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No signs of progressive beta cell damage during 20 years of prospective follow-up of autoantibody-negative diabetes

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Abstract

Aims Both type 1 (T1D) and type 2 (T2D) diabetes are considered to be associated with different degrees of progressive beta cell damage. However, few long term studies have been made. Our aim was to study the clinical course of 20 years of diabetes disease, including diabetes progression, co-morbidity and mortality in a prospectively studied cohort of consecutively diagnosed diabetic patients.

Methods Among all 233 patients diagnosed with diabetes during 1985-1987 in Malmö, Sweden, 50 of 118 surviving patients were followed-up after 20 years. The age at diagnose was 42.3±23.1 and 57.5±13.6 yrs for antibody positive and antibody negative patients, respectively. HbA1c and plasma lipids were analysed with regard to metabolic control.

Results Islet antibody negative patients at diagnosis had highly preserved C-peptide levels after 20 years in contrast to antibody positive patients (antibody-negative: C-peptide 0yrs 0.78±0.47 and 20yrs 0.70±0.46 (nmol/l), p=0.51 and antibody-positive: C-peptide 0yrs 0.33±0.35 and 20yrs 0.10±0.18; p<0.001. Islet antibodies but not age, BMI or C-peptide at diagnosis were predictors of C-peptide levels at 20 years when analysed by logistic regression (p<0.05). HbA1c did not differ between the groups after 20 years. The 20-year mortality was higher among antibody-negative patients, dependent on the higher age at diagnosis in this group (number of deaths: antibody-positive: 18 of 56 vs. antibody-negative: 109 of 188, p<0.001). Of the deceased, 79 % had died from diseases or complications that may be associated to diabetes**.**

Conclusion We found no progressive beta cell damage in autoantibody negative diabetes at a 20 year follow-up of the clinical course of diabetes.

Keywords C-peptide, Diabetes mellitus, Follow-up, HbA1c, Long term effects

Abbreviations GADA, glutamic acid decarboxylase antibodies, IA-2A, protein tyrosine phosphatase-like antibodies, IAPP, islet amyloid polypeptide and ICA, islet cell antibodies.

Introduction

Type 1 and type 2 diabetes are different in terms of aetiology and clinical course but have both been proposed to be associated with progressive beta cell damage [1-3]. However, this notion has been questioned. Antigen-unspecific islet cell antibodies (ICAs) and antigen-specific glutamic acid decarboxylase 65 antibody (GADA) are markers of islet autoimmunity present in most patients at the diagnosis of type 1 diabetes [4] and in latent autoimmune diabetes in adults (LADA) patients [5]. Like GADA, protein tyrosine phosphatase-like protein antibodies (IA-2As) are antigen-specific islet antibodies. IA-2As are detected in a high frequency at diagnosis in type 1 diabetic children [6], whereas the frequency is lower in adult-onset type 1 diabetic patients [7,8] and in LADA patients [9]. In addition, it has been suggested that IA-As could be of value for identifying LADA patients [10] It was previously described from our group that low levels of GADA, ICA or IA-2A may indicate a preserved beta cell function [4].

Different groups of type 1 and type 2 diabetes patients have been followed in interventional studies [11,12]. However, these have often involved selected patients, which makes it difficult to draw conclusions for the general diabetes population. In this context, the present cohort is unique since it involves patients of different background which have been followed already from the diagnosis of diabetes.

Our aim was to investigate beta cell function, diabetes treatment, levels of HbA1c, and plasma lipids, micro- and macrovascular complications and mortality after 20 years of follow-up of all new consecutively diagnosed adult diabetic patients in the city of Malmö, Sweden. We set out to study whether there are any differences in the disease course at the level of beta cell function between patients that are antibody positive and antibody negative at diagnosis and if we could find anything that could serve as a predictor for future C-peptide levels. In addition, we wanted to evaluate whether presence of antibodies at diagnosis has any implication for diabetes complications, mortality and causes of death. To our knowledge, the present study is the only non-interventional 20 year follow-up study of the clinical course of diabetes in a total material of consecutively diagnosed adult onset diabetic patients [13].

