Pediatric Diabetes

WILEY

Research Article

Open Access

https://doi.org/10.48130/pedi-0026-0012

Pediatric Diabetes **2026**, 2026: e012

Identifying children and adolescents with preclinical type 1 diabetes among first-degree relatives of type 1 diabetes patients: a Polish pilot study (pre-d1abetes study)

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Abstract

Clinical type 1 diabetes (T1D) is often preceded by preclinical stages with the presence of islet autoantibodies (IAb). The aim of the study was to conduct an early screening programme for preclinical T1D in children and adolescents with a family history of T1D. In total, 1,275 first-degree relatives of T1D patients from 14 clinical centers across Poland participated in the study. Serum samples were tested via the 3-Screen ICA^{TM} enzyme-linked immunnosorbent assay (ELISA) for combined detection of autoantibodies to glutamic acid decarboxylase (GADAb), islet antigen IA-2 (IA-2Ab), and zinc transporter 8 (ZnT8Ab). All positive samples (104 subjects, 8.1%) were assayed for the individual autoantibodies (via ELISAs) and insulin antibodies (IAAs) (via radioimmunoassay), all using equipment from RSR Ltd, Cardiff, UK. The presence of two or more antibodies identified 69 subjects (5.4%) with preclinical T1D. Among IAb-positive participants, GADAb antibodies were the most frequent, whereas IAAs were the least frequent. Metabolic staging with an oral glucose tolerance test and glycated hemoglobin (HbA_{1c}) was carried out in the consenting subjects. T1D was diagnosed in 10 patients with multiple antibody positivity, of whom none presented with diabetic ketoacidosis (DKA). The results of our study highlight the importance of IAb screening in individuals with already increased risk of T1D developing. Early detection of IAb identifies preclinical T1D prior to the onset of dysglycemia, with subsequent follow-up allowing for timely intervention preventing the development of DKA and its severe manifestations. Implementation of screening programs in routine clinical practice may open up opportunities for innovative therapeutic interventions in individuals at a high risk of T1D.

Keywords: Type 1 diabetes, Screening, Islet autoantibodies, Relatives, Children and adolescents

Citation: Noiszewska K, Jamiołkowska-Sztabkowska M, Zasim A, Polkowska A, Mazur A, et al. 2026. Identifying children and adolescents with preclinical type 1 diabetes among first-degree relatives of type 1 diabetes patients: a Polish pilot study (pre-d1abetes study). *Pediatric Diabetes* 2026: e012 https://doi.org/10.48130/pedi-0026-0012

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Introduction

Type 1 diabetes (T1D) is ranked among the most common chronic metabolic diseases in the pediatric population and accounts for 90% of all diabetes cases in children and adolescents[1,2]. In the last 20 years, there has been an increase in the incidence of T1D, especially among very young children, who present a greater risk of life-threatening complications[3,4]. An increase in the disease's incidence has also been observed in the Polish pediatric population[5,6].

The clinical picture of T1D in the pediatric population at the time of diagnosis may vary, with slow development of the typical symptoms of diabetes in some individuals or with quick progression to life-threatening diabetic ketoacidosis (DKA) in others[7–9]. In particular, younger children aged < 5 years are at risk of more rapid disease progression and are at a greater risk of developing DKA[10]. Currently in Poland, T1D in children is mostly diagnosed at the onset of clinical symptoms, and about one-third of hospitalized patients present with DKA at the time of T1D diagnosis[11,12].

With dynamic changes in T1D's epidemiology, rapid improvements in insulin-based treatment have been observed in recent decades. As the goals of effective insulin treatment have become more easily achieved, a new challenge for pediatric diabetology has arisen: detecting and treating the developing disease as early as possible. Early awareness of a risk of developing the disease allows patients and their families to learn how to monitor blood glucose at home, recognize the early signs of T1D, follow a healthy lifestyle, and thus help prevent DKA and its serious consequences[13–16]. Moreover, recognizing the early stages of T1D opens up opportunities to implement targeted therapies to delay or prevent the onset of clinically overt disease before the need to administer exogenous insulin[17–20]. New hope for at-risk individuals arose in 2019 with a clinical trial reporting that the onset of T1D in autoantibody-positive relatives can be delayed by a median of 2 years[21,22].

In the natural history of T1D, islet cell autoantibodies (IAbs), including glutamic acid decarboxylase antibodies (GADAbs), insulin autoantibodies (IAAs), islet antigen 2 antibodies (IA-2Abs), and zinc transporter 8 antibodies (ZnT8Abs), are markers of T1D's development[23,24]. The presence of IAb gives warning of autoimmune inflammation in the islet cells, sometimes long before a decrease in insulin secretion is observed. According to the current classification, there are four stages of the development of T1D. Stage 1 is characterized by the presence of at least two IAbs with a normal glycemic profile; Stage 2 is defined by the presence of at least two IAbs with dysglycemia. Both Stage 1 and Stage 2 are considered preclinical stages of the disease[25,26]. Stage 3 is a clinical phase of the disease with hyperglycemia, and Stage 4 is long-standing T1D. There is a 44% 5-year risk and an 80%–90% 15-year risk of developing overt disease for Stage 1, a 75% 5-year risk, and a nearly 100% lifetime risk for Stage 2[27–29]. A retrospective study from Finland revealed that 95% of children who developed T1D by puberty presented with IAbs by the age of 5 years[30].

