Barbara Głowińska-Olszewska*, Justyna Michalak, Włodzimierz Łuczyński, Maria del Pilar Larosa, Shu Chen, Jadwiga Furmaniak, Bernard Rees Smith and Artur Bossowski

Organ-specific autoimmunity in relation to clinical characteristics in children with long-lasting type 1 diabetes

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Abstract

Background: The aim of this study was to assess the prevalence of diabetes and other organ-specific autoantibodies (Ab) associated with various autoimmune conditions, in Polish children with type 1 diabetes mellitus (T1DM).
Methods: In this study 114 patients, aged 13.4 years, with mean diabetes duration 5.2 years were included. Ab to islet cell antigens: glutamic acid decarboxylase (GAD), insulinoma antigen 2 (IA-2), zinc transporter 8 (ZnT8), together with thyroid peroxidase Ab (TPO Ab), thyroglobulin Ab (Tg Ab), tissue transglutaminase Ab (tTG Ab) and 21-hydroxylase Ab (21-OH Ab) were measured.
Results: The prevalence of at least one diabetes associated Ab was found in 87%, with the highest prevalence of 64% for ZnT8 Ab. In patients with disease duration <5 years, at least one antibody was present in 90%, the most prevalent was ZnT8 Ab (72%). In patients with duration >10 years, 50% had at least one antibody. The prevalence of other than islet cell autoimmunity was high (34%). Thyroid Ab were detected in 26% patients, 42% in girls vs. 8% in boys, $p<0.001$. tTG Ab were found in 11% patients, with a greater prevalence in children with early onset ($p=0.01$). 21-OH Ab were found in 2.6% T1DM patients.
Conclusions: Islet Ab were found in most T1DM children and remained positive even 10 years after onset. ZnT8 Ab emerged as an important marker for the diagnosis of T1DM in the Polish children. Screening for non-diabetes Ab in T1DM may be helpful in identifying subclinical cases of autoimmune thyroid, celiac or Addison's disease (AD).

Keywords: Addison autoimmunity; celiac; children; islet autoimmunity; thyroid; type 1 diabetes.

***Corresponding author: Barbara Głowińska-Olszewska,** MD PhD, Department of Pediatrics, Endocrinology, Diabetology with Cardiology Division, Medical University of Białystok, 17 Waszyngtona Street, 15-274 Bialystok, Poland, Phone/Fax: +48 85 7450 730, E-mail: bglowinska@poczta.onet.pl
Justyna Michalak, Włodzimierz Łuczyński and Artur Bossowski: Department of Pediatrics, Endocrinology, Diabetology with Cardiology Subdivision, Medical University of Białystok, Poland
Maria del Pilar Larosa, Shu Chen, Jadwiga Furmaniak and Bernard Rees Smith: FIRS Laboratories, RSR Ltd. based at Parc Ty Glas, Llanishen, Cardiff, UK

Introduction

Type 1 diabetes mellitus (T1DM) is a HLA-linked disease resulting from immune-mediated destruction of the insulin-producing β-cells in pancreatic islets [1, 2]. Autoantibodies (Ab) to islet cell antigens such as glutamic acid decarboxylase (GAD), insulinoma antigen 2 (IA-2), insulin and the more recently identified zinc transporter 8 (ZnT8) [3] are serological markers of T1DM. Measurement of these specific Ab has an important role in clinical practice including classification of diabetes, identification of individuals at risk of developing T1DM and as endpoints in observation studies [4]. The prevalence of islet cell Ab in T1DM varies in relation to patient's age and ethnic origin, as well as to disease duration [5–8]. In our study, we have assessed the prevalence of diabetes associated Ab in Polish children with T1DM. Also, the relationship between Ab positivity and body mass index (BMI), glycosylated haemoglobin, patient's age, disease onset and duration was analysed.

T1DM often occurs together with other autoimmune diseases, including autoimmune thyroid disease (AITD), celiac disease (CD) and Addison's disease (AD) [9–12]. In particular, T1DM, AITD and AD present as a clinical triad associated with autoimmune polyendocrine syndrome type II (APS type II). Consequently, we also assessed the prevalence of non-diabetic organ-specific autoimmunity in the children.

Materials and methods

Patients

We studied 114 individual children and adolescents with T1DM, who were seen in the Department of Pediatrics, Endocrinology, Diabetology with Cardiology Division, Medical University of Białystok from March 2012 through to June 2013. The diagnosis of T1DM was made in accordance with American Diabetes Association criteria [13]. The mean age of the patients was 13.4±3.8 years, with mean age at onset 8.2±4.1 years, mean diabetes duration 5.2±3.6 years and mean hemoglobin A_{1c} (HbA_{1c}) 8.0%±1.5% (46% male, 54% female; Table 1). The control group was recruited from children hospitalised with mild heart diseases. Serum samples from 51 children who had no autoimmune conditions and no family history of autoimmunity were included in the control group. The mean age was 13.3±3.6 years (51% male, 49% female).