Patients and methods

Patients

All new consecutively diagnosed adult diabetic patients (>15 years of age, n=233) between September 1985 and August 1987 in the city of Malmö, Sweden, were included in this prospective study [4,13-16]. The diabetes definition based on fasting plasma glucose ≥7.8 mmol/l or a 2 hr plasma glucose after oral glucose tolerance test (OGTT) ≥11.1 mmol/l was used and 13% of all diabetes patients were clinically considered to have type 1 diabetes, 62% type 2 diabetes and 6% were classified as suspected type 2 diabetes but with islet antibodies. All surviving patients were invited to a follow-up study after 20 years. Among the 233 adult patients diagnosed (1985-1987), 115 had died at the time of follow-up. This was the case for 68 patients (mean age 53±16 years). Among the 118 survivors, 50 participated in the study. Among the non-participants, three patients had moved from the area and the rest felt too old or tired to participate. From the original cohort, 12 additional patients died during the time of the present study.

The study was approved by the Ethics Committee at Lund University (LU 327/2006).

Clinical and laboratory measurements

Blood samples for fasting P-C-peptide measurements were obtained after an overnight fast from 216 patients at diagnosis, 150 patients after 3 years, 148 patients after 5 years, 107 patients after 12 years and from 50 patients after 20 years to evaluate endogenous beta cell function. Blood samples for fasting P-C-peptide was only analysed at diagnosis if the patients had been fully fasting overnight. Blood samples for HbA1c, cholesterol and triglycerides were analysed in 176 patients after 5 years and in 50 patients after 20 years to evaluate metabolic control.

Patients were regarded as islet antibody positive if they had one or more types of antibodies, i.e. GADA, IA-2A and ICA. GADA and IA-2A were determined by radioligand binding assays [17,18]. The GADA assay was based on 125I-labeled human recombinant GAD65. Both the sensitivity and specificity of this GADA assay were 100% when compared with a 35S-GADA assay evaluated in the Diabetes Autoantibody Proficiency Testing Program for GADA(no. 2, 24 samples tested). A value>1.9 units/ml (97.5% percentile of 199 nondiabetic control subjects) was considered abnormal [14]. The IA-2A assay was based on 35S-methionine-labeled human recombinant in vitro transcribed-translated intracellular domain of IA-2 as described in detail [4]. The ICAs were determined by a prolonged immunoflourence assay. In the latest Diabetes Autoantibody Profiency Program (no. 13, 20 samples tested) the ICA assay had 100% sensitivity and 100% specificity. In the present study, the detection limit was 3 Juvenile Diabetes Foundation (JDF) units for the used pancreas, i.e. an ICA value ≥33 JDF units was considered abnormal.

Serum total cholesterol, HDL subfractions (after precipitation), and triglycerideconcentrations were measured on a Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland).

The detection limit for the fasting C-peptide assay was 0.10 nmol/l [19]. HbA1c was determined by a high-performance liquid chromatography method with reference values of 3.90-5.30% for healthy individuals [20].

To study long term effects on metabolic control, blood samples were analysed for HbA1c, cholesterol and TG at 5 and 20 years since stress related to the onset of diabetes may be a confounder when these variables are measured at diagnosis.

Statistics

Data are presented as means±SD for normally distributed data. Univariate analysis of variance was used to test significance of difference between groups. Non parametric methods were used for not normally distributed data. Data were analysed using the SPSS statistical software 14.0 (SAS Institute, Cary, NC, USA).