The risk of T1D in the general population is estimated to be 0.4% and a large proportion of newly diagnosed patients have no family history of the disease[31]. However, similar to other autoimmune conditions, T1D is likely to occur in individuals and families already affected by autoimmunity[32], with the association of T1D with Class II HLA (human leukocyte antigen) already being known[33]. Children with T1D-affected parents or siblings have an increased risk of developing the disease compared with the general population[34–38] and a first-degree relative has a 15-fold increased risk of T1D[25]. The aggregate risk of T1D is about 4% in the offspring of a person with T1D who had a diagnosis at the age of 16–39 years. Moreover, the risk rises up to 70% in monozygotic twins[39,40].

The history of active screening for individuals with presymptomatic T1D dates back to the 1990s. At the beginning, it focused primarily on children with a positive family history of the disease[41–44]. Initially, the main goal of screening and monitoring heathy children from T1D-affected families was to prevent DKA at disease diagnosis. The first attempts at identifying children susceptible to T1D's occurrence covered both genetic (HLA-linked) and immunological testing, performed mostly in the healthy relatives of T1D patients[45–48], as those individuals may benefit the most from early disease detection.

The purpose of the current study was to conduct a structured screening program for preclinical stages of T1D among Polish children and adolescents with a positive family history of T1D. We aimed to analyze the prevalence of individual IAbs in this population and potential influencing factors. We also attempted to search for factors influencing participation in the screening program.

Material and methods

Between April 2019 to December 2023, we enrolled healthy children and adolescents (younger than 18 years old) from 14 clinical centers who met the criterion of having at least one first-degree relative with T1D. Through an informative campaign, we recruited family members (parents and/or children) of individuals with T1D who were already receiving care at the clinical centers. The study protocol was approved by the Bioethics Committee of the Medical University of Białystok (R-I-002/616/2018) on 20 December 2018, and written consent was obtained from all participants and/or their parents/caregivers before sample collection.

Venous blood was collected from the participants: 2.7 mL from children under 5 years of age and 5 mL from participants over 5 years of age. After sample centrifugation, the serum was aliquoted, frozen at −20 °C, and sent to FIRS Laboratories RSR Ltd (Cardiff, UK) for a screening test using a 3 Screen ICA™ enzyme-linked immunosorbent assay (ELISA) that allows the detection of the combined presence of antibodies against the three key islet antigens (GAD, IA-2, and ZnT8) in a single serum sample. The test has proven very useful in identifying individuals at high risk in several studies, including large screening programs such as FR1DA[49–53].

The results for the 3 Screen ICA™ ELISA were expressed as an index value using a sample's optical density (OD) read by the ELISA reader as a percentage of a kit reference preparation's OD with the manufacturer's recommended cut-off index of 30. All samples that were positive in the 3 Screen ICA™ ELISA were then tested for individual autoantibodies in the relevant single-antibody ELISAs (all from RSR Ltd, Cardiff, UK). For each ELISA, the cut-offs recommended by the instructions for use were used: GADAb, 5.0 U/mL[54]; IA-2Ab, 7.5 U/mL[55]; and ZnT8Ab, 15 U/mL[56]. The laboratory's ELISAs' reported sensitivity and specificity in Islet Autoantibody Standarization Program (IASP) 2020 were as follows, respectively: 96% and 100% for 3 Screen ICA™, 88% and 98.9% for GADAb, 72% and 100% IA-2Ab, and 74% and 98.9% for ZnT8Ab. All samples that were positive in the 3 Screen ICA™ test were also tested for IAAs using the radioimmunoassay (RIA) from RSR Ltd (Cardiff, UK), with a cut-off of ≥ 0.4 U/mL[57] as per the manufacturer's instructions, and with 32%–38% sensitivity and 96%–99% specificity reported by the manufacturer (based on Diabetes Antibody Standarization Program DASP 2007 and DASP 2009). These values are in line with data available from IASP 2020, showing a median of 32% sensitivity and a median of 97.8% specificity for all analyzed IAAs' RIA/RBA (radiobinding assay)[58]. Samples positive for two or more antibodies in the individual ELISAs and/or the IAA RIA were considered to be positive for the preclinical T1D stages.

Alongside sample collection, the participants were asked to complete a questionnaire detailing their age, gender, height, and weight. It also included optional questions regarding their relative with T1D, such as the relative's relationship to the participant (sibling or parent), disease duration, and age at diagnosis. Once anonymized, the data were forwarded to the coordinating center.

Subjects with a positive screening result were then enrolled into the care of a local diabetes outpatient clinic for metabolic staging using standard methods: anthropometric measurements, glycated hemoglobin (HbA_{1c}), fasting C-peptide measurements, and the oral glucose tolerance test (OGTT). In the OGTT, fasting glucose and insulin levels were measured at 30, 60, 90 and 120 min after receiving a 1.75 g/kg body weight glucose load (maximum = 75 g of glucose).

Depending on the OGTT result, further follow-up was scheduled, For subjects with normal glucose levels in the OGTT (Stage 1), visits were planned for every 6 months with repeat OGTT testing every 12 months; for subjects with abnormal fasting glucose and/or abnormal glucose tolerance (Stage 2), visits were scheduled every 6 months with another OGTT in 6 months; and subjects with blood glucose levels fulfilling the criteria for diabetes (Stage 3) were referred to the hospital for care immediately.

Each participant received a glucometer and was advised to perform measurements of both fasting glucose and 2-h postprandial glucose at least once a week. In addition, participants also received urine ketone tests to carry out once a month. Participants and their families were educated about the symptoms indicating hyperglycemia (polyuria, polydipsia, nocturia, and weight loss) and were advised to contact a medical professional in the case of abnormal glycemic values, the presence of disease symptoms, and/or the presence of ketones in the urine test. Psychological support was also offered as a part of the structured care.