All patients with T1DM were evaluated for: age, sex, height, weight, body mass index (BMI) and BMI standardized deviation score (SDS) (Table 1). Furthermore disease-associated parameters were considered including disease duration, age at disease onset and glycosylated hemoglobin levels (HbA_{1c}; mean of previous year measurements) (Table 1). Patients with HbA_{1c} values ≤7.5% were considered to have adequate metabolic control [14]. The collected data were referenced against the most recently updated normal ranges for Polish children, including weight centile charts according to sex and age (Polish nationwide OLAF project). Normal weight was defined as BMI-SDS>–1 and <1, overweight as ≥1 and <2 and obesity as ≥2 [15].

Autoimmune diseases other than T1DM were assessed by clinical symptoms and signs and/or pathology results followed by screening for the disease related Ab. The diagnosis of clinical autoimmune (Hashimoto's) thyroiditis was based on the elevated level of thyroid-stimulating hormone (TSH), positivity for thyroid peroxidase (TPO) and/or thyroglobulin Ab (Tg Ab) and thyroid ultrasound presentation (Immunodiagnostic test Varelisa – Variable Enzyme Linked Immuno Sorbent Assay, Pharmacia Upjohn Diagnostics, GmbH & Co. KG., Freiburg, Germany. The results were read on a photometer (STAT FAX 303 PLUS, ANALCO-GBG, Krakow, Poland)). None of our patients was diagnosed with Graves' disease. Serum levels of free thyroxine (fT4), free triiodothyronine (fT3) and TSH were determined by electrochemiluminescence "ECLIA" with Cobas e 411 analyzer (Roche Diagnostics, Warsaw, Poland). Normal values for fT4 ranged between 0.71 and 1.55 ng/dL, for fT3 between 2.6 and 5.4 ng/dL and for TSH between 0.32 and 5.0 (mIU/mL). Patients with positive tissue transglutaminase Ab (tTG Ab) had CD diagnosis confirmed by duodenal biopsy.

AD was diagnosed based on the presence of clinical signs and symptoms of adrenal insufficiency, low plasma cortisol levels, increased ACTH levels and with Synacthen test. The patient with AD was started on substitutive therapy for hypoadrenalism at the time of the diagnosis. However, 21-hydroxylase Ab (21-OH Ab) test result was not available at the time of diagnosis. In this patient, AD presented a year before T1DM.

Ab assays

On the day of sample collection, fasting venous blood was drawn, serum separated and stored at –80 °C.

GAD Ab were measured by ELISA [16], using kits from RSR Ltd. (Cardiff, UK) and values of GAD Ab of ≥5.0 WHO units/mL (National Institute for Biological Standards and Control; NIBSC 97/550) were considered positive following the manufacturer's recommendations. IA-2 Ab were measured using an immunoprecipitation assay (IPA) based on ^{125}I-labeled IA-2 [17] (kits from RSR Ltd.). IA-2 Ab levels >125 WHO units/mL were considered positive in this assay as recommended in the kit instructions. Ab to ZnT8 were measured by ELISA [18] using kits from RSR Ltd. and values of ZnT8 Ab of ≥15 units/mL were considered positive, following the manufacturer's recommendations. In

Table 1: General characteristics of the entire study group and comparison between boys and girls.

	Total study group	Boys	Girls	χ^{2a}	p-Value
Number	114	52 (45.6)	62 (54.4)		
Age, years	13.4±3.8	14.0±4.1	13.0±3.5		0.19
Diabetes duration, years	5.2±3.6	5.4±4.1	5.1±3.17		0.70
Age of onset, years	8.2±4.1	8.5±4.4	7.8±3.8		0.36
HbA_{1c}, %	8.0±1.5	8.2±1.8	7.8±1.29		0.19
HbA_{1c}<7.5%	50 (44)	20 (38)	30 (48)	0.29	0.58
BMI, kg/m^2	20.2±3.8	20.6±4.1	19.6±3.5		0.27
SDS-BMI	0.4±1.1	0.4±1.3	0.35±0.9		0.55
Overweight/obesity	27 (23.6)	13 (25)	14 (23)	0.35	0.54
GAD Ab+	70 (61)	34 (65)	36 (58)	0.6	0.40
IA-2 Ab+	55 (48.2)	25 (48)	30 (48)	0.001	0.97
ZnT8 Ab+	73 (64)	34 (65)	39 (62)	0.007	0.78
At least one diabetes Ab+	99 (86.8)	44 (84)	55 (88)	0.41	0.51
Thyroid disease	26 (22.8)	3 (5.77)	23 (37)	**15.7**	**<0.001**
Thyroid Ab+	30 (26.3)	4 (7.69)	26 (41.9)	**17**	**<0.001**
Celiac disease	9 (7.8)	2 (3.85)	7 (12.3)	1.25	0.26
tTG Ab+	12 (10.8)	4 (7.69)	8 (13.5)	0.47	0.49

[a]Differences between boys and girls in χ^2 test. Data are presented as mean±SD for individual parameters, or number of patients (%). HbA_{1c}, hemoglobin A_{1c}; BMI, body mass index; GAD, glutamic acid decarboxylase; Ab, autoantibody; IA-2, insulinoma antigen 2; ZnT8, zinc transporter 8; tTG Ab, tissue transglutaminase Ab. Bold values indicate statistically significant difference.

the Islet Autoantibody Standardization Program 2013 (IASP, 2013), the GAD Ab ELISA showed 78% sensitivity and 98% specificity, IA-2 Ab IPA 66% sensitivity and 98% specificity and ZnT8 Ab ELISA 74% sensitivity and 99% specificity. Ab to insulin were measured using an immunoprecipitation assay based on ^{125}I-labeled insulin [17] (kits from RSR Ltd.). Insulin Ab levels >0.4 units/mL were considered positive in the assay as suggested in the kit instruction. However, majority of patients in the study had started insulin therapy, consequently insulin Ab results were not included in the analysis.

