular complications [11]. Our study adds to the existing literature that measuring DAs over time does not appear to be helpful as a predictor of the clinical course, but it can be helpful in correctly classifying the type of diabetes which in turn will prompt appropriate screening for diabetes complications or autoimmune co-morbidities.

This study has many strengths. It has the largest number of participants and one of the longest duration of diabetes reported in the literature with repeated measured DA status after diagnosis. The study population is racially and ethnically diverse which makes it overall representative of the U.S. population, but perhaps not generalizable to a specific location

because the clinical sites differed by ethnic distribution. It is one of the few studies that assessed longitudinal changes in ZnT8 and evaluated both the status and the titers of 3 different DAs. The limitations of the study include that about 38% of baseline participants did not have data at follow up which reduced the size of the longitudinal follow up cohorts. While the number of white, black and Hispanic participants was large, the number of native Americans and Asian/Pacific Islanders was small and not sufficient to draw conclusion. The baseline DA status was determined within a year from diagnosis (mean of 10 months) and not immediately at diagnosis; while not much is known about how DA change during the first year of diabetes diagnosis, it is likely they persist and evidence suggests that DA negative patients with clinical type 1 diabetes can convert to positive within a year from diagnosis [24]. DKA and severe hypoglycemia episodes were self-reported. The DAs were not measured at even intervals and ZnT8 was not obtained at the intermediate follow up visits due to funding limitation (only at baseline and ≥5 year visits) contributing to inconsistent number of DAs at different diabetes durations.

In summary, this study showed that DA status is likely to remain stable in the first 7 years after diagnosis for the majority of patients. Despite the study limitations, we observed that having only one positive DA at baseline, younger age at diagnosis and longer diabetes duration were associated with less persistence of the DAs for those with DA data at follow up. Only a small percentage of individuals with negative baseline DAs converted to positivity at follow-up. The change in the number of positive DAs did not correlate with the clinical course or the risk of diabetes complications for those with DA and complications data at follow up. Therefore, measuring the DAs may not be useful in the clinical setting beyond diabetes classification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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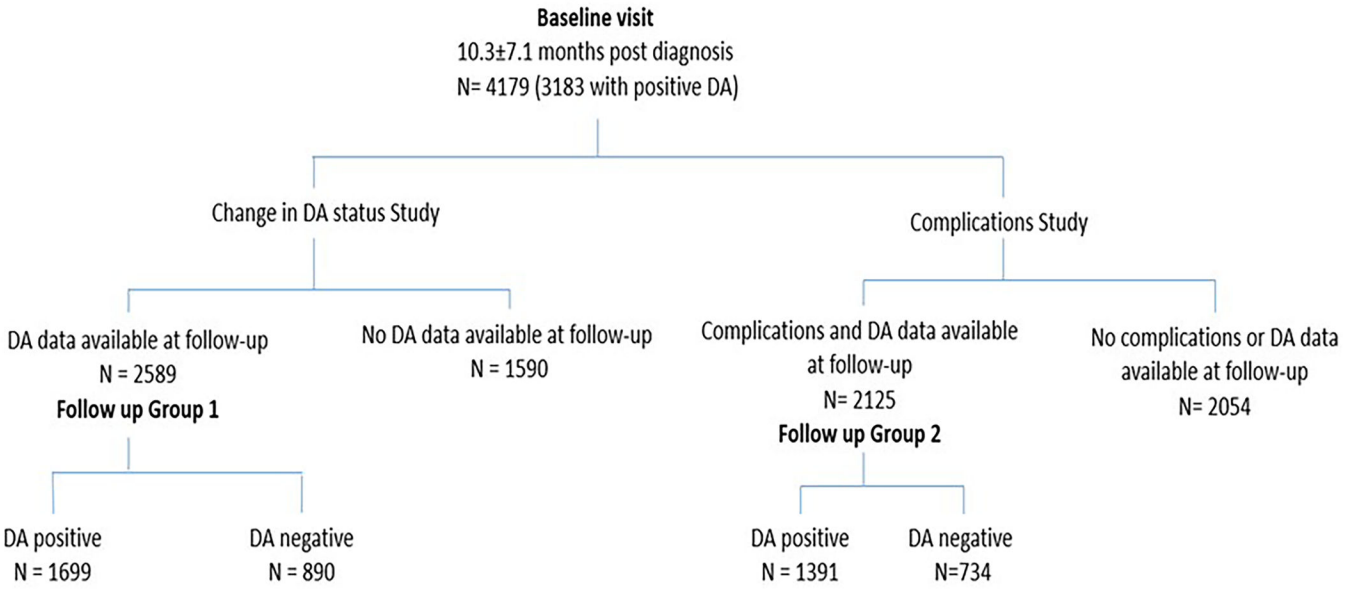

Figure 1: Flow chart of the number of participants and their percentage of positive DA at each visit.

