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Islet antibodies and remaining β-cell function 8 years after diagnosis of diabetes in young adults: a prospective follow-up of the nationwide Diabetes Incidence Study in Sweden

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Abstract. Schölin A, Björklund L, Borg H, Arnqvist H, Björk E, Blohmé G, Bolinder J, Eriksson JW, Gudbjörnsdottir S, Nyström L, Östman J, Karlsson AF, Sundkvist G (Uppsala University Hospital, Uppsala; Malmö University Hospital, Malmö; Linköping University Hospital, Linköping; South Stockholm General Hospital, Stockholm; Huddinge University Hospital, Huddinge; Umeå University Hospital, Umeå; Sahlgrenska University Hospital, Gothenburg; Umeå University, Umeå, Sweden). Islet antibodies and remaining β-cell function 8 years after diagnosis of diabetes in young adults: a prospective follow-up of the nationwide Diabetes Incidence Study in Sweden. *J Intern Med* 2004; **255:** 384–391.

Objectives. To establish the prevalence of remaining β-cell function 8 years after diagnosis of diabetes in young adults and relate the findings to islet antibodies at diagnosis and 8 years later.

Design. Population-based cohort study.

Setting. Nationwide from all Departments of Medicine and Endocrinology in Sweden.

Subjects. A total of 312 young (15–34 years old) adults diagnosed with diabetes during 1987–88.

Main outcome measure. Plasma connecting peptide (C-peptide) 8 years after diagnosis. Preserved β-cell function was defined as measurable C-peptide levels. Three islet antibodies – cytoplasmic islet cell antibodies (ICA), glutamic acid decarboxylase antibodies and tyrosine phosphatase antibodies – were measured.

Results. Amongst 269 islet antibody positives (ab^+) at diagnosis, preserved β-cell function was found in 16% (42/269) 8 years later and these patients had a higher body mass index (median 22.7 and 20.5 kg m^{-2}, respectively; $P = 0.0003$), an increased frequency of one islet antibody (50 and 24%, respectively; $P = 0.001$), and a lower prevalence of ICA (55 and 6%, respectively; $P = 0.007$) at diagnosis compared with ab^+ without remaining β-cell function. Amongst the 241 patients without detectable β-cell function at follow-up, 14 lacked islet antibodies, both at diagnosis and at follow-up.

Conclusions. Sixteen per cent of patients with autoimmune type 1 diabetes had remaining β-cell function 8 years after diagnosis whereas 5.8% with β-cell failure lacked islet autoimmunity, both at diagnosis and at follow-up.

Keywords: C-peptide, diabetes, GADA, IA-2A, ICA, young adults.

Introduction

Islet antibodies [cytoplasmic islet cell antibodies (ICA), glutamic acid decarboxylase antibodies (GADA) and tyrosine phosphatase antibodies (IA-2A)] are markers of autoimmune type 1 diabetes found in most children (95–98%) at diagnosis of diabetes [1–3]. In young adults with type 1 diabetes,

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the prevalence of islet antibodies at diagnosis is lower (83%) [4]. However, islet antibodies are also found at diagnosis in 25% of young adults considered to have type 2 diabetes [5]. Follow-up has shown that islet antibodies in patients with clinical features of type 2 diabetes are associated with future clinical type 1 diabetes and β-cell failure, although it might take up to 12 years until it occurs [6, 7]. Hence, patients with phenotypic type 2 diabetes but with islet antibodies should be considered as having type 1 diabetes.

Albeit β-cell failure can be considered as the definite criterion for type 1 diabetes [8], the Diabetes Control and Complication Trial (DCCT) showed that there are patients classified as type 1 diabetes with remaining β-cell function, as indicated from residual plasma C-peptide concentrations, several years after diagnosis [9]. This seems to be of clinical importance. Patients with measurable concentrations of plasma C-peptide have a lower frequency of hypoglycaemia, lower HbA1c values and less microvascular complications than those without [10]. Hence, it is of interest to estimate the prevalence of preserved β-cell function in patients with type 1 diabetes in a population-based unbiased patient group. In this context, it is of interest to explore whether there are differences in the association between the individual antibodies versus preserved β-cell function. In an immunosuppression trial, patients with IA-2A were found to have less chance to restitute β-cell function [11].