Statistical analyses were performed using Statistica 14.1 (StatSoft Krakow, Poland). Continuous variables were tested by the Kolmogorov–Smirnov with Lilliefors correction and Shapiro–Wilk tests for normal distribution. As the studied parameters were not normally distributed, descriptive statistics were calculated as median with the interquartile range (IQR). To compare continuous variables, Mann–Whitney U-tests and Kruskal–Wallis analysis of variance (ANOVA) tests were used, and the χ^2-test with Yates correction was used to compare categorical variables between groups. *Post hoc* analysis for the χ^2-test using Bonferroni correction was also applied. Correlations between studied variables were assessed using Spearman's rank correlation coefficients. Logistic regression was performed to evaluate independent predictors; p-values < 0.05 were considered to be statistically significant. To assess the robustness of our findings in the presence of missing demographic data, a sensitivity analysis was conducted. The primary analysis was based on complete cases (CCA). As a sensitivity check, missing values were imputed using multiple imputation by chained equations under the missing at random assumption, generating imputed datasets. Statistical models were then re-run on the imputed data, and pooled estimates were compared with the primary analysis.

Results

In total, 1,275 children under the age of 18 years with at least one first-degree relative with T1D from 13 regions of Poland (14 medical centres) participated in the study, as shown in Fig. 1.

The characteristics of the study population are presented in Table 1. Although 1,275 children were initially recruited to the study, detailed demographic data for analysis were only available for 778 subjects. Furthermore, because the data were reported from multiple study centers and COVID-19 pandemic occurred during the study, the data collected for those 778 subjects were also incomplete (as stated in the table).

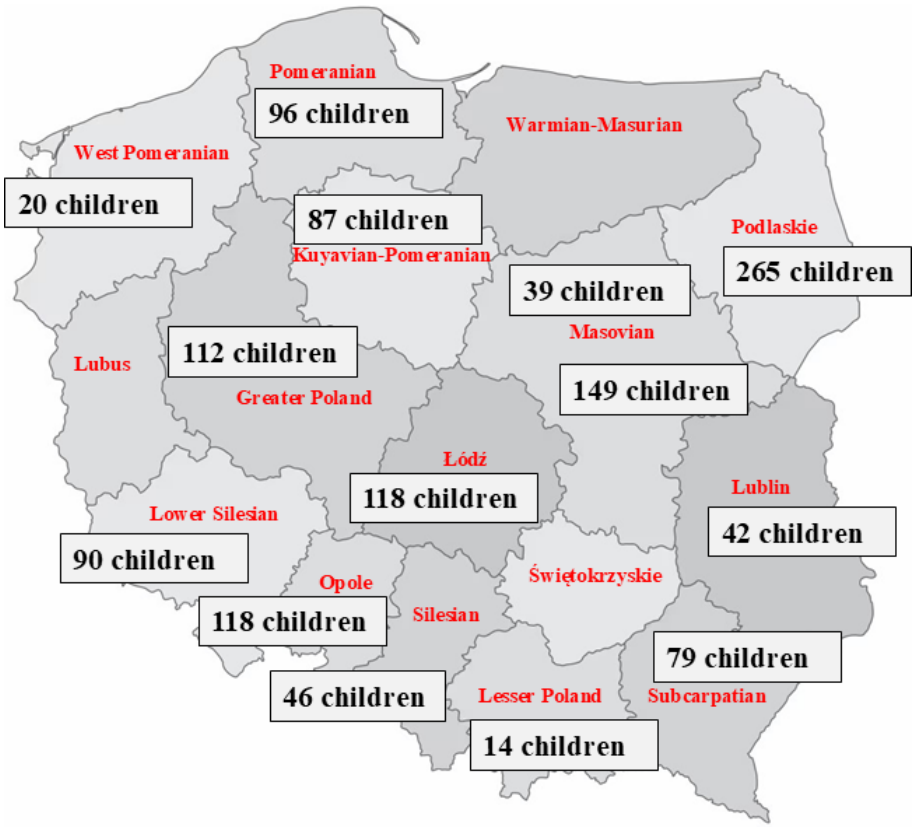


Fig. 1 Number of samples obtained from participating clinical centers in Poland.

Table 1. Characteristics of the study group.

Category	*n* (%)	Any positive IAb, *n* (%)	Single positive IAb, *n* (%)	≥ 2 positive IAbs, *n* (%)
Screening result	1,275	104 (8.1%)	35 (2.7%)	69 (5.4%)
Gender	778			
Female	372 (47.81%)	32 (8.60%)	12 (3.23%)	20 (5.38%)
Male	406 (52.19%)	28 (6.90%)	7 (1.72%)	21 (5.17%)
Age	757			
0–6 years	321 (42.40%)	15 (8.88%)	5 (1.56%)	10 (3.12%)
7–12 years	267 (35.27%)	31 (11.6%)	8 (2.99%)	23 (8.61%)
13–18 years	169 (22.33%)	15 (4.67%)	6 (3.55%)	9 (5.33%)
First-degree relative with T1D	721			
Sibling	582 (80.72%)	49 (8.42%)	15 (2.58%)	34 (5.84%)
Parent	139 (19.28%)	9 (6.47%)	3 (2.16%)	6 (4.32%)
No. of first-degree relatives with T1D	721			
One relative	699 (96.95%)	58 (8.30%)	18 (2.58%)	40 (5.72%)
Multiple relatives	22 (3.05%)			
Relative's age at diagnosis	560			
Child	510 (91.07%)	41 (8.04%)	12 (2.35%)	29 (5.69%)
Adult	50 (8.93%)	2 (4.00%)		2 (4.00%)