Follow-up group 1 includes participants with longitudinal DA data only. Follow-up group 2 includes participants with both longitudinal DA data and complications measures at ≥ 5 years.

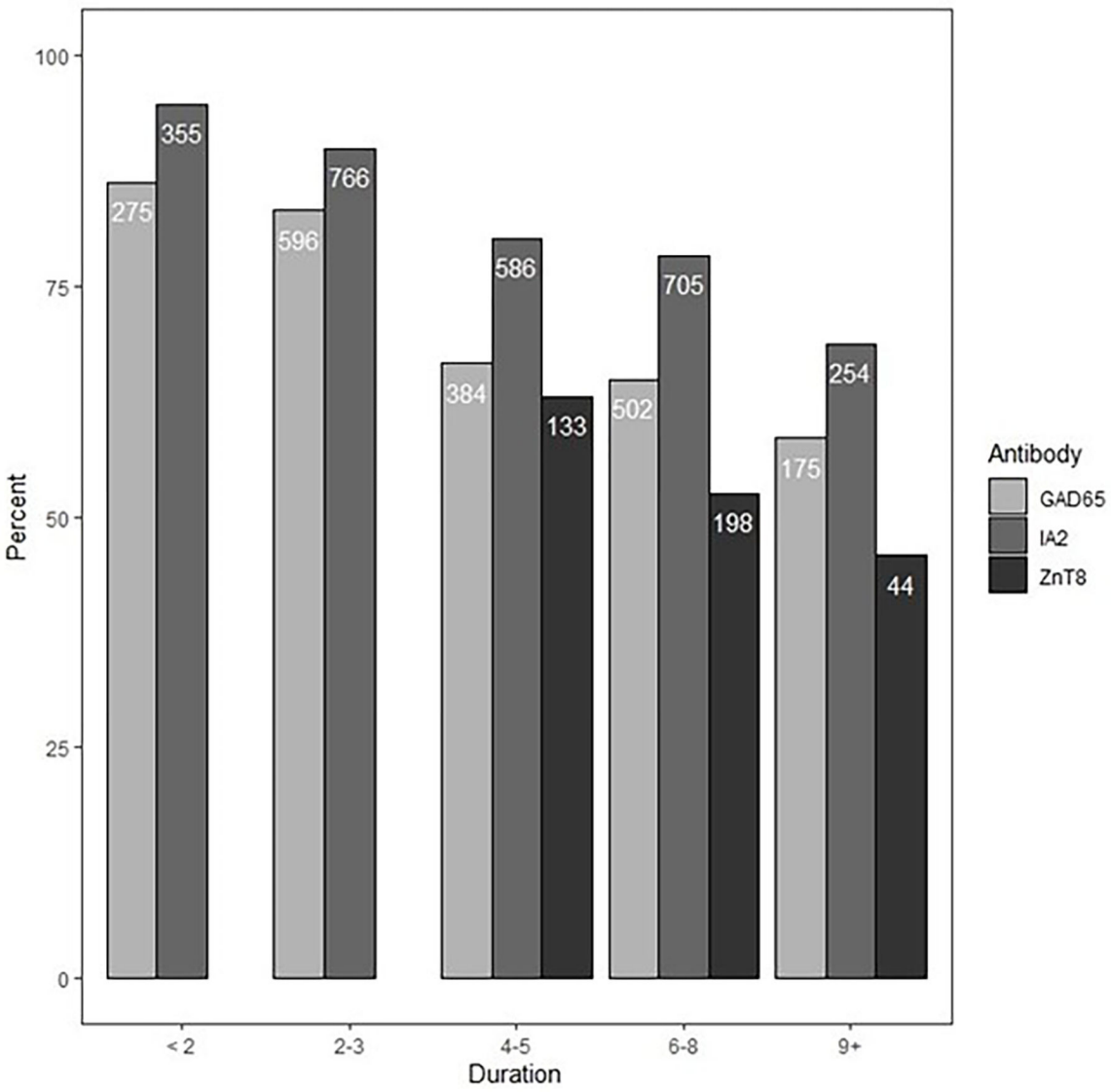

Figure 2: The percentage of participants with baseline positive DA who remained DA positive at different diabetes durations.