Type 2 diabetes is the most plausible cause of diabetes in young adults with diabetes negative for islet antibodies (ab^-). However, idiopathic type 1 diabetes has also to be considered in this context [8]. Idiopathic type 1 diabetes has been found in patients of African or Asian origin [12] and in a few Caucasian children from Italy [13]. The prevalence of idiopathic type 1 diabetes amongst Caucasian young adult patients has to be established in a large population-based patient group.

To clarify these issues, we have followed up a cohort of young adults in Sweden who at diagnosis of diabetes were evaluated with regard to ICA prevalence [5]. The aims of the current study were to (i) follow-up the prevalence of islet antibodies 8 years after diagnosis, (ii) estimate the prevalence of preserved β-cell function 8 years after diagnosis, (iii) relate β-cell function 8 years after diagnosis to islet antibodies and clinical characteristics at diagnosis, and (iv) estimate the prevalence of idiopathic type 1 diabetes, defined as β-cell failure without islet antibodies amongst young adult Caucasian patients.

Materials and methods

Study population

Since 1983, all newly diagnosed 15–34-year-old patients with diabetes mellitus in Sweden are prospectively registered at diagnosis in the nationwide population-based Diabetes Incidence Study in Sweden (DISS) [14]. The ascertainment level for patients with type 1 diabetes in DISS is 0.86 [15]. During a 2-year period (1987–88), 839 patients with diabetes mellitus were registered. At diagnosis, these patients were asked to deliver a blood sample for measurement of islet antibodies and 636 of 839 (76%) accepted (Fig. 1). Eight years later, all patients (including those without a blood sample in the first study) were invited to a second, follow-up study of blood sampling, and 400 of 839 accepted (312 of them had blood sample both at diagnosis and follow-up). Blood samples at follow-up were taken 8.6 ± 0.6 years after diagnosis. In this nationwide study conducted at all Departments of Medicine and Endocrinology in Sweden, it was decided that to achieve optimal compliance the follow-up sample could be taken at anytime of the day (random). Table 1 shows that the cohort of patients who participated in the follow-up did not

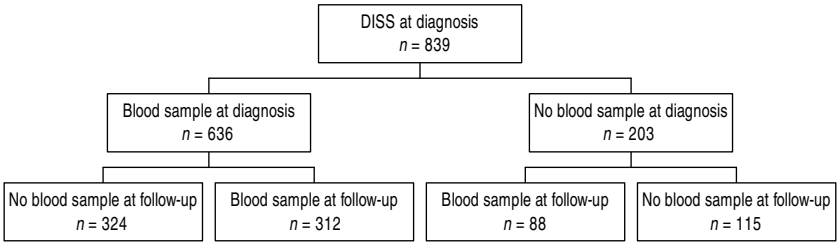

Fig. 1 Flow chart of blood samples in the DISS 87/88 cohort at diagnosis and follow-up.

Table 1 Characteristics at diagnosis of patients in the DISS 1987/1988 cohort, and subgroups with available blood samples at diagnosis, follow-up, or both