Serum 21-OH Ab were measured by an immunoprecipitation assay based on ^{125}I-labeled recombinant 21-OH [19] (kits from RSR Ltd.). 21-OH Ab levels >1.0 units/mL were considered positive in the assay as suggested in the kit instructions. This assay was assessed in the 1st International Standardization Program for determination of 21-OH Ab and reported sensitivity was 91.3%, specificity 98% and area under the ROC curve 0.95.

TPO Ab and Tg Ab were measured in all samples by electrochemiluminescence "ECLIA" with a Modular Analytics E170 analyzer (Roche Diagnostics, Poland). The negative values for thyroid Ab were: 0–34 IU/mL for TPO Ab and 0–115 IU/mL for Tg Ab. Ab to tTG Ab were measured by ELISA, with values <10 IU/mL considered as normal (INOVA, Biocom).

The study was approved by the Bioethics Committee, Medical University of Białystok, Poland. Caregivers and children were informed about the purpose and nature of the study. The caregivers gave written consent, whereas the children expressed spoken consent before examination.

Statistical analysis

Data were analysed using Statistica program version 10.0. The unpaired Student's test was performed for comparison of means between two groups, ANOVA test was used to compare means between more than two groups for continuous variables. χ^2-test was used for comparison of percentages among different subgroups of patients for categorical variables. Statistical significance difference was defined as $p<0.05$

Results

Out of 114 T1DM children studied, 62 (54%) were female. Mean age at onset of diabetes was 8.2 years (ranged from 1 to 17 years), mean diabetes duration was 5.2 years (ranged from 0 to 18 years) and mean age at sample collection was 13.4 (range from 3 to 18 years). Fifty of 114 patients (44%) had optimal glycemic control, while 27 (24%) were overweight or obese (Table 1).

GAD Ab positivity was detected in 70 (61%) T1DM patients, IA-2 Ab positivity in 55 (48%) patients while ZnT8 Ab positivity was the highest and found in 73 (64%) patients. Ninety-nine (87%) patients tested were positive for at least one diabetes associated Ab and there was no difference in the prevalence for GAD Ab, IA-2 Ab and ZnT8 Ab between males and females (Table 1). Out of all 114 T1DM patients in the study, 30 (26%) patients were positive for TPO Ab and/or Tg Ab and 26 of these were diagnosed with autoimmune thyroid disease. Twelve (11%) patients were positive for tTG Ab of whom nine had clinical CD confirmed by duodenal biopsy. Three patients (2.6%) were positive for 21-OH Ab with levels of 2.7, 4.6 and 90.3 unit/mL, respectively. The patient with a 21-OH Ab level of 4.6 unit/mL was diagnosed with AD. Taken together, 34% (n=39) patients were positive for Ab other than diabetes associated Ab, i.e. thyroid Ab (TPO and/or Tg), tTG Ab or 21-OH Ab. Six patients (5%) were positive for two types of these Ab. None were positive for all three types (thyroid, gut and adrenal) Ab. Thyroid Ab were more prevalent in females than males (42% vs. 8%; $p<0.001$), while no gender differences were observed for the prevalence of tTG Ab or 21-OH Ab (Table 1).

GAD Ab positive patients had shorter diabetes duration (4.5±2.9 years vs. 6.5±4.2 years, $p=0.004$) and were older at the age of onset (9.2±3.8 vs. 6.5±4.2, $p<0.001$) compared to GAD Ab negative patients (Table 2). IA-2 Ab positive patients had shorter disease duration (4.0±3.1 years vs. 6.4±3.7 years, $p=0.001$), and were younger (12.4±4 vs. 14.4±3.2, $p=0.004$) than IA-2 Ab negative patients (Table 2). ZnT8 Ab positive patients had shorter disease duration: 4.7±3.3 years vs. 6.2±3.9 years ($p=0.003$) compared to patients negative for ZnT8 Ab (Table 2). Ninety-nine (87%) patients who were positive for at least one diabetes Ab had shorter disease duration (4.8±3.2 vs. 8.0±4.9 years, $p=0.001$) and were older at diabetes onset (8.6±3.9 vs. 5.1±4.1 years, $p=0.001$) compared to 15 (13%) diabetes Ab negative patients. In this study four (5%) of diabetes associated Ab positive patients had clinically recognized CD, compared to four (26%) Ab negative patients ($\chi^2=5.1$, $p=0.001$). There was no relationship between diabetes associated Ab positivity and thyroid Ab or tTG Ab positivity, BMI or HbA_{1c} level. However, we found that the prevalence of obesity was higher in ZnT8 Ab positive patients (30% vs. 13%, $p=0.04$) (Table 2).