Series of cross-sectional data with number of participants for each DA and diabetes duration included. These participants were all DA positive at baseline.

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Table 1.

The longitudinal change in number of positive DAs between baseline and follow-up group 1 with participants' demographic and limited clinical characteristics: (Participants with negative baseline DAs are also included)

		N (%)									
		Overall[†] N = 1900	Negative[‡] N = 443	Baseline 1 DA[§]		Baseline ≥2 DA[§]			Chi square P-values		
Characteristics				Follow-up negative N = 102	Follow-up ≥1 DA N = 166	Follow-up negative N = 94	Follow-up 1 DA N = 329	Follow-up ≥2 DA N = 766	BL 1 DA vs. BL ≥2 DA	BL 1 DA: FU 0 vs. ≥ 1 DA	BL ≥2 DA: FU 0 vs. 1 vs. ≥2 DA
Sex	Male	907 (47.7)	174 (39.3)	51 (35.7)	92 (64.3)	54 (9.2)	169 (28.6)	367 (62.2)	0.269	0.388	0.165
	Female	993 (52.3)	269 (60.7)	51 (40.8)	74 (59.2)	40 (6.7)	160 (26.7)	399 (66.6)			
Race / ethnicity	White	1235 (65.0)	177 (40.0)	62 (36.3)	109 (63.7)	64 (7.2)	255 (28.7)	568 (64.0)	0.015	0.833	0.176
	Black	302 (15.9)	146 (33.0)	17 (40.5)	25 (59.5)	11 (9.6)	33 (28.9)	70 (61.4)			
	Hispanic	290 (15.3)	83 (18.7)	18 (39.1)	28 (60.9)	16 (9.9)	34 (21.1)	111 (68.9)			
	Asian / Pacific Islander	35 (1.8)	10 (2.3)	3 (60.0)	2 (40.0)	2 (10.0)	4 (20.0)	14 (70.0)			
	Native American	30 (1.6)	23 (5.2)	1 (50.0)	1 (50.0)	0 (0.0)	3 (60.0)	2 (40.0)			
	Other	7 (0.4)	4 (0.9)	0 (0.0)	1 (100.0)	1 (50.0)	0 (0.0)	1 (50.0)			
Age (years) at diagnosis	< 5	111 (5.8)	12 (2.7)	12 (63.2)	7 (36.8)	11 (13.8)	28 (35.0)	41 (51.3)	0.978	0.059	< 0.001
	5–10	681 (35.8)	69 (15.6)	43 (38.4)	69 (61.6)	54 (10.8)	146 (29.2)	300 (60.0)			
	11–15	812 (42.7)	231 (52.1)	39 (37.1)	66 (62.9)	22 (4.6)	114 (23.9)	340 (71.4)			
	16+	296 (15.6)	131 (29.6)	8 (25.0)	24 (75.0)	7 (5.3)	41 (30.8)	85 (63.9)			
Clinical diagnosis at baseline	Type 1 diabetes	1600 (84.2)	181 (40.9)	94 (37.0)	160 (63.0)	90 (7.7)	324 (27.8)	751 (64.5)	0.011	0.023	0.326
	Type 2 diabetes	282 (14.8)	251 (56.7)	8 (72.7)	3 (27.3)	4 (20.0)	4 (20.0)	12 (60.0)			
	Other/ unknown	18 (0.9)	11 (2.5)	0 (0.0)	3 (100.0)	0 (0.0)	1 (25.0)	3 (75.0)			
Diabetes duration (years) at most recent visit	< 2 Years	20 (1.1)	6 (1.4)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	11 (100.0)	0.020	0.003	< 0.001