Results

Patients without islet antibodies at diagnosis showed highly preserved fasting C-peptide levels after 20 years in contrast to patients with antibodies at diagnosis (antibody-negative: C-peptide 0yrs 0.78±0.47 (nmol/l) vs. C-peptide 20yrs 0.70±0.46 (nmol/l), p=0.51 and antibody-positive: C-peptide 0yrs: 0.33±0.35 (nmol/l) vs. C-peptide 20yrs 0.10±0.18 (nmol/l), p<0.001). Among the antibody positive patients, four had lost their antibodies after 20 years and three antibody negative patients had become antibody positive, which tended to have an impact on C-peptide levels at the 20 year follow-up (antibody negative at diagnosis 1.55±0.24 (nmol/l), loss of antibodies 0.36±0.46 (nmol/l), antibody development 0.10±0.09 (nmol/l), antibody positive at diagnosis 0.05±0.05 (nmol/l). The C-peptide levels after 20 years was not affected by the type of diabetes treatment that had been used for the first five years after diagnosis, i.e. diet, oral antidiabetic treatment or insulin (C-peptide 20 yrs: 1.32±1.96 vs. 0.73±0.54 vs. 0.38±0.34 for diet, oral antidiabetic treatment and insulin, respectively, p=0.14). The analysis was only performed in the subgroup of patients that were antibody negative at diagnosis since all antibody positive patients except one were treated with insulin at an early stage. Islet antibodies but not age, BMI or C-peptide at diagnosis were predictors of C-peptide levels at 20 years when analysed by logistic regression (p<0.05).

After 20 years, antibody negative patients were more often treated with oral antidiabetic drugs (66% vs. 6%; p<0.001) and antihypertensive agents (80% vs. 41%; p<0.001) compared to antibody positive patients, whereas insulin treatment was less common (57% vs. 100%; p<0.001) (table 2). The use of ASA, beta-blockers and lipid lowering substances did not differ between the groups even though there was a tendency towards a higher use of lipid lowering substances in patients with islet antibodies at diagnosis (table 2).

The antibody positive patients had improved their HbA1c at follow-up compared to at diagnosis, whereas the metabolic control was unchanged in antibody negative patients (antibody-positive: HbA1c 5yrs 10.0±2.1% vs. HbA1c 20 yrs 7.8±1.9%; p=0.001, antibody negative 5yrs: 7.9±2.1% vs. antibody-negative 20yrs: 7.2±1.6 (%). The HBA1c did not differ between the groups at follow-up after 20 years (table 2).

Plasma lipids remained stable throughout the years in antibody positive patients, whereas a significant improvement was found regarding total and LDL cholesterol and triglycerides (TG) in antibody negative patients (total cholesterol 5yrs 5.6±1.4 mmol/l and after 20yrs 3.9±1.0 mmol/l, p<0.001, TG 5yrs 2.1±1.5 mmol/l and after TG 20yrs 1.4±0.7 mmol/l, p<0.01). Lower plasma lipids were seen in antibody negative patients compared to antibody positive subjects at the 20 year follow-up (table 2).

Diabetic complications like acute myocardial infarction (17% vs. 0%; p=0.06) and diabetic nephropathy (80% vs. 20%, p=0.07) tended to be more common among antibody negative patients compared to antibody positive subjects. Antibody negative patients had lower estimated GFR after 20 years (68±4 vs. 85±3; p=0.008 (ml/min)). Nine antibody negative and one antibody positive patients had developed nefropathy when defined as GFR<60 ml/min. One patient was treated with hemodialysis at follow-up. No difference was seen between the different patient groups regarding reports of stroke or stable angina pectoris.

The 20-year mortality was higher among antibody-negative patients, most probably dependent on the higher age at diagnosis in this group (number of deaths: antibody-positive: 18 vs. antibody-negative: 109; $p<0.001$) (Fig. 1). Among 103 deceased patients in whom the cause of death had been registered, 79 % had died from diseases and complications which might have been associated to diabetes (one from renal failure, two from cardiovascular disease, three from stroke, six from hyper- or hypoglycemia and five from infection) (table 3). At the time of the study, the cause of death had not yet been registered for 22 deceased individuals.

Discussion

The present study is the first 20-year follow-up of the clinical course of diabetes in a consecutively diagnosed material. We found that patients with autoantibody negative diabetes have a highly preserved beta cell function after 20 years in contrast to antibody positive patients. This is well in line with what has previously been described for T1D [1,2] but in contrast to the general view of progressive beta cell damage in T2D [3].