Patients were divided into three groups based on the disease duration (Table 3). Metabolic control (based on HbA_{1c} results) showed a tendency to decrease as the disease duration increased (7.5%, 8.5% and 8.7% for disease duration <5 years, 6–10 years and >10 years, respectively). The positivity for GAD Ab and IA-2 Ab decreased significantly in patients with longer disease duration (Table 3). In particular, in T1DM children with disease duration <5 years, at least one Ab was present in 55 out of 61 patients (90%), the most prevalent were ZnT8 Ab (72%) followed by GAD Ab (67%) and IA-2 Ab (59%). Similar diabetes Ab positivity was observed for the patients with disease duration of 6–10 years. However, in the case of T1DM children with

Table 2: Comparison between diabetes associated antibody positive and negative patients.

	GAD Ab+	GAD Ab−	χ^2	p-Value	IA-2 A+	IA-2 Ab−	χ^2	p-Value	ZnT8 Ab+	ZnT8 Ab−	χ^2	p-Value
Number	70 (61)	44 (39)	1.8	0.20	55 (48)	59 (52)			73 (64)	41 (35)		
Age, years	13.7±3.7	13±3.9		0.35	12.4±4	14.4±3.2		**0.004**	13.1±3.9	14.1±3.6		0.1
Duration, years	4.5±2.9	6.5±4.2		**0.004**	4.0±3.1	6.4±3.7		**0.001**	4.7±3.3	6.2±3.9		**0.003**
Age of onset, years	9.2±3.80	6.5±4.0		**<0.001**	8.4±4.1	8.0±4.1		0.63	8.3±4.1	7.8±4.2		0.52
HbA_{1c}, %	8.0±1.6	7.9±1.4		0.80	7.8±1.4	8.1±1.6		0.30	8.0±1.6	8.0±1.3		0.90
HbA_{1c}<7.5%	33 (48)	17 (39)	0.8	0.35	28 (51)	22 (37)	1.9	0.16	32 (43)	18 (47)	0.12	0.72
BMI, kg/m^2)	20±3.6	20±4.08		0.50	19.4±4.0	20.9±3.4		**0.03**	20.3±3.5	2.01±3.9		0.80
SDS-BMI	0.27±1.2	0.63±1.1		0.10	0.35±1.1	0.47±1.1		0.56	0.25±1.2	0.5±1.1		0.26
Overweight/obesity	14 (20)	13 (30)	1.3	0.24	14 (25)	13 (22)	0.14	0.70	22 (30)	5 (13)	**3.9**	**0.04**
Thyroid disease	13 (18)	13 (29.5)	1.8	0.20	12 (21.8)	14 (23)	0.05	0.8	16 (21)	10 (24)	0.09	0.76
Thyroid Ab+	15 (21)	15 (34)	2.2	0.13	12 (21)	18 (30.5)	1.1	0.29	20 (27)	10 (24)	0.12	0.72
Celiac disease	3 (4.3)	6 (13)	3.2	0.14	3 (5.4)	6 (10.1)	0.8	0.35	3 (4)	6 (14.6)	2.6	0.10
tTG Ab+	3 (4.2)	9 (20.4)	**7.4**	**0.001**	5 (9)	7 (11.8)	0.26	0.60	9 (12)	3 (7.8)	0.5	0.40

Data are presented as mean±SD for individual parameters, or number of patients (%). HbA_{1c}, hemoglobin A_{1c}; BMI, body mass index; GAD, glutamic acid decarboxylase; Ab, autoantibody; IA-2, insulinoma antigen 2; ZnT8, zinc transporter 8; tTG Ab, tissue transglutaminase Ab. Bold values indicate statistically significant difference.

Table 3: Analysis of various parameters for 114 T1DM patients based on disease duration and age of onset.