		N (%)									
		Overall† N = 1900	Negative‡ N = 443	Baseline 1 DA§		Baseline ≥2 DA§			Chi square P-values		
Characteristics				Follow-up negative N = 102	Follow-up ≥1 DA N = 166	Follow-up negative N = 94	Follow-up 1 DA N = 329	Follow-up ≥2 DA N = 766	BL 1 DA vs. BL ≥2 DA	BL 1 DA: FU 0 vs. ≥ 1 DA	BL ≥2 DA: FU 0 vs. 1 vs. ≥2 DA
	2–3 Years	99 (5.2)	38 (8.6)	0 (0.0)	16 (100.0)	0 (0.0)	0 (0.0)	45 (100.0)			
	4–5 Years	441 (23.2)	85 (19.2)	23 (36.5)	40 (63.5)	20 (6.8)	70 (23.9)	203 (69.3)			
	6–8 Years	916 (48.2)	199 (44.9)	58 (39.5)	89 (60.5)	44 (7.7)	178 (31.2)	348 (61.1)			
	9+ Years	424 (22.3)	115 (26.0)	21 (53.8)	18 (46.2)	30 (11.1)	81 (30.0)	159 (58.9)			
Body mass index (Z-score)	<1	1036 (55.6)	120 (27.6)	68 (38.0)	111 (62.0)	63 (8.5)	203 (27.5)	471 (63.9)	0.398	0.240	0.350
	1–<2	525 (28.2)	111 (25.6)	23 (33.8)	45 (66.2)	19 (5.5)	92 (26.6)	235 (67.9)			
	≥ 2	302 (16.2)	203 (46.8)	10 (55.6)	8 (44.4)	8 (9.9)	24 (29.6)	49 (60.5)			
Mean BMI Z-score (SD)		0.8 (1.2)	1.5 (1.2)	0.5 (1.1)	0.4 (1.1)	0.6 (1.0)	0.6 (1.0)	0.6 (1.1)	0.036	0.267	0.863
IS score	< 4	156 (8.6)	126 (29.0)	6 (54.5)	5 (45.5)	1 (5.3)	6 (31.6)	12 (63.2)	0.022	0.523	0.423
	4 - < 8	450 (24.9)	171 (39.4)	17 (37.8)	28 (62.2)	12 (5.1)	69 (29.5)	153 (65.4)			
	8+	1202 (66.5)	137 (31.6)	76 (37.4)	127 (62.6)	76 (8.8)	233 (27.0)	553 (64.2)			
Mean IS score (SD)		6.4 (2.9)	4.9 (3.1)	6.7 (3.2)	7.2 (2.8)	7.0 (2.9)	6.5 (2.6)	6.9 (2.6)	0.223	0.258	0.107
HbA1c	< 7.5	1012 (53.5)	289 (65.2)	44 (33.6)	87 (66.4)	47 (7.9)	151 (25.5)	394 (66.6)	0.003	0.205	0.323
	7.5 - < 9	579 (30.6)	85 (19.2)	35 (46.1)	41 (53.9)	36 (8.6)	120 (28.7)	262 (62.7)			
	9+	302 (16.0)	69 (15.6)	23 (38.3)	37 (61.7)	11 (6.4)	57 (32.9)	105 (60.7)			
HLA	High risk	932 (54.7)	106 (30.5)	51 (34.7)	96 (65.3)	59 (8.7)	193 (28.4)	427 (62.9)	0.025	0.100	0.565
	Moderate risk	562 (33.0)	120 (34.6)	31 (39.7)	47 (60.3)	22 (6.0)	90 (24.7)	252 (69.2)			
	Low risk	108 (6.3)	44 (12.7)	0 (0.0)	10 (100.0)	4 (7.4)	15 (27.8)	35 (64.8)			
	Protective	101 (5.9)	77 (22.2)	4 (40.0)	6 (60.0)	1 (7.1)	4 (28.6)	9 (64.3)			

†Because ZnT8 data was not available for all visits, only 1900 participants could be classified into one of six categories: DA negative at both visits, baseline 1 DA and follow-up DA negative, baseline 1 DA and follow-up ≥1 DA, baseline ≥2 DA and follow-up DA negative, baseline ≥2 DA and follow-up 1 DA, and baseline ≥2 DA and follow-up ≥ 2 DA.

‡Percentages of participants with negative DAs per characteristic are presented as column percentage.