It is clear that more long term studies of the clinical course of antibody-negative diabetes are needed. On one hand, we share the view that some patients with antibody-negative diabetes appear to have a good metabolic control with lifestyle changes initially but that they later will require oral antidiabetic drugs or insulin treatment [3]. On the other hand, most mechanisms that have been suggested to underlie a putative progressive beta cell damage in T2D may not be relevant for the clinical situation or have been difficult to show *in vivo*, e.g. glucotoxicity due to uncontrolled diabetes and cytotoxicity by islet amyloid polypeptide (IAPP) [21,22] [23]. Mechanistically, it has also been discussed whether chronic hyperglycemia and different genetic variants of the insulin receptor may affect the clinical course of diabetes [24-26].

To study long term effects on metabolic control, blood samples were analysed for HbA1c, cholesterol and TG at 5 and 20 years. In our opinion, such an approach gives a better understanding of long term effects of the disease than to study these variables at diagnosis when stress related to the onset of diabetes may be a confounder. The plasma lipid concentrations remained unchanged in antibody positive patients, whereas a significant improvement was found regarding total and LDL cholesterol and TG in antibody negative patients. Lower plasma lipids were seen in antibody negative patients than antibody positive subjects at the 20-year follow-up, probably reflecting a higher use of lipid lowering drugs in the first group of patients. The vast majority of the patients met the Swedish national guidelines for lipid control (National Guidelines for the care and treatment of diabetes mellitus. Stockholm: National Board of Health and welfare; 1999).

The majority of the studied patients had higher HbA1c levels than those that will soon become recommended by the national guidelines in Sweden, i.e. HbA1c<6% (www.sos.se). The antibody positive patients had improved their HbA1c at follow-up compared to at diagnosis, whereas the metabolic control remained unsatisfactory in antibody negative patients. One may speculate that the introduction of insulin glargin and determir has contributed to the improvement in metabolic control during the last years, particularly in autoantibody positive diabetes. However, the HbA1c did not differ between the groups of patients at follow-up. In general, the HbA1c levels were in the same range as the in 10-year follow-up study of the United Kingdom Prospective Diabetes Study (UKPDS) [12].

Diabetes complications and co-morbidity, e.g. acute myocardial infarction tended to be more common among antibody negative compared to antibody positive patients. This was also reflected by a higher use of antihypertensive treatment in the antibody negative group.

Interestingly, the presence of antibodies at diagnosis had a significant impact on mortality. Among the deceased where the cause of death had been registered, 79 % had died from diseases and complications that may be associated to diabetes (i.e. renal failiure, cardiovascular disease, stroke, hyper- or hypoglycemia and infection). Furthermore, among the antibody negative patients, ten had died from malignancies. Both the increased mortality and increased prevalence of malignancies might of course be due to older age in this group (table 3).

We conclude that the present 20-year prospective study of consecutive diabetes patients is a unique set-up for studying the clinical course of diabetes. Our data indicates no progressive beta cell damage at autoimmune negative diabetes.

Competing interests

None to declare.

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Legends to the tables

Figure 1. Kaplan-Meier curve of all studied patients during 20 years of follow-up. Ab - denotes patients without islet antibodies and Ab + denotes patients with islet antibodies. Time is expressed in years.

Table 1. Clinical characteristics of patients at the diagnosis of diabetes according to the presence of islet antibodies. T1D and T2D denote type 1 and type 2 diabetes.

Table 2. Clinical characteristics 20 years after the diagnosis of diabetes according to the presence of islet antibodies. LDL, HDL and ASA indicate low density lipoprotein, high density lipoprotein and acetylsalisylic acid, respectively.

Table 3. Registered causes of deaths and number of deceased individuals.

