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Original Article

Multinational study in children and adolescents with newly diagnosed type 1 diabetes: association of age, ketoacidosis, HLA status, and autoantibodies on residual beta-cell function and glycemic control 12 months after diagnosis

HB Mortensen, PGF Swift, RW Holl, P Hougaard, L Hansen, H Bjoerndalen, CE de Beaufort, M Knip. Multinational study in children and adolescents with newly diagnosed type 1 diabetes: association of age, ketoacidosis, HLA status, and autoantibodies on residual beta-cell function and glycemic control 12 months after diagnosis.
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Objective: To identify predictors of residual beta-cell function and glycemic control during the first 12 months after the diagnosis of type 1 diabetes (T1D).
Subjects and Methods: Clinical information and blood samples were collected from 275 children. HbA1c, antibodies, HLA typing and mixed meal-stimulated C-peptide levels 1, 6, and 12 months after diagnosis were analyzed centrally.
Results: Mean age at diagnosis was 9.1 yr. DKA with standard bicarbonate <15 mmol/L was associated with significantly poorer residual beta-cell function 1 ($p = 0.004$) and 12 months ($p = 0.0003$) after diagnosis. At 12 months, the decline in stimulated C-peptide levels compared with the levels at 1 month was 69% in the youngest age group and 50% in patients 10 yr and above ($p < 0.001$). Stimulated C-peptide at 12 months was predicted by younger age ($p < 0.02$) and bicarbonate levels at diagnosis ($p = 0.005$), and by stimulated C-peptide ($p < 0.0001$), postmeal blood glucose ($p = 0.0004$), insulin antibodies (IA; $p = 0.02$) and glutamic acid decarboxylase antibodies (GADA; $p = 0.0004$) at 1 month. HbA1c at 12 months was predicted by HbA1c at diagnosis ($p < 0.0001$), GADA at 1 month ($p = 0.01$), and non-white Caucasian ethnicity ($p = 0.002$).
Conclusions: Younger age, ketoacidosis at diagnosis, and IA and GADA 1 month after diagnosis were the strongest explanatory factors for residual beta-cell function at 12 months. Glycemic control at 12 months was influenced predominantly by ethnicity, HbA1c at diagnosis, and GADA at 1 month.

Henrik B Mortensen[a], Peter GF Swift[b], Reinhard W Holl[c], P Hougaard[d], Lars Hansen[a], Hilde Bjoerndalen[e], Carine E de Beaufort[f], Michael Knip[g] and Hvidoere Study Group on Childhood Diabetes[h]

[a]Department of Pediatrics, Glostrup University Hospital, Glostrup, Denmark; [b]Leicester Royal Infirmary Children's Hospital, Leicester, UK; [c]University of Ulm, Ulm, Germany; [d]Department of Statistics, University of Southern Denmark, Odense, Denmark; [e]Department of Pediatrics, Ulleval University Hospital, Oslo, Norway; [f]Clinique Pediatrique, Centre Hospitalier de Luxembourg, Luxembourg; [g]Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland; and [h]a list of the Hvidoere Study Group appears in the Appendix

Key words: C-peptide – pancreatic autoantibodies – partial remission phase – residual beta-cell function

Corresponding author:
Henrik B. Mortensen
Department of Pediatrics
Glostrup University Hospital
2600 Glostrup
Denmark.
Tel: +45 43232967;
fax: +45 43233964;
e-mail: hbmo@glo.regionh.dk

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Introduction

Type 1 diabetes (T1D) is the end result of immune-mediated beta-cell destruction. T-cells (autoreactive CD8+ T effector cells and CD4+ T regulatory cells) together with dendritic cells play a major pathogenic role in islet cell infiltration and destruction that together with cytokines/chemokines (IL-10, IL-1, IFNγ,TNFα) are the constituents of the autoimmune processes (1). It is generally accepted that at the time of T1D diagnosis, an individual has lost most (60–80%) of the beta-cell function but as many as 10–20% are still capable of insulin production. Many children and adolescents with newly diagnosed T1D experience a transient remission period ('honeymoon') starting shortly after insulin treatment is initiated when the requirement for exogenous insulin treatment declines (partial remission) or even becomes non-existent (2, 3). It is reasonable to consider that the partial remission phase is a period of relative beta-cell recovery. The pathogenesis of this has been the subject of discussion (4) but is likely to be a combination of factors such as an amelioration of glucose toxicity (5), a reduction in the immune inflammatory conditions in the islets (insulitis) (6), improvement of peripheral insulin sensitivity (7), or even a still regenerating beta-cell mass (8). The duration of the remission period depends at least partly on the recovery of the beta-cell function, which may be assessed by measurement of stimulated C-peptide secretion (9, 10).

At diagnosis, pediatric patients with T1D present with varying degrees of metabolic derangement. The rate and severity of diabetic ketoacidosis (DKA) at diagnosis vary considerably between countries and regions (11, 12). Ketoacidosis at diagnosis has been related in some studies to lower C-peptide values, higher insulin requirements, and higher HbA1c values during the first 2 yr of diabetes (13) but not in others (14). Other factors affecting the remission phase have been discussed and there remains some controversy regarding the impact of various factors influencing C-peptide secretion (4, 15).

This prospective study was initiated by the Hvidoere Study Group on Childhood Diabetes (HSG), a multinational collaboration of pediatric diabetes centers, to describe the demography, patterns of clinical and laboratory characteristics at diagnosis and to explore the metabolic disturbance, genetic background, and immune activity as predictors of residual beta-cell function and glycemic control.