Variables at diagnosis	All patients in DISS 1987–88 ($n = 839$)	Patients from the DISS 1987–88 cohort with blood samples		
		At diagnosis ($n = 636$)	At follow-up ($n = 400$)	Both at diagnosis and follow-up ($n = 312$)
Age (years)[a]	25.1 (10.0)	24.9 (9.8)	24.8 (10.2)	24.8 (9.5)
Gender				
Male	522 (62%)	397 (62%)	237 (59%)	182 (58%)
Female	317 (38%)	239 (38%)	163 (41%)	130 (42%)
BMI (kg m^{-2})[b]	22.9 ± 5.4	22.8 ± 5.4	22.1 ± 4.3	22.0 ± 4.4
Classification[c]				
IDDM	599 (72%)	468 (75%)	313 (79%)	254 (81%)
NIDDM	130 (16%)	82 (13%)	44 (11%)	30 (10%)
Unclassifiable	90 (11%)	72 (11%)	36 (9%)	27 (9%)
Secondary	8 (1%)	6 (1%)	3 (1%)	1 (0%)

[a]Median (IQR). [b]Mean ± SD. [c]Twelve of 839 cases were not classified by the local doctor.

differ in clinical features available at diagnosis from those with blood sample at diagnosis, except that insulin-dependent diabetes mellitus (IDDM or type 1 diabetes) was more frequent in patients with a follow-up sample (81 vs. 75%; $P = 0.02$). In agreement, the prevalence of patients positive for islet antibodies (ab^+; ICA and/or GADA and/or IA-2A) was slightly higher in the patients delivering blood samples both at diagnosis and at follow-up than in the complete material (272/311 vs. 518/631; $P = 0.038$). In the final analysis, we used the 312 patients with blood samples both at diagnosis and at follow-up.

Samples taken at diagnosis were initially tested only for ICA [5] but later for GADA and IA-2A except in one patient due to lack of specimen of serum. The samples were stored in −20 °C until assayed. Repeated tests of control samples showed that antibody levels were unaffected by this storage. The follow-up samples were tested for ICA, GADA, IA-2A, random plasma C-peptide, and HbA1c. As a result of haemolysis, islet antibodies and plasma C-peptide could not be measured in two of the follow-up samples. In addition, due to lack of specimen GADA and plasma C-peptide could not be measured in a third and ICA in a fourth follow-up sample. Hence, complete data regarding islet antibodies were available in 307 patients (Table 2). At follow-up, blood glucose was measured at the local hospital immediately after blood samples had been taken.

The classification of the type of diabetes at diagnosis was based on clinical judgement as reported by the diagnosing clinician to the DISS registry [IDDM, noninsulin-dependent diabetes mellitus (NIDDM), unclassifiable diabetes or diabetes secondary to other causes]. Height, weight, gender, and age were also reported on the registration form. Using a standardized questionnaire, the patient's local diabetes nurse or doctor obtained clinical information in the follow-up study. The clinical type of diabetes (type 1, type 2, unclassifiable, or secondary diabetes), based on clinical judgement at time for follow-up, was reported. The nomenclature in Sweden has changed during the study period. Type 1 diabetes had replaced IDDM and type 2 diabetes NIDDM. Results regarding islet antibodies and C-peptide were not provided to the clinicians when the patients were classified with regard to their type of diabetes.

Assay methods

GADA and IA-2A, respectively, were determined by radioligand binding assays based on ^{35}S-methionine-labelled human recombinant *in vitro* transcribed-translated GAD 65 and IA-2, respectively [16, 17]. The GADA and IA-2A results, respectively, are presented as indexes calculated according to the formula $100 \times (u - n)/(p - n)$, where u is mean radioactivity (CPM) of the unknown sample, n is CPM of the negative control and p is CPM of positive control. The cut-off limit for abnormality was the 97.5th percentile of 165 healthy controls in the age of 1–34 years (GADA index >4.6 and an IA-2A index >1.0, respectively) [16, 17]. In the first Diabetes Autoantibody proficiency Standardization

Table 2 Islet antibody pattern in serum samples available at diagnosis and follow-up