	Diabetes duration			χ^2	p-Value	Diabetes onset			χ^2	p-Value
	<5 years	6–10 years	>10 years			<5 years	6–10 years	>10 years		
Number	61 (53)	43 (37)	10 (8.77)			30 (26)	45 (40)	39 (34)		
Age, years	12.3±4.4	14.2±2.5	15.4±1.9		**<0.001**	10.5±4.5	13±3	16±1.8		**<0.001**
Duration, years	2.5±1.7	7.3±1.2	12.7±2.2		**<0.001**	7.6±4.7	5.4±2.9	3.3±2.1		**<0.001**
Age of onset, years	9.7±4.2	7.2±2.8	2.8±1.7		**<0.001**	2.9±1.2	7.6±1.4	12.9±1.6		**<0.001**
HbA_{1c}, %	7.5±0.8	8.5±2.0	8.7±1.6		**0.002**	7.9±1.5	8.1±1.8	7.8±1.2		0.74
HbA_{1c}<7.5%	31 (52)	16 (38)	3 (30)	3.0	0.21	13 (44)	21 (47)	16 (42)	0.26	0.87
BMI, kg/m^2	19.8±4.3	20.44±2.9	21.4±3.9		0.44	18.7±3.6	19.5±3.6	22.2±3.7		**<0.001**
SDS-BMI	0.5±1.2	0.7±0.9	0.4±1.3		0.54	0.45±1.2	0.24±0.98	0.58±1.2		0.38
Overweight/obesity	19 (32)	7 (16.6)	1 (10)	4.4	0.10	6 (21)	7 (16)	14 (36)	5.1	0.07
GAD Ab+	41 (67)	27 (62)	2 (20)	8.1	**0.01**	11 (37)	31 (65)	28 (73)	9.5	**0.008**
IA-2 Ab+	36 (59)	18 (41)	1 (10)	9.2	**0.009**	14 (48)	21 (44)	20 (52)	0.53	0.76
ZnT8 Ab	44 (72)	25 (58)	4 (40)	4.8	**0.08**	18 (62)	28 (59)	27 (71)	1.2	0.53
At least one diabetes Ab+	55 (90)	39 (90)	5 (50)	13	**0.001**	20 (68)	43 (91)	36 (94)	**11**	**0.003**
Thyroid disease	11 (18)	12 (27)	3 (30)	1.7	0.42	8 (27)	11 (23)	7 (18)	0.8	0.67
Thyroid Ab+	13 (21)	14 (32)	3 (30)	1.7	0.40	9 (31)	16 (34)	5 (13)	5.1	0.07
Celiac disease	4 (6.5)	3 (6.9)	2 (20)	2.2	0.30	2 (6.9)	7 (14)	0 (0)	6.4	**0.03**
tTG Ab+	6 (10)	3 (7)	3 (30)	4.4	**0.01**	3 (10)	9 (20)	0 (0)	8.8	**0.01**

Data are presented as mean±SD for individual parameters, or number of patients (%). HbA_{1c}, hemoglobin A_{1c}; BMI, body mass index; GAD, glutamic acid decarboxylase; Ab, autoantibody; IA-2, insulinoma antigen 2; ZnT8, zinc transporter 8; tTG Ab, tissue transglutaminase Ab. Bold values indicate statistically significant difference.

disease duration >10 years, only five out of 10 patients (50%) had at least one antibody with ZnT8 Ab being the most prevalent (40%) followed by GAD Ab (20%) and IA-2 Ab (10%). We also observed that tTG Ab positive patients were more likely to be found in patients with a longer duration of diabetes (>10 years vs. <10 years, p=0.01), while thyroid Ab positive patients were equally distributed in all groups (Table 3). When the age at onset was also taken into consideration, ZnT8 Ab were the most prevalent (62% compared to 48% for IA-2 Ab and 37% for GAD Ab) in children who were younger at onset (<5 years of age) while in older children (>10 years) GAD Ab and ZnT8 Ab showed similar prevalence (73% and 71%, respectively) compared to IA-2 Ab at 52%. tTG Ab was more prevalent in children who were younger at onset (<10 years) than in older patients (p<0.05) while thyroid Ab were equally distributed in all children irrespective of age (Table 3).

When patients were divided into four groups based on their age at the time of the sample collection for this study (0–5, 6–10, 11–15 and >15 years; Table 4), the patients who

Table 4: Study parameters in patients of different ages at the time of sample collection.

	Patient age				χ^2	p-Value
	0–5 years	6–10 years	11–15 years	>15 years		
Number	5 (4)	18 (15)	51 (44)	40 (35)		
Age, years	4.2±0.8	7.7±1.1	13.5±1.4	17.1±0.8		**<0.001**
Diabetes duration, years	0.6±0.5	2.8±2.4	5.9±3.5	6.1±3.7		**<0.001**
Age of onset, years	3.6±0.5	4.8±2.7	7.6±3.6	11.0±3.7		**<0.001**
HbA_{1c}, %	7.8±1.2	7.1±0.7	7.9±1.4	8.5±1.9		**0.029**
HbA_{1c}<7.5%	2 (40)	13 (72)	22 (43)	13 (33)	9.0	**0.02**
BMI, kg/m^2	15.6±0.8	16.9±2.5	19.7±2.9	22.9±3.5		**<0.001**
SDS-BMI	−0.03±0.6	0.5±1.2	0.2±0.9	0.6±1.3		0.17
Overweight/obesity	0 (0)	6 (33)	6 (12)	15 (38)	11.0	0.01
GAD Ab+	3 (60)	10 (55)	30 (58)	27 (67)	1.5	0.65
IA-2 Ab+	4 (80)	12 (67)	24 (47)	15 (38)	7.0	0.06
ZnT8 Ab+	3 (60)	14 (78)	32 (62)	24 (60)	2.9	0.4
At least one diabetes Ab+	4 (80)	15 (83)	46 (90)	34 (85)	0.23	0.97
Thyroid disease	0 (0)	3 (17)	17 (33)	6 (15)	5.5	0.13
Thyroid Ab+	0 (0)	5 (28)	18 (58)	7 (17)	4.8	0.18
Celiac disease	0 (0)	1 (5.5)	6 (11)	2 (5)	1.7	0.60
tTG Ab+	0 (0)	4 (22)	6 (11)	2 (5)	4.8	0.18

Data are presented as mean±SD for individual parameters, or number of patients (%). HbA_{1c}, hemoglobin A1c; BMI, body mass index; GAD, glutamic acid decarboxylase; Ab, autoantibody; IA-2, insulinoma antigen 2; ZnT8, zinc transporter 8; tTG Ab, tissue transglutaminase Ab. Bold values indicate statistically significant difference.