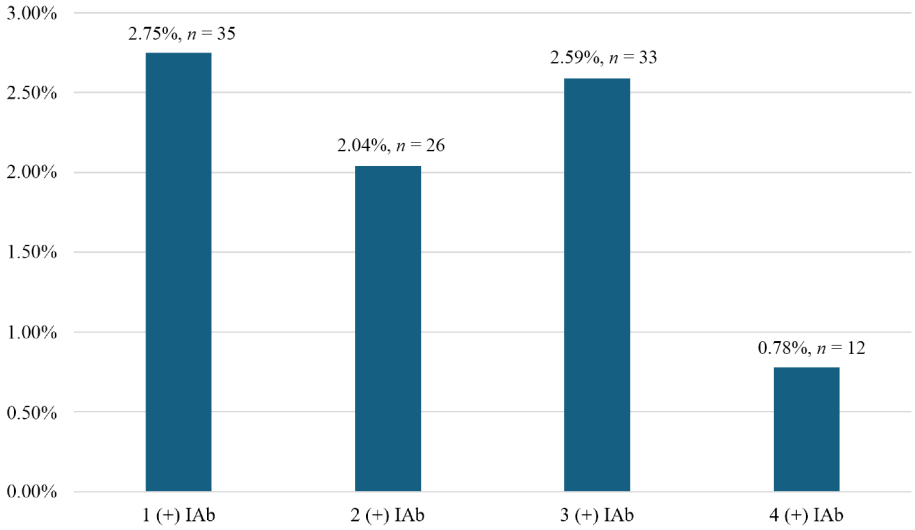


Fig. 2 Number of positive islet cell autoantibodies (IAbs), presented as percentages and the absolute number of subjects within the whole study group (n = 1,275).

For the 757 participants for whom the age information was available, the age ranged from 9 months to 18 years (median: 8.0 years, IQR: 4.0–12.0 years).

The observed median age of T1D onset in relatives (known for 557 participants) was 9 years (IQR: 5.0–12.0 years). The median duration of the relatives' disease (known for 564 participants) was 2 years (IQR: 0.5–9.0 years). In most cases, it was less than 12 months and it was < 1 month for 29.25% (n = 165), 1–6 months for 36.35% (n = 205), and 6–12 months for 16.84% (n = 95) cases. In only 11.88% (n = 67) of participants, the relative's disease duration was 12–24 months, and in 5.67% (n = 32), it was > 24 months. Among the group with available data, individuals whose relatives were diagnosed with T1D as adults appeared to participate in the screening at a younger age than the ones with relatives diagnosed with T1D during childhood (6.1 years vs 7.9 years; p = 0.030619). Moreover, according to the available data, participants whose parents were affected with T1D were statistically younger than those with affected siblings (6.4 years vs 8.1 years; p = 0.000756).

Among the whole study cohort, 8.1% (n = 104) were positive in the 3 Screen ICA^{TM} test: 2.7% presented with a single positive IAb (n = 35), and 5.4% (n = 69) presented with multiple positive IAbs (Fig. 2). There was a full agreement between the positivity in the 3

Table 2. Comparison of the participants' characteristics based on the IAb results.

Category	Negative vs positive IAbs	Single vs multiple IAbs	1 vs 2 vs 3 vs 4 positive IAbs
Gender (male/female)	p = 0.37306	p = 0.29908	p = 0.37444
Age (years)	p = 0.60960	p = 0.81950	p = 0.72100
First-degree relative with T1D (sibling/parent)	p = 0.44888	p = 0.87116	p = 0.67268
Number of first-degree relatives with T1D (single/multiple relatives)	p = 0.15885	p = 1.0000	p = 0.73849

Age was compared using Mann–Whitney U-test, categorical variables using the χ^2-test.

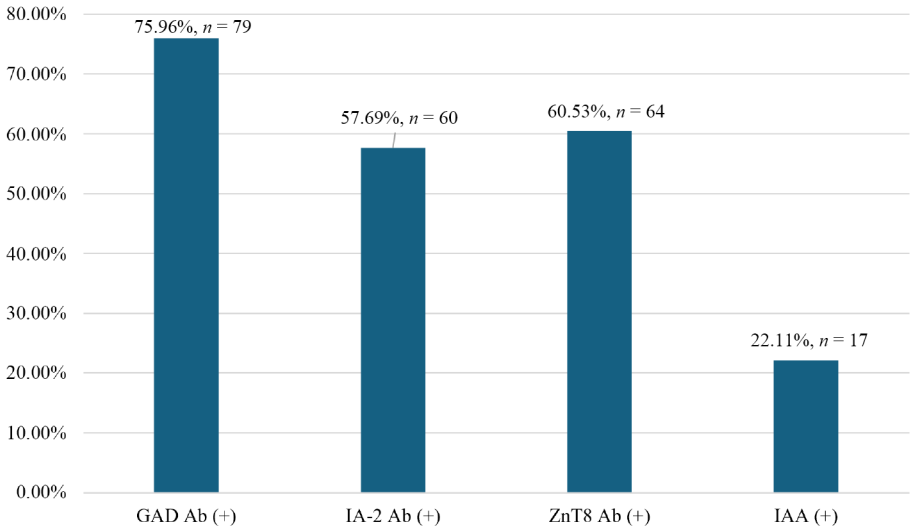


Fig. 3 Prevalence of IAb types presented as percentages and absolute number of subjects with positive islet autoimmunity (n = 104). GADAb, glutamic acid decarboxylase antibody; IAA, insulin autoantibody; IA-2Ab, islet antigen 2 antibody; ZnT8Ab, zinc transporter 8 antibody.

Table 3. Logistic regression analysis regarding the influence of participants' age on individual IAb positivity.