		At follow-up						
	At diagnosis	Ab$^-$	ICA only	GADA only	IA-2A only	ICA and IA-2A	ICA and GADA	All three aab
Ab$^-$	39 (13)	37 (95)	0 (0)	1 (3)	1 (3)	0 (0)	0 (0)	0 (0)
ICA only	16 (5)	13 (81)	0 (0)	0 (0)	0 (0)	0 (0)	2 (13)	1 (6)
GADA only	53 (17)	18 (34)	0 (0)	33 (62)	0 (0)	0 (0)	2 (4)	0 (0)
IA-2A only	7 (2)	2 (29)	0 (0)	0 (0)	5 (71)	0 (0)	0 (0)	0 (0)
ICA and IA-2A	14 (4)	4 (29)	0 (0)	1 (7)	4 (29)	2 (14)	2 (14)	1 (7)
ICA and GADA	56 (18)	6 (11)	0 (0)	33 (59)	0 (0)	0 (0)	17 (30)	0 (0)
GADA and IA-2A	13 (4)	1 (8)	0 (0)	10 (77)	2 (15)	0 (0)	0 (0)	0 (0)
All three aab	109 (35)	0 (0)	0 (0)	53 (49)	10 (9)	3 (3)	10 (9)	33 (30)
Total number	307 (100)	81 (26)	0 (0)	131 (43)	22 (7)	5 (2)	33 (11)	35 (11)

Values are expressed as *n* (%). ICA, islet cell autoantibodies; GADA, glutamic cell decarboxylase antibodies; IA-2A, tyrosine phosphatase antibodies; aab, islet autoantibodies.

Program (DASP), the GADA assay performed with 80% sensitivity and 96% specificity and the IA-2A assay with 58% sensitivity and 100% specificity. ICAs were determined by a prolonged immunofluorescence assay [18]. In the last Diabetes Autoantibody Proficiency Program (No. 13; 20 samples tested), the ICA assay performed with 100% sensitivity and 100% specificity, respectively. ICA are not included in the DASP program. Plasma C-peptide was measured by a radioimmunoassay [19]. The detection limit was 0.10 nmol L^{-1} and normal reference interval was 0.25–0.75 nmol L^{-1}. C-peptide concentrations <0.1 nmol L^{-1} were considered to indicate complete β-cell failure and concentrations ≥0.1 nmol L^{-1} was considered to indicate preserved β-cell function [20]. HbA1c was measured by a high-performance liquid chromatography method [21]. The normal reference interval was 4.0–5.2%.

Statistics

Data with normal distribution are given as mean ± SD, whereas data with a non-normal distribution are given as median and interquartile ranges. Fisher's exact test was used for comparisons between two groups for categorical parameters. Mann–Whitney *U*-test was used for comparisons of non-normally distributed values between two groups. Spearman's rank correlation coefficient was calculated to assess the association between non-normal distributed continuous variables and Wilcoxon signed rank test was used to evaluate paired differences. *P*-values <0.05 were considered as significant.

The study was approved by the Ethics Committee at the Karolinska Institute, Stockholm, Sweden.

Results

Islet antibodies at diagnosis and follow-up 8 years later

At diagnosis, 88% (272/311) of patients were islet antibody positive (ab$^+$) compared with 73% (226/308) at follow-up ($P < 0.0001$); ICA were found in 64% (199/312), GADA in 76% (235/311) and IA-2A in 46% (143/311), respectively (Fig. 2). Eight years later, the prevalence of ICA had substantially decreased from 64 to 24% (73/309; $P < 0.0001$). In contrast, the prevalence of GADA had decreased less and were still noted in 65% (200/309) at follow-up ($P = 0.03$) when IA-2A were found in 34% (106/310; $P = 0.003$ compared with diagnosis). Table 2 shows that 35% (109/311) of the patients had all three islet antibodies at diagnosis whilst only 11% (35/308; $P < 0.0001$) showed so at follow-up. In agreement, the prevalence of patients without antibodies (ab$^-$) had increased from 13% (39/311) at diagnosis to 27% (82/308; $P < 0.0001$) 8 years later.