were older had longer diabetes duration (0.6±0.5, 2.8±2.4, 5.9±3.5, 6.1±3.7 years, respectively, p<0.001). Furthermore, fewer of the older patients had HbA_{1c} <7.5% (40%, 72%, 43% and 33%, respectively, p=0.02). Percentages of overweight and obese patients increased with age and reached 38% in patients aged over 15 years, while no patients younger than 5 years were obese (p<0.01). IA-2 Ab were found in more children below the age of 10 years at the time of testing but the positivity for all diabetes Ab was comparable in all age groups. None of five patients younger than 5 years had thyroid Ab and tTG Ab whereas the percentage of patients with thyroid Ab and tTG Ab increased with age; group 6–10 years (28% and 22%, respectively) and group 10–15 years (58% and 11%, respectively). However the prevalence was lower in age group >15 years (17% and 5%, respectively) (Table 4).

T1DM patients who were diagnosed with thyroid disease (n=26) or CD (n=9) were predominantly female (89% and 78%, respectively) (Table 1). However, the presence of thyroid and CD was not significantly associated with patient age, duration of diabetes, BMI and HbA_{1c} levels (data not shown).

One patient, positive for 21-OH Ab (4.6 unit/mL) had T1DM for one year and was also diagnosed with AD. The other two patients were positive for 21-OH Ab (2.7 and 90.3 unit/mL, respectively) both 12 years old, had diabetes for 6 and 11 years, respectively without clinical symptoms of AD.

Analysis of the association between treatment of diabetes including metabolic control, body weight and organ-specific autoimmunity status is summarised in Table 5. Compared to children with poor metabolic control (HbA_{1c}>7.5%), children with good metabolic control (HbA_{1c} <7.5%) were younger (mean age of 12.6±3.9 years) (p<0.05), tended to have shorter diabetes duration (4.5±3.2 years) but there was no relationship between diabetes Ab positivity and the prevalence of AITD or CD. Overweight or obese T1DM children tended to be older at the onset of diabetes (9.5±4.2 years compared to 7.8±1.0 years) and had worse metabolic control (HbA_{1c} 8.4%±1.8% compared to 7.8%±1.4%). The positivity of ZnT8 Ab but not GAD Ab and/or IA-2 Ab was significantly higher in obese patients (81% vs. 60%, P = 0.04) compared to normal weight patients. None of the CD patients were overweight or obese (Table 5).

Out of the 51 control children tested for GAD Ab, IA-2 Ab, ZnT8 Ab, insulin Ab and 21-OH Ab, only one out of 51 was positive for GAD Ab and two out of 51 were positive for ZnT8 Ab, while the remaining 48 were negative for all the Ab studied (data not shown).

Discussion

We have studied the prevalence of diabetes associated Ab and their relationship to patient's age, the age at onset,

Table 5: Study parameters in patients with optimal vs. poor glycemic control and in obese patients vs. patients with normal weight.

	HbA_{1c}<7.5	HbA_{1c}>7.5	χ^2	p-Value	Obese/overweight	Normal weight	χ^2	p-Value
Number	50 (44)	64 (56)			27 (24)	87 (76)		
Boys/girls	20 (42)/30 (47)	30 (46)/34 (53)			13 (27)/14 (23)	39 (73)/48 (78)		
Age, years	12.6±3.9	14.2±3.68		**0.03**	14.4±4.1	13.1±3.7		0.10
Duration, years	4.5±3.2	5.9±3.9		0.16	4.9±2.9	5.3±3.8		0.57
Age of onset, years	8.1±4.2	8.2±4.1		0.88	9.5±4.2	7.8±1.0		0.06
HbA_{1c}, %	6.9±0.49	8.9±1.61		**0.001**	8.4±1.8	7.8±1.4		0.08
BMI, kg/m^2	19.5±3.4	20.8±4.1		0.07	24.5±3.5	18.8±2.7		**<0.001**
SDS-BMI	0.4±0.9	0.4±1.2		0.90	1.9±0.7	−0.07±0.7		**<0.001**
Overweight/obesity	10 (20)	17 (27)	0.9	0.39	27 (100)	0 (0)		
HbA_{1c}<7.5%	50 (100)	0 (0)			10 (37)	40 (47)	0.92	0.43
GAD Ab+	33 (66)	35 (54)	0.86	0.35	14 (51)	54 (64)	1.3	0.20
IA-2 Ab+	28 (56)	26 (41)	1.9	0.16	14 (51)	40 (47)	0.14	0.70
ZnT8 Ab+	32 (64)	41 (64)	0.12	0.72	22 (81)	51 (60)	3.9	**0.04**
Thyroid disease	12 (24)	14 (22)	0.28	0.59	6 (21)	20 (23)	0.1	0.70
Thyroid Ab+	12 (24)	18 (28)	0.02	0.80	9 (32)	21 (24)	0.54	0.46
Celiac disease	3 (6)	6 (9.3)	0.02	0.56	0 (0)	9 (10)	3.4	0.06
tTG Ab+	6 (12)	6 (9.8)	0.003	0.95	2 (7.4)	10 (11)	0.42	0.50