Table 1

Type of diabetes	**Antibody positive N=49**	**Antibody negative N=184**	***p* value**
Age at diagnose (Yrs)	42.3±23.1	57.5±13.6	<0.001
Clinical diagnose (%) (T1D/T2D)	96/4	0/100	<0.001
BMI (kg/m2)	22.3±4.9	28.7±5.1	<0.001
HbA1c (%)	9.6±2.1	8.0±2.2	<0.001
fPCpeptide (nmol/l)	0.34±0.30	0.90±0.55	<0.001

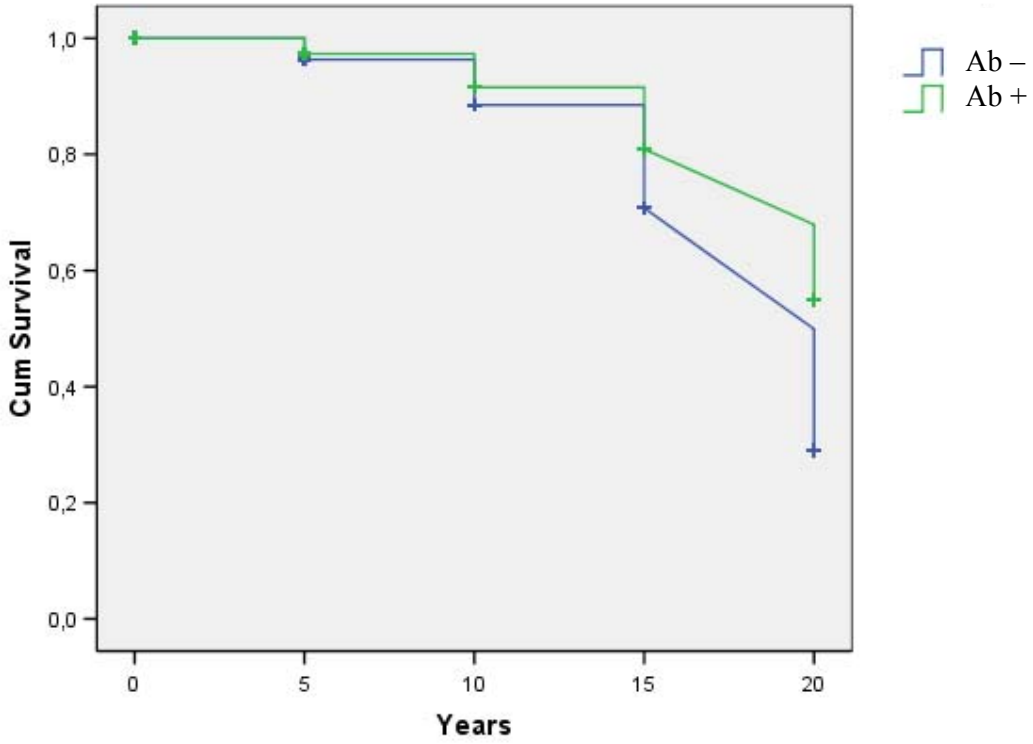
Table 2

Type of diabetes	Antibody positive N=17	Antibody negative N=33	*p* value
BMI (kg/m2)	24.8±2.8	27.1±4.2	<0.05
Systolic blood pressure (mmHg)	137±15	150±17	<0.05
Diastolic blood pressure (mmHg)	81±7	80±9	0.87
HbA1c (%)	7.8±1.9	7.1±1.6	0.26
fPCpeptide (nmol/l)	0.10±0.18	0.97±1.34	<0.01
Total cholesterol (mmol/l)	4.6±0.4	3.9±1.0	<0.05
LDL-cholesterol mmol/l)	2.8±0.5	2.2±0.8	<0.05
HDL-cholesterol (mmol/l)	1.3±0.4	1.1±0.3	<0.05
Triglycerides (mmol/l)	1.0±0.7	1.4±0.7	<0.05
Diabetes treatment (Diet / Oral / Insulin) (%)	0/6/100	2/66/57	<0.01
Lipid lowering treatment (%)	35	60	0.07
Anti hypertensive treatment (%)	41	80	<0.01
ASA treatment (%)	18	51	<0.05

Table 3

Type of diabetes	Antibody positive N=15	Antibody negative N=88
Age at death (years)	77±10	73±10
Renal insufficiency	0	1
Cardiovascular diseases	8	42
Stroke	2	7
Diabetes	2	13
Infections	2	5
Pulmonary diseases	0	1
Malignancies	1	10
Other	0	9

Figure 1



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