Amongst patients negative for islet antibodies (ab$^-$) at diagnosis, only two of 39 had converted to ab$^+$ at follow-up (Table 2). One patient had developed GADA (index 94.1) together with IA-2A (index 1.4) and a second only IA-2A (index 8.7). Amongst patients with all three antibodies at diagnosis, only 30% (33/109) had three antibodies at follow-up, however, all were still ab$^+$ and GADA were most frequent (88%; 96/109). Similarly, amongst patients with GADA in combination with ICA or IA-2A at diagnosis, 87% (60/69) were still positive for GADA at follow-up. Amongst patients with ICA in combination with IA-2A at diagnosis, most (71%;

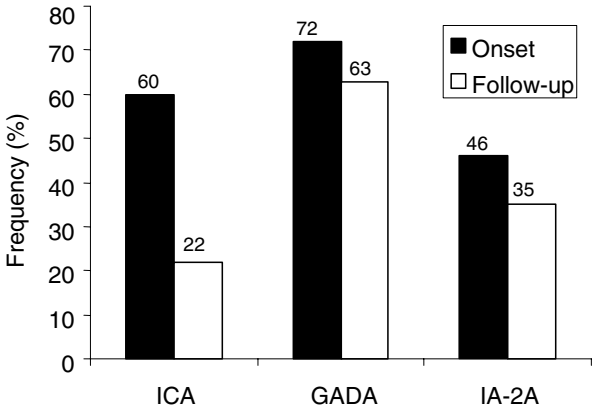

Fig. 2 The prevalence of islet antibodies at diagnosis (white bars) compared with status at follow-up 8 years later (black bars). The decrease in prevalence was most profound for cytoplasmic islet cell antibodies (ICA) ($P < 0.0001$).

10/14) were ab$^+$ at follow-up but only a few (29%; 4/14) had developed GADA (alone or in combination) at follow-up (Table 2).

β-cell function at follow-up in patients ab$^+$ at diagnosis

Amongst ab$^+$ at diagnosis ($n = 269$; C-peptide could not be assessed in three of 272 ab$^+$), 16% (42/269) had measurable plasma C-peptide concentrations (≥0.10 nmol L^{-1}) at follow-up. In 18/42 (43%) of these patients, plasma C-peptide concentration was within the normal reference range (≥0.25 nmol L^{-1}). Patients ab$^+$ at diagnosis with measurable C-peptide at follow-up showed significantly higher body mass index (BMI; $P = 0.0003$), a higher frequency of only one islet antibody ($P = 0.001$), more rarely two islet antibodies ($P = 0.03$), and a lower frequency of ICA ($P = 0.007$) at diagnosis than those with immeasurable C-peptide at follow-up (Table 3). Further, amongst ab+ at diagnosis, patients with measurable C-peptide at follow-up were more often considered to have type 2 diabetes ($P = 0.002$ at diagnosis and $P = 0.0003$ at follow-up, respectively), more often converted to ab$^-$ ($P = 0.003$) and showed lower HbA1c values [6.8% (2.4) vs. 7.5% (2.1); $P = 0.03$] compared with those with immeasurable C-peptide at follow-up. There were no significant correlations between plasma C-peptide concentrations at follow-up versus the levels of ICA, GADA or IA-2A, respectively, neither at diagnosis nor at follow-up.

β-cell failure at follow-up in ab$^-$ patients

There were 241/309 (78%) with immeasurable C-peptide at follow-up. Most of them were ab$^+$ at diagnosis; however, 14/241 (5.8%) of them were ab$^-$. If type 1 diabetes was defined as β-cell failure at follow-up, the prevalence of idiopathic (ab$^-$) type 1 diabetes was 5.8%. Noteworthy, 79% (11/14) of ab$^-$ patients with β-cell failure, were classified as with type 1 diabetes (both at diagnosis and follow-up), all 14 ab$^-$ were insulin treated, none had converted to ab$^+$ at follow-up, and BMI was low [21.9 (2.0) kg m^{-2}] at diagnosis.