Data are presented as mean±SD for individual parameters, or number of patients (%). HbA_{1c}, hemoglobin A_{1c}; BMI, body mass index; GAD, glutamic acid decarboxylase; Ab, autoantibody; IA-2, insulinoma antigen 2; ZnT8, zinc transporter 8; tTG Ab, tissue transglutaminase Ab. Bold values indicate statistically significant difference.

diabetes duration, metabolic control status and patient's weight in a cohort of Polish T1DM children and adolescents. The prevalence of some other Ab in particular thyroid Ab, tTG Ab and 21-OH Ab was also studied. Such comprehensive Ab screening, including the new diabetes marker ZnT8 Ab, has not been reported before in Polish T1DM children below 18 years of age.

In our study, ZnT8 Ab was assessed alongside GAD Ab and IA-2 Ab in 114 T1DM children with different diabetes duration. 87% of the patients had at least one diabetes Ab detected. ZnT8 Ab positivity was the highest (72%) in children with short disease duration (<5 years) and this is consistent with the prevalence (60%–80%) reported in previous studies [3, 20–23]. IA-2 Ab positivity was the lowest irrespective of diabetes duration, the age at onset or patient age at the time of testing. In 43 patients with diabetes duration between 5 and 10 years, 90% had at least one diabetes associated Ab detected, with GAD Ab, ZnT8 Ab and IA-2 Ab positivity at 62%, 58% and 41%, respectively. This is in agreement with previous observations that 65% to 85% of diabetic patients tested 4–10 years after the diagnosis had detectable levels of islet-cell Ab [5–7]. However, only five out of 10 (50%) patients with disease duration longer than 10 years were positive for at least one diabetes associated Ab. This is consistent with the reports that diabetes associated Ab levels decline in long-lasting disease [5–8].

In the current study we found that ZnT8 Ab prevalence in Polish T1DM children is the highest among all studied Ab, although not significantly higher compared to GAD Ab. Before the era of ZnT8 Ab, many studies reported the highest prevalence of GAD Ab. The discussion on the frequency and relation of ZnT8 Ab to diabetes features in open. In Italian multicentre study on paediatric patients it was proved, that ZnT8 Ab disclosed the same sensitivity (61%) at disease onset as GAD Ab (61%) [24]. Some authors prove almost 10% higher frequency of ZnT8 Ab compared to GAD Ab [25]. In our study we found that ZnT8 positive patients were younger at disease onset, while GAD positivity was more characteristic for older onset. Quite opposite are the results of Finnish studies, where ZnT8 Ab positivity was associated with older age at diagnosis (mean 8.2 years vs. 7.5 years, $p<0.001$). In this observation diabetic ketoacidosis at diagnosis was less common among subjects with ZnT8 Ab than among those without (16% vs. 20%, $p=0.012$). The prevalence of ZnT8 Ab was decreased in DR3/DR4 heterozygotes when compared with those with other DR combinations ($p<0.001$). Subjects with the neutral DR13-DQB1*0604 haplotype tested more frequently positive for ZnT8 Ab than the rest of the population ($p<0.001$) [26]. The frequency of ZnT8 Ab may be related to some HLA differences, and be similar in similar populations, as in the study of Polish neighbours, Czech T1DM children, the prevalence of this Ab was similar to our results [18]. Interestingly, in Chinese T1D subject ZnT8 Ab and IA-2 Ab were less prevalent than in Caucasian populations, but similar to Japanese [27].

It has been reported that ZnT8 Ab positivity together with GAD Ab positivity increase the diagnostic sensitivity

for detection of autoimmunity in juvenile-onset T1DM [28]. In our group of patients, nine out of 114 (8%) patients were negative for both GAD Ab and IA-2 Ab, but positive for ZnT8 Ab. Whereas only four out of 114 (4%) patients were negative for GAD Ab and ZnT8 Ab but positive for IA-2 Ab. Overall in our study both ZnT8 Ab and GAD Ab were found in 92 out of 96 (96%) of Ab positive patients.

Diabetes associated Ab or 21-OH Ab were negative in the control children except for one out of 51 for GAD Ab and two out of 51 for ZnT8 Ab. A somewhat lower prevalence for GAD Ab and ZnT8 Ab in children without diabetes has been reported previously [18, 20].

In T1DM patients, other autoimmune diseases are often silent or asymptomatic, and are detected by serological screening. The recent extensive Ab screening study in patients with T1DM has raised awareness of the association of T1DM with organ-specific autoimmunity [29]. The reported prevalence of thyroid autoimmunity in young T1DM patients ranges between 3% and 50% [10, 30–33], while it is 3.4%–7% in the general paediatric population [34, 35]. The association of T1DM and AITD is highly dependent on age, sex and ethnicity [36]. Approximately 10% of T1DM patients are affected by CD with prevalence ranging from 0.6% to 16.4% reported in different studies [10, 37–39] and that is significantly higher than in non-diabetic children (1%–2% of the population). CD and T1DM may develop in parallel however usually T1DM precedes the onset of CD [40]. AD and autoimmune gastritis are less common in children with T1DM [10, 41].