IAb	B coefficient	Standard error	Odds ratio	95% confidence interval	p-value
GADAb	0.059	0.031	1.061	0.999–1.128	0.05007
IA2Ab	0.036	0.037	1.037	0.964–1.114	0.32896
ZnT8Ab	0.052	0.033	1.054	0.988–1.123	0.10959
IAA	0.053	0.071	1.055	0.918–0.121	0.44878

B stands for "B coefficient" - a parameter of logistic regression analysis.

Screen ICA™ test and the individual antibody ELISAs. All samples found to be positive in the 3 Screen ICA™ test were positive in at least one of the individual ELISAs. There were 26 children with two antibodies, 33 with three antibodies, and 10 with four antibodies. A statistically significant positive correlation of the 3 Screen ICA™ results with GADAb (n = 104; p = 0.75), IA-2Ab (n = 104; p = 0.58), ZnT8Ab (n = 104; p = 0.51), and IAA (n = 104; p = 0.36) was observed; for all IAbs, $p < 0.05$.

On the basis of the available demographic data, we have not found any statistical differences between individuals with positive versus negative screening results regarding gender, age, and both the number and type (parent/sibling) of relatives with T1D. Among antibody-positive individuals with known age, gender, and relatives' status, no statistically significant differences were identified among the participants with a specific number of positive IAbs, or between subjects with single and multiple positive IAbs (Table 2).

GADAbs were the most frequently reported (76.0% of antibody-positive participants, n = 79), whereas IAA were the least frequent (22.1% of antibody-positive participants, n = 23), as shown in Fig. 3. Considering the whole study group, GADAbs and ZnT8Abs were the most prevalent among participants aged 7–12 years (p = 0.13 and p = 0.16, respectively), whereas other IAbs showed a similar prevalence in all age groups. However, logistic regression analysis did not show statistically significant influence of age on any individual IAbs' prevalence (Table 3). The sensitivity analysis for the influence of age on the individual IAbs' prevalence is presented in Supplementary Tables S1, S2.

To analyze the frequency of individual IAbs among subjects with positive islet autoimmunity, we started with establishing different IAb configurations among participants with ≥ 2 positive IAbs. We have observed that among individuals with ≥ 2 positive IAbs, the combination of positive GADAbs, IA2-Abs, and ZnT8Abs was the most prevalent (n = 29, 42%) followed by all four IAbs (n = 10, 14.5%) and the combinations of positive GADAbs and ZnT8Abs (n = 9, 13.0%), and GADAbs and IA-2Abs (n = 8, 11.6%), as shown in Fig. 4.

Then we analyzed the frequency of antibodies' co-occurrence using the χ^2-test, as shown in Table 4. Only co-occurrence of IA2Abs and ZnT8Abs presented statistical significance.

Regarding the group with a single positive autoantibody (n = 35), GADAbs were observed to be the most frequent (45.7%, n = 16), whereas the least frequent in this group were IAAs (14.3%, n = 5) (Fig. 5).

Out of all children with multiple IAbs, 40 out of 69 (58%) individuals participated in the clinical staging with 60% (n = 24) of these were identified as Stage 1, 15% (n = 6) as Stage 2, and 25% (n = 10) as Stage 3. The median HbA_{1c} in these participants was 5.3%, and there was a significant difference among the stages, with a gradual increase in concentration from Stage 1 to Stage 3. The median fasting C-peptide was 1.4 ng/dL and was the highest in individuals at Stage 1 (1.5 ng/dL). Median OGTT results in the whole group were 87.0 mg/dL for fasting glucose, 124.5 mg/dL for 60-min glucose, and 111.0 mg/dL for 120-min glucose; detailed OGTT results are presented in Table 5.

Individuals diagnosed with Stage 3 presented at a median age of 8 years, 33% were boys, of whom 60% had siblings and 40% had parents with T1D. All were positive for multiple IAbs (40% were positive for two IAbs, 40% for three IAbs, and 20% for four IAbs). Furthermore, 90% of these patients were positive for GADAb, 80% for IA-2Ab, 70% for ZnT8Ab, and 20% for IAA. Notably, none of the patients with Stage 3 developed DKA at the time of the diagnosis.

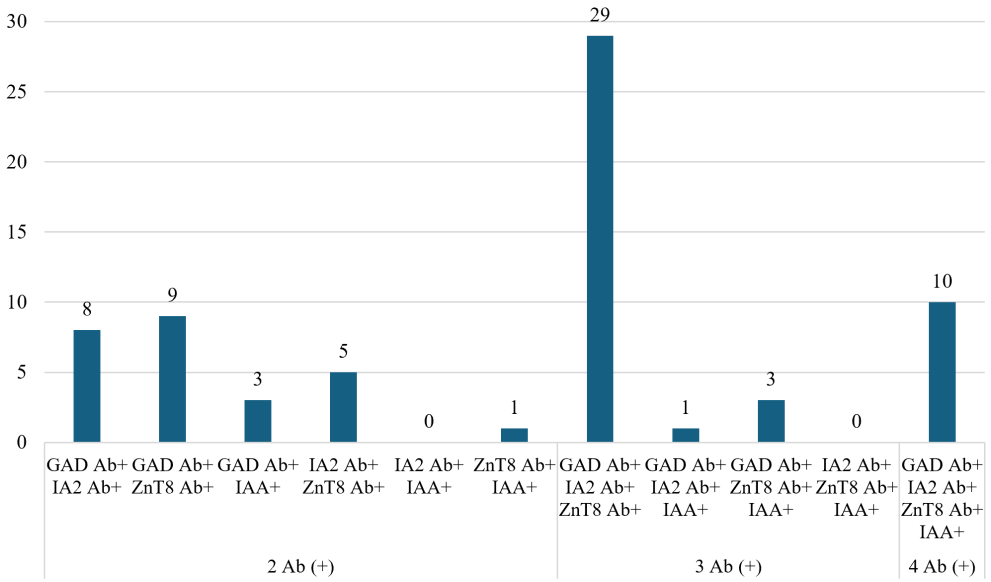


Fig. 4 Analysis of IAbs' prevalence in subjects with multiple positive autoantibodies: two positive IAbs (2Ab+), three positive IAbs (3Ab+), and four positive IAbs (4Ab+), presented as the number of individuals. GADAb, glutamic acid decarboxylase antibody; IAA, insulin autoantibody; IA-2Ab, islet antigen 2 antibody; ZnT8Ab, zinc transporter 8 antibody.