Discussion

This follow-up study showed that in young adult patients 15–34 years of age at diagnosis of diabetes, islet antibodies were shown in 88% at diagnosis and the prevalence was almost as frequent (73%) 8 years after diagnosis. Only two of 39 patients ab$^-$ at diagnosis had converted to ab$^+$ 8 years later. Amongst patients ab$^+$ at diagnosis, preserved β-cell function, as inferred from detectable plasma C-peptide concentrations, was found in 16% 8 years later. Indeed, almost half of ab$^+$ patients with measurable C-peptide 8 years after diagnosis had C-peptide concentrations within the normal range. Higher BMI, lower prevalence of ICA, and higher prevalence of only one islet antibody were characteristics of ab$^+$ patients with preserved β-cell function compared with those without. In addition, β-cell failure at follow-up was shown in 5.8% of patients without islet antibodies.

Islet antibodies are considered as markers of the immunopathogenic process against β-cells [22]. This concept is supported by the current study. Patients ab$^+$ at diagnosis who had become ab$^-$ at follow-up had higher plasma C-peptide concentrations at follow-up compared with those who remained ab$^+$. Further, patients with only one islet antibody at diagnosis had higher C-peptide concentrations at follow-up compared with those with several islet antibodies. Hence, islet antibodies appear in parallel to the cellular destruction associated with insulitis, as seen in the recurrence of type 1 diabetes after pancreas transplantation [23, 24]. In addition, our study as well as the DCCT [9, 10] indicate that the type 1 autoimmune β-cell destruc-

Table 3 Characteristics of patients islet antibody positive (ab^+) at diagnosis, at follow-up divided into groups with or without detectable C-peptide levels

	C-peptide at follow-up		
	≥0.1 nmol L^{-1} (n = 42)	<0.1 nmol L^{-1} (n = 227)	*P*-value
At diagnosis			
Age (years)*	25.4 (12.0)	24.5 (9.0)	n.s.
BMI (kg m^{-2})*	22.7 (5.6)	20.5 (3.7)	0.0003
Male/Female	27/15	131/96	n.s.
Titres*			
ICA	200 (174)	200 (334)	n.s.
GADA	40.3 (124.8)	55.4 (107.6)	n.s.
IA-2A	93.6 (98.0)	81.1 (100.5)	n.s.
Individual antibodies n (%)			
ICA (alone or in combination)	23 (55%)	173 (76%)	0.007
GADA (alone or in combination)	33 (78%)	199 (88%)	n.s.
IA-2A (alone or in combination)	21 (50%)	122 (54%)	n.s.
Numbers of islet antibodies n (%)			
One	21 (50%)	55 (24%)	0.001
Two	7 (17%)	77 (34%)	0.03
Three	14 (33%)	95 (42%)	n.s.
Classification n (%)			
IDDM	25 (60%)	204 (90%)	<0.0001
NIDDM	8 (21%)	10 (4%)	0.002
Unclassified	9 (21%)	13 (6%)	0.002
At follow-up			
BMI (kg m^{-2})*	24.6 (3.8)	24.0 (3.7)	0.04
HbA1c (%)*	6.8 (2.4)	7.5 (2.1)	0.03
Individual antibodies n (%)			
ICA (alone or in combination)	9 (21%)	64 (28%)	n.s.
GADA (alone or in combination)	23 (55%)	176 (78%)	0.004
IA-2A (alone or in combination)	18 (43%)	86 (38%)	n.s.
Numbers of islet antibodies n (%)			
None	14 (33%)	30 (13%)	0.003
One	12 (32%)	96 (42%)	n.s.
Two	10 (24%)	71 (31%)	n.s.
Three	6 (16%)	29 (13%)	n.s.
Classification n (%)			
Type 1 diabetes	31 (76%)	208 (95%)	0.0003
Type 2 diabetes	8 (20%)	7 (3%)	0.0006
Unclassified	2 (5%)	4 (2%)	n.s.
Treatment n (%)			
Insulin	35 (85%)	216 (99.5)	<0.0001
Oral hypoglycaemic agents (OHA)	2 (5%)	0 (0%)	
Diet	2 (5%)	0 (0%)	
Insulin and OHA	2 (5%)	1 (0.5%)	