[§] Percentages of participants with baseline 1 DA or ≥2 DAs are presented as row percentage (follow-up negative and follow-up ≥1 DA/baseline 1 DA) and (follow-up negative, follow-up 1 DA and follow-up ≥2 DA/baseline ≥2 DA).

Table 2:

The longitudinal change in number of antibodies using follow-up group 2 and clinical parameters including complications:

	Mean (SD) or N (%)								
	Baseline Negative N = 443	Baseline 1 DA		Baseline ≥2 DA			P-values		
Clinical Parameters at Follow Up		Follow-up negative N = 102	Follow-up ≥1 DA N = 132	Follow-up negative N = 94	Follow-up 1 DA N = 329	Follow-up ≥2 DA N = 653	BL 1 DA vs. ≥2 DA	BL 1 DA: FU 0 vs. ≥1 DA	BL ≥2 DA: FU 0 vs. 1 vs. ≥2 DA
Hba1c (%)	8.9 (2.8)	9.6 (2.1)	9.2 (1.9)	9.2 (1.8)	9.3 (1.9)	9.0 (1.8)	0.019	0.219	0.033
Insulin dose (unit / kg)	0.8 (0.4)	0.9 (0.5)	0.8 (0.3)	0.9 (0.6)	0.9 (0.3)	0.8 (0.3)	0.126	0.153	0.014
Frequency of DKA admissions in past 6 months[†]	2.0 (11.5)	0.7 (1.9)	0.7 (1.7)	1.1 (6.6)	2.8 (8.2)	3.6 (10.6)	0.676	0.374	0.06
Frequency of severe Hypoglycemia in past 6 months[†]	0.7 (4.8)	0.7 (4.2)	0.1 (0.5)	0.1 (0.6)	4.0 (30.4)	3.8 (31.0)	0.386	0.085	0.199
C-peptide (ng/ml)[‡]	2.31 (2.04)	0.26 (0.79)	0.20 (0.63)	0.12 (0.27)	0.10 (0.17)	0.10 (0.14)	0.005	0.802	0.632
Microalbuminuria – Yes	43 (14.2%)	8 (50.0%)	8 (50.0%)	8 (13.1%)	15 (24.6%)	38 (62.3%)	0.482	0.532	0.362
No	260 (85.8%)	81 (42.0%)	112 (58.0%)	78 (8.6%)	278 (30.8%)	547 (60.6%)			
Hypertension – Yes	150 (40.3%)	21 (58.3%)	15 (41.7%)	12 (7.1%)	61 (35.9%)	97 (57.1%)	0.902	0.051	0.237
No	222 (59.7%)	80 (40.8%)	116 (59.2%)	82 (9.1%)	268 (29.7%)	553 (61.2%)			
Arterial stiffness – Yes	117 (38.5%)	12 (66.7%)	6 (33.3%)	4 (4.4%)	30 (33.3%)	56 (62.2%)	0.675	0.049	0.284
No	187 (61.5%)	87 (42.6%)	117 (57.4%)	86 (9.4%)	282 (31.0%)	543 (59.6%)			
Retinopathy - Yes	33 (9.1%)	4 (50.0%)	4 (50.0%)	2 (5.6%)	12 (33.3%)	22 (61.1%)	0.983	0.697	0.767
No	329 (90.9%)	96 (43.0%)	127 (57.0%)	90 (8.9%)	309 (30.5%)	613 (60.6%)			
Cardiac autonomic neuropathy - Yes	55 (16.4%)	9 (36.0%)	16 (64.0%)	14 (11.8%)	35 (29.4%)	70 (58.8%)	0.836	0.371	0.530
No	280 (83.6%)	85 (45.5%)	102 (54.5%)	73 (8.6%)	258 (30.4%)	517 (61.0%)			
Peripheral neuropathy - Yes	57 (15.9%)	6 (54.5%)	5 (45.5%)	3 (4.4%)	22 (32.4%)	43 (63.2%)	0.349	0.448	0.433
No	302 (84.1%)	94 (42.9%)	125 (57.1%)	89 (9.0%)	303 (30.5%)	600 (60.5%)			

Total number of participants included in this table is 1753 and included those with baseline DA data and follow up DA, clinical and complication data.

[†]Reported as frequency per 100 patients' years

‡A log transformation was applied to c-peptide data analysis due to the skewed distribution.