In our study, in agreement with previous studies we have found high prevalence of additional organ-specific autoimmune manifestations in children with T1DM. 34% of 114 patients were positive for thyroid Ab, and/or tTG Ab and/or 21-OH Ab. In particular, 26% of patients were positive for TPO Ab and/or Tg Ab and 87% of these were diagnosed with autoimmune thyroid disease. 11% were positive for tTG Ab and 75% of these had clinical CD. 2.6% were positive for 21-OH Ab and one of them had clinical AD. Younger female than male patients were affected by autoimmune thyroid disease (37% and 6%, respectively). Although it has been reported that females are more predisposed to develop CD [37, 42, 43], this could not be assessed in our study due to the low number of patients who had associated CD. We observed that the children with T1DM developed AITD and/or CD irrespective of age at diabetes onset, duration of the disease or diabetic Ab status. However, autoimmune thyroid disease and/or CD presented predominantly in T1DM patients in the age group 11–15 years old (33% for thyroid disease and 11% for CD), and in none of five children younger than 5 years old. Our observations are in agreement with previous studies [42–44] reporting that among T1DM children, thyroid Ab positivity was higher in older children (at mean age of 16.6 years) compared to mean age of 12.0 years for thyroid Ab negative children ($p=0.027$).

Of the three patients who were positive for 21-OH Ab, one had AD. Adrenal function tests were not available for the other two, asymptomatic patients, at the time of writing this report. However, the 21-OH Ab positive children will be followed up carefully [45].

Children with HbA_{1c} below 7.5% were younger and tended to have shorter diabetes duration. We have not observed any difference in the diabetes associated Ab profile for children with optimal or poor glycemic control. This is in agreement with previous studies that found no correlation between single and/or combined diabetes Ab positivity and diabetes control where some pancreatic function was preserved [39, 40, 46]. However, interestingly it was proved recently, that autoimmune thyroiditis accompanying T1DM is associated with worse glycemic control and lipid profile [47].

Exogenous obesity is known to be associated with T1DM [48–50] often together with autoimmune thyroid disease and other autoimmune conditions [51, 52]. A recent cross-sectional study has shown a higher prevalence of hypothyroidism and TPO Ab positivity in obese patients. This suggests, that obesity may increase susceptibility to thyroid autoimmunity [53, 54]. In our study children with T1DM and coexisting CD were thinner and the difference was significant in girls for BMI and SDS-BMI. None of the children in the group of overweight/obese patients had CD. These observations are consistent with the previous reports that diabetic children with tTG Ab who had small bowel villous atrophy and were asymptomatic for CD had lower body weight [37, 55]. In our study, obese children tended to have poor metabolic control compared to children with normal weight, but no significant differences in Ab profiles except for ZnT8 Ab were observed among the two groups of children. In our study ZnT8 Ab were significantly more prevalent in children with excessive body weight compared to slim children ($p<0.05$). Zinc is necessary for structural stability of stored insulin in the β-cells. In pancreatic β-cells, the transport of zinc into the cytoplasm and insulin secretory granules is accomplished by ZnT8 [56] and ZnT8 Ab is one of the major markers for T1DM [57]. A possible association between ZnT8 expression in different tissues and metabolic control in experimental animals has been reported. Very interesting results come from experimental study where β-cell-specific ZnT8 Ab knockout (Ins2Cre:Znt8loxP/loxP) and whole body ZnT8 knockout [Cre-:Znt8(−/−)] mice were placed on a high fat, high caloric diet for 16 week. Ins2Cre:Znt8loxP/loxP mice

had similar body weights throughout the study but displayed impaired insulin biosynthesis and secretion and were glucose intolerant. In contrast, Cre-:Znt8(−/−) mice became remarkably obese, hyperglycemic, hyperinsulinemic, insulin resistant and glucose intolerant. These data show that β-cell ZnT8 alone does not considerably aggravate weight gain and glucose intolerance during metabolic stress imposed by an HFHC diet. However, global loss of ZnT8 is involved in exacerbating diet-induced obesity and resulting insulin resistance, and this may be due to the loss of ZnT8 activity in a tissue other than the β-cell [58, 59]. Probable real and precise role of ZnT8 Ab in the pathogenesis of T1DM and the relationship with the obesity requires further study.

Overall in our study, GAD Ab, ZnT8 Ab and IA-2 Ab were found in the majority of pediatric patients with T1DM with different disease duration and remained positive for as long as 10 years after onset. ZnT8 Ab emerged as an important marker for the diagnosis of T1DM in Polish children. The observation of a relationship between ZnT8 Ab positivity and obesity in T1DM children requires further study. The prevalence of non-islet autoimmunity among T1DM children was relatively high with thyroid specific Ab more prevalent in females than males, while celiac autoimmunity presented regardless of the gender. Both autoimmune thyroid disease and/or CD were more prevalent in T1DM patients in age group of 11–15 years old than in the younger children. Screening for non-diabetes Ab in T1DM may be helpful in identifying subclinical cases of AITD or CD or AD and improvement in patient's care by regular monitoring the children at risk of developing overt forms of these diseases and enabling early treatment interventions.

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