ICA, islet cell autoantibodies; GADA, glutamic cell decarboxylase antibodies; IA-2A, tyrosine phosphatase antibodies; n.s., not significant. *Median (interquartile range).

tive process might be partial or slowly progressive [7], at least in young adults.

As previously shown both in adults and children [25–27], our study confirms that a low frequency of ICA at diagnosis amongst ab^+ is associated with high plasma C-peptide concentrations later after diagnosis. Although the occurrence of ICA is related to high GADA and/or IA-2A levels [7], in contrast to studies conducted a few years after diagnosis [28, 29], we found no associations between the levels of GADA and/or IA-2A at diagnosis versus plasma C-peptide concentrations at follow-up 8 years later. This indicates that GADA and IA-2A levels only are associated with C-peptide concentrations during the first years after diagnosis of type 1 diabetes when there is an active autoimmune destruction of the

β-cells. Therefore, ICA might be associated with β-cell damage unrelated to GADA or IA-2A as supported by the fact that ICA had disappeared at follow-up in most of our patients ICA+ at diagnosis. In this context, it might be mentioned that the presence of ICA at diagnosis was not mentioned to the patient's clinician. Hence, it is unlikely that awareness of ICA affected the ascertainment at follow-up.

There is a positive correlation between BMI and plasma C-peptide concentrations in healthy subjects [30]. Our observation, that BMI at diagnosis was higher in ab^+ with preserved β-cell function at follow-up, is another evidence for that high plasma C-peptide concentrations at diagnosis of diabetes are protective for decreases [28]. This is in line with the finding that BMI at diagnosis of type 1 diabetes was a predictor of the duration of remission in another cohort of DISS patients diagnosed during the years 1992–93 [31]. Low BMI at diagnosis might reflect an aggressive early phase of diabetes with little preserved β-cell function at clinical diagnosis. However, high BMI could be associated with a high number of β-cells, as seen in the ob/ob obese mice [32], leading to a less progressive course of β-cell failure.

Amongst patients ab^- at diagnosis, 95% were still ab^- 8 years later. Hence, islet antibody negativity was a persistent finding in our study. Despite the absence of islet antibodies, 36% (14/39) of patients ab^- at diagnosis lacked detectable plasma C-peptide concentrations 8 years later. Some of these patients might have high-risk human leucocyte antigen haplotypes [33], antibodies against antigens not tested for [34], or islet antibodies disappearing before diagnosis. Another option is that islet antibodies appearing after diagnosis, occurring in about 10% of ab^- young adults [7, 35], might have disappeared later. To address this issue, in the ongoing DISS study, we have included measurements of islet antibodies 4–6 months and 12–18 months after diagnosis. Until these results are available, ab^- patients with β-cell failure after diagnosis might be considered as having idiopathic type 1 diabetes according to recent American Diabetes Association criteria [8]. This type of diabetes has previously been reported in patients of African or Asian origin [12] and only in a few Caucasian children [13]. Our study shows that as many as 5.8% of young adult Caucasian patients with β-cell failure might have idiopathic type 1 diabetes.

In conclusion, 8 years after diagnosis of autoimmune diabetes in young adult diabetic patients, 16% had preserved β-cell function. High BMI, a low prevalence of ICA, the presence of only one islet antibody at diagnosis, and conversion to ab^- at follow-up were characteristics of patients with autoimmune diabetes and preserved β-cell function. In addition, idiopathic type 1 diabetes was seen in 5.8% of the patients with type 1 diabetes.

Conflict of interest statement

No conflict of interest was declared.

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