Table 4. Antibody co-occurrence analysis among participants with ≥ 2 positive IAbs.

Antibody co-occurrence	*n* (%)	χ^2	Degrees of freedom	*p*-value
GADAb+ and IA-2Ab+	48 (46.2%)	1.266670	1	0.26039
GADAb+ and ZnT8Ab+	51 (49.0%)	1.265114	1	0.26069
GADAb+ and IAA+	17 (16.4%)	0.067865	1	0.79447
IA-2Ab+ and ZnT8Ab+	**44 (42.3%)**	**8.335757**	1	**0.00389** *
IA-2Ab+ and IAA+	11 (10.6%)	1.177716	1	0.27782
ZnT8Ab+ and IAA+	14 (13.5%)	0.005582	1	0.94044

Here, *p*-values < 0.05 are in bold. Statistical significance with *post hoc* Bonferroni correction is marked with *. GADAb, glutamic acid decarboxylase antibody; IAA, insulin autoantibody; IA-2Ab, islet antigen 2 antibody; ZnT8Ab, zinc transporter 8 antibody.

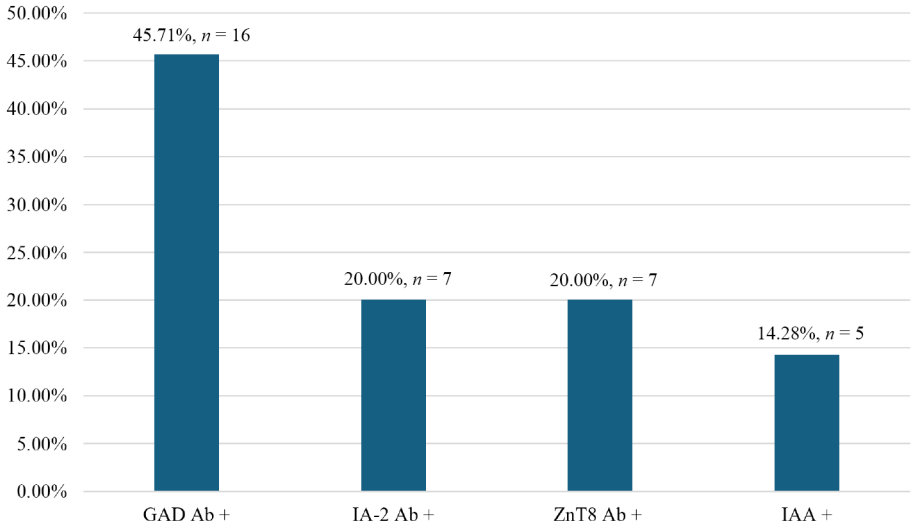


Fig. 5 Prevalence of individual IAbs, presented as percentages and absolute numbers of subjects with a single positive IAb (n = 35). GADAb, glutamic acid decarboxylase antibody; IAA, insulin autoantibody; IA-2Ab, islet antigen 2 antibody; ZnT8Ab, zinc transporter 8 antibody.

Discussion

To our knowledge, this is the first study aiming at the early detection of preclinical T1D in the population of Polish children and adolescents with a first-degree family history of T1D. The multicenter study design allowed for the enrollment of a large cohort of individuals with increased risk of T1D developing.

The most important finding of the current study is the identification of multiple positive IAbs in 5.4% of the study population. Furthermore, 2.7% participants were positive for a single positive IAb, and, overall, 8.1% of the screened population were positive for single or multiple autoantibodies. Our results can be compared with IAbs' presence in about 5% of first-degree relatives of T1D patients demonstrated in the TrialNet study[59] and with 6.5% of genetically

Table 5. Metabolic staging results.

	Stage 1 (n = 24; 60%)	Stage 2 (n = 6; 15%)	Stage 3 (n = 10; 25%)	p-value
Gender (% of boys)	41.7%	83.3%	30.0%	0.101
Age [years]	8.5 (1.0–18.0)	16.0 (5.0–18.0)	8.0 (3.0–11.0)	0.53
HbA_{1c} (%)	5.1 (4.8–6.0)	5.5 (5.1–5.9)	6.0 (5.57–10.5)	**< 0.001**
OGTT – fasting (mg/dL)	86.0 (68.0–94.0)	102.0 (83.0–114.0)	98.0 (80.0–101.0)	**< 0.007**
OGTT – 60 min (mg/dL)	115.4 (74.0–168.0)	216.0 (188.0–226.0)	224.0 (127.0–467.0)	**< 0.003**
OGTT – 120 min (mg/dL)	104.0 (71.0–128.0)	153.0 (117.0–184.0)	239.0 (105.0–247.0)	**< 0.001**
Fasting C-peptide (ng/dL)	1.5 (0.6–2.0)	1.1 (0.6–1.6)	1.1 (0.1–1.6)	0.4

Data are presented as the median and min–max; data with $p < 0.05$ are in bold.

predisposed children followed in the TEDDY study[28]. The study performed by Battaglia et al. showed a lower prevalence of both multiple and single IAb positivity (2.7% and 1.83%, respectively) among a large group of relatives of T1D subjects; however, the study cohort included both children and adults[31]. Another study reported any positive IAb in 5.5% children and adolescents (all with a documented family history of T1D) and multiple IAbs in about half of these individuals; however, the study protocol did not include the assessment of ZnT8Abs[60]. Ghalwash et al. described the presence of ≥ 1 positive IAb in 23.4% of a multinational cohort of adolescents with an increased T1D risk, with 13.9% of the participants developing T1D before the age of 18 years[61]. In a recent Italian study, first- and second-degree relatives of T1D patients were screened at a median age of 17.6 years and 4.9% were found to be positive, with 3.1% presenting with a single IAb and 1.8% presenting with multiple IAbs. During a median 10-year follow-up, 1.6% of the participants progressed to Stage 3, mostly in those positive for multiple IAbs[62].

In our study, the highest percentage of individuals with ≥ 2 positive IAbs was observed in the group of 7–12-year-olds, followed by the youngest group (aged 1–6 years). As islet autoimmunity precedes the disease's onset by months or even years, our finding is consistent with the reported peak incidence of T1D occurring at the ages of 5–7 years, and a second peak between the ages of 10–12 years[63].

Metabolic staging of 40 individuals with ≥ 2 positive IAbs revealed preclinical Stage 1 in 60% of subjects, preclinical Stage 2 in 15% of subjects, and another 25% subjects presented with glycemic values consistent with an early diagnosis of clinical diabetes. Importantly, among all the patients with Stage 3, none developed DKA at the time of the diagnosis.

T1D is reported to be more common in the male gender, in contrast to other autoimmune diseases, which are more commonly described in girls[64]. However, in our cohort, percentages of those with multiple positive IAbs were similar in both genders, and we observed a numerical superiority of girls in individuals who were then diagnosed with Stage 3. We have not found statistically significant differences in the number of positive IAbs regarding gender and age (in the cohort with available demographic data), and regarding the number and type (parent/sibling) of relatives with T1D.

In the Type 1 Diabetes TrialNet Pathway to Prevention Study over 220,000 individuals were screened, and at least one IAb was found in about 5% of healthy T1D relatives and multiple IAbs in half of these[31]. Another study conducted on a large cohort of relatives reported GADAb and IAA as the most frequently found[65]. The frequent occurrence of GADAb at the time of T1D diagnosis (30%–80%) and their reported presence in the preclinical phase of the disease (91%) make them important indicators of disease progression[66–68]. Our data support the previous observation that GADAbs are the most frequent IAb in individuals with a family history of T1D[65–68]. In our cohort, IAAs were the least frequent; however, this might have been a result of testing for IAAs only in the individuals already found to be positive by the 3 Screen ICA™ ELISA, further influenced by the sensitivity of the RIA. In a separate study, we carried out IAb screening in 1–9-year-old school children in the general population and assessed IAAs at the time of testing for GADAb, IA-2Ab and ZnT8Ab in via the 3 Screen ICA™ ELISA. In that study, IAAs were one of the most frequently reported antibodies (observed in about 50% of IAb-positive participants)[69]. It should be noted that the methods used in our two studies to detect IAAs were different (RIA in the current study versus ELISA in the general population study). Additionally, the wide age range of the participants and the inclusion of adolescents up to 18 years of age in the current study might have affected the observed prevalence of IAAs. IAAs are considered to be the least persistent of all antibodies, and once insulin therapy is initiated, they lose their diagnostic value, since antibodies against exogenous insulin are difficult to distinguish from autoantibodies. Their presence before the age of 5 years heralds the early onset of clinically overt diabetes, with rapid progression of the disease[70]. In our analysis, however, we did not observe a statistically significant influence of age on any of the IAbs' presence. The relatively high frequency of ZnT8Abs in our study population is in agreement with our recent results of screening children aged 1–9 years in the general population, where GADAbs and ZnT8Abs were the most prevalent in participants with a family history of T1D[69]. As the history of testing for ZnT8Ab is the shortest among all IAbs[71–73], their presence might have been underestimated in earlier studies. Notably, the presence of ZnT8Ab also predisposes individuals to the development of autoimmunity in other organs including endocrine glands[74]. Given the increased occurrence of other autoimmune conditions (hypothyroidism, celiac disease, Addison's disease) in patients with T1D, this finding should emphasize the need to screen for additional autoimmunity in individuals screened for T1D[75].

Numerous studies have shown that IA-2Ab positivity is closely related with the disease's pathogenesis, particularly with the faster progression to clinical T1D[76]. In our study, 42% of the children with ≥ 2 positive IAbs presented with the GADAb–IA-2Ab–ZnT8Ab configuration; however, only the co-occurrence of IA2Ab and ZnT8Ab was statistically significant. This finding is very interesting, as previous observations indicated positivity for IA-2Ab and ZnT8Ab to be highly predictive for a rapid progression of the disease[77].

In one study 3.5% of HLA-predisposed children recruited from the general population and monitored from birth had progressed to T1D by the age of 15.5 years, with IAA and ZnT8Ab appearing as the first IAb before 2 years of age. During the follow-up, IA-2Ab and GADAb positivity increased in those of preschool age, and GADAb continued to appear steadily until the age of 10–15 years. Interestingly, the presence of ZnT8Ab in the first positive sample was associated with a higher 10-year risk of clinically overt disease[78]. In contrast, in our cohort, we did not observe any relationship between the age and IAb occurrence; however, this could be related to an overall smaller number of screened individuals.

The early identification of individuals prone to developing T1D is considered to be one of the most important challenges of modern

diabetology. Individuals found to be at the preclinical stages of T1D may benefit from shorter paths to clinical trials and strategies aimed at delaying or preventing the onset of the disease[79]. Currently, significant emphasis is put on T1D screening in the general population as part of standard clinical care[80,81]. However, in the countries where general population screening programmes are not yet available or where public awareness of the disease is low, families with a history of T1D may result in better compliance with screening and follow-up procedures. In our cohort, we observed that most of the participants were siblings to children with T1D with a relatively short disease duration. This may indicate that families with the trauma of a recent diagnosis of the disease in one family member are more liable to participate in screening and may actively seek disease-modifying therapy for other children. Given that the long-term goal of T1D screening programs is to identify individuals at a risk of or with preclinical T1D and offer them treatment options to delay or prevent the condition, screening children and adolescents from affected families appears fully justified.

Limitations of the study

Although 1,275 individuals were tested for IAb, the collected demographic data and details regarding T1D-affected relatives were unfortunately incomplete. The multicenter design of the study and the disruption caused by the COVID-19 pandemic introduced unexpected challenges into the data collection process. Furthermore, follow-up data were available only for 55.5% of subjects with multiple positive IAbs. This could be explained by the limited access to specialist medical care during the COVID-19 pandemic, as well as the migration of families to other parts of the country during follow-up. We also hypothesize that some families declined to proceed with the OGTT out of parental concerns regarding the requirement for multiple blood samples. A further limitation is related to the testing protocol for IAbs. As per the study protocol, only one blood sample was collected from the participants and sent to FIRS Laboratories RSR Ltd in Cardiff for testing. Consequently, the confirmation tests for individual IAbs were carried out using the same blood sample as the 3 Screen ICA™ ELISA. In the ideal scenario, a confirmatory test would be done on a separate blood sample. However, this would increase a burden and volume of the blood sampling at screening. A second sample to confirm the persistence of the IAbs also was not collected, as our study was designed prior to the publication of the guidance for general population screening[80,81]. Furthermore, positive samples were retested using the same assay platform (ELISA), rather than alternative IAb assays based on different methodologies. In addition, the contribution of transient or low-affinity IAbs to positive ELISA results could not be ruled out. The abovementioned limitations in antibody testing could have led to overestimation of the IAb positivity rate. Moreover, the prevalence of IAA positivity may be underestimated, as the IAA was not analyzed in all participants but only in individuals with a positive 3 Screen ICA™ result. Selecting a more sensitive method for detecting IAA could also increase the observed prevalence of these specific IAbs. Combining IAb screening with a genetic risk score could also enhance the predictive sensitivity of our program.

Conclusions

The results of our study highlight the importance of IAb screening programmes, especially in individuals with an already increased risk of T1D development. Early detection of IAbs by 3 Screen ICA™ ELISA allows for the identification of preclinical T1D before dysglycemia occurs. Further follow-up consisting of metabolic staging, early education, and multidirectional diabetes care can prevent the occurrence of DKA associated with severe clinical manifestations. Implementation of screening programs in routine clinical practice may pave the way for innovative therapeutic interventions in individuals with a high risk of the disease.

Ethical statements

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Bioethics Committee of Medical University of Białystok (R-I-002/616/2018) on 20 December 2018. Before sample collection, written consent was obtained from all participants and/or their parents/caregivers.

During the preparation of this work, the authors confirm that artificial intelligence (AI) tools were not used for writing, data analysis, statistical computation, or figure/image preparation.

Author contributions

The authors confirm their contributions to this study as follows: Noiszewska K: conceptualized and designed the study, contributed to funding acquisition, formal analysis and investigation, drafted the manuscript; Jamiołkowska-Sztabkowska M: conceptualized and designed the study, contributed to formal analysis and investigation, drafted, reviewed and edited the manuscript; Zasim A, Polkowska A: contributed to patient recruitment and data acquisition, performed clinical follow-up; Mazur A, Brzuszek M, Niechciał E, Wysocka-Mincewicz M, Myśliwiec M, Szmigierko-Kawko M, Noczyńska A, Zubkiewicz-Kucharska A, Chobot A, Górska-Flak K, Szadkowska A, Wyka K, Pietrzak I, Robak-Kontna K, Żbikowska-Bojko M, Jarosz-Chobot P, Rusak E, Beń-Skowronek I, Nazim J, Groele L, Walczak M, Marcinkiewicz K, Horodnicka-Józwa A: contributed to patient recruitment and data acquisition, performed clinical follow-up, and approved the version to be published; Bossowski F, Bossowska M, Nesterowicz M, Anikiej K: assisted in data acquisition; Powell M, Furmaniak J, Amoroso M, Chen S, Smith BR: performed laboratory analyses, guided study methodology, revised the manuscript critically for important intellectual content, and approved the version to be published; Bossowski A: conceptualized and designed the study, contributed to funding acquisition, provided oversight and coordination for the project, critically reviewed and revised the manuscript, gave final approval of the version to be published. All authors reviewed the results and approved the final version of the manuscript.

Data availability

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

Acknowledgments

We would like to thank RSR Ltd, Cardif, UK, which provided diabetes-associated autoantibody diagnostic kits and carried out diabetes-associated antibody tests.

Funding

The study was funded by the Polish Diabetes Association as part of a research grant awarded in 2019 to Klaudyna Noiszewska and by the Medical University of Białystok (funding number B.SUB.25.420).

Conflict of interest

Michael Powell, Marie Amoroso, Shu Chen, Jadwiga Furmaniak, and Bernard Rees Smith are/were employees of RSR Ltd. RSR Ltd is a developer of medical diagnostics equipment, including kits for measuring islet cell autoantibodies. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary information accompanies this paper online at: https://doi.org/10.48130/pedi-0026-0012.

Dates

Received 29 September 2025; Revised 11 August 2026; Accepted 3 September 2026; Published online 28 October 2026

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