Material and methods

Subjects

The Hvidoere Remission Phase Study is a prospective, long-term observational study conducted in 18 centers representing 15 countries in Europe and Japan. Two hundred and seventy-five children aged less than 16 yr with newly diagnosed diabetes presenting to the pediatric departments of the participating centers between August 1999 and December 2000 were included in the study.

Exclusion criteria were suspected non-T1D [maturity-onset diabetes of the young (MODY), secondary diabetes, etc.], and patients initially treated outside of the centers for more than 5 d. Patients were diagnosed according to the World Health Organization criteria. A number of eligible patients or parents declined to participate. The study was performed according to the criteria of the Helsinki II Declaration and was approved by the local ethics committee in each center. All the patients and their parents or guardians gave informed consent.

Clinical descriptives on each patient were collected at the first hospital visit, including date of birth, gender, duration of symptoms, height, and weight. Detailed hospital admission data, metabolic status (blood glucose, pH, standard bicarbonate, and urinary ketones were determined locally by quality controlled standard laboratory methods), and insulin therapy were recorded. Blood samples for centralized measurement of HbA1c, genetics, immunology, and stimulated C-peptide were collected prospectively.

Ketoacidosis was defined by a standard bicarbonate value $\leq$15 mmol or – if no standard bicarbonate was available – by a pH-value $\leq$7.3. Mild DKA: standard bicarbonate $>$15 and $\leq$22 mmol/L or pH $>$7.3. No DKA: standard bicarbonate $>$22 mmol/L or pH $>$ 7.4.

Insulin

Insulin regimens were recorded 1, 3, 6, 9, and 12 months after diagnosis. After 12 months 52.9% of the children were on twice insulin daily, 25% on three times and 18.5% on four or more injections. Only a few children (3.3%) received one insulin injection daily. A premixed form of insulin was used in 72.3% of the children on twice daily insulin. Only three children used an insulin infusion pump while 13% were treated with a rapid acting insulin analogue. Mean daily insulin dose: 0.72 ± 0.28 U/kg (mean $\pm$ SD).

HbA1c

Capillary samples for HbA1c analysis were collected at diagnosis and after 1, 3, 6, 9, and 12 months at each center and mailed to the Steno Diabetes Centre (Denmark) using the Bio-Rad HbA1c sample preparation kit (Bio-Rad Laboratories, Munich, Germany) as previously described (16). HbA1c analysis was performed by automatic high-pressure liquid chromatography with the same calibrator lots as used in The

Diabetes Control and Complications Trial (DCCT) to facilitate comparisons. Normal range for the Steno method was 4.4–6.3% (about 0.3% higher than the DCCT method).

C-peptide

After 1, 6, and 12 months of diabetes, a liquid meal challenge was utilized to stimulate endogenous C-peptide release. There were 133 girls and 129 boys, who contributed with at least one measurement (95% of total). The test was in the morning after fasting for at least 8 h, the morning insulin dose being omitted. Boost (formerly Sustacal; Mead Johnson, Evansville, IN, USA) was ingested in less than 10 min given at a dose of 6 mL/kg (max: 360 mL). In agreement with the DCCT protocol, capillary glucose was measured at time 0 and venous C-peptide and postmeal blood glucose at 90 min after ingestion of the liquid meal (15).

Serum samples were labeled and frozen at –20°C until shipment on dry ice to Steno Diabetes Center for the determination of C-peptide. Samples were thawed only once for the C-peptide assay. Serum C-peptide was analyzed by a fluoroimmunometric assay (AutoDELFIA™ C-peptide, PerkinElmer Life and Analytical Sciences Inc., Turku, Finland). The sensitivity was below 1 pmol/L, intra-assay coefficient of variation below 6% at 20 pmol/L, and recovery of standard, added to plasma before extraction, about 100% when corrected for losses inherent in the plasma extraction procedure.

HLA

Typing of the HLA class II *DRB1* locus was performed by direct sequencing of exon 2 of *DRB1* according to the Immuno Histocompatibility Working Group (17). *DR 03/04* and *DR 04/04* were defined as high-risk genotypes (n = 97), while *DR 03/03* and *DR 04/08* were considered to convey moderate risk (n= 31). All other genotypes were classified as low risk (n = 130).

Antibody Measurements at 1, 6, and 12 months

Islet cell antibodies. Islet cell antibodies (ICA) of the IgG class were detected by indirect immunofluorescence using commercial Primate Pancreas slides from INOVA Diagnostics, Inc. (San Diego, CA, USA). All initially ICA-positive samples are re-tested to confirm antibody positivity. The sera were screened at a dilution of 1:2 and FITC-labeled anti-human IgG (Dako, Copenhagen, Denmark) was used as conjugate and grouped as negative 0–0.5 U (Department of Autoimmunology, Statens Serum Institut, Copenhagen, Denmark).

GAD antibodies. Glutamic acid decarboxylase antibodies (GADA). Antibodies to the 65 kD isoform of GAD were quantified by a direct radioimmunoassay (Diamyd Anti-GAD-65 RIA; Diamyd Diagnostics, Stockholm, Sweden) according to the protocol provided by the manufacturer. Sera were run in duplicate, and the results were read on a gamma counter (Wizard 1470; Wallac/PerkinElmer, Turku, Finland) and calculated from a standard curve. The cut-off limit was 10 units/mL and the intra- and interassay coefficients of variation were 2.4 and 3.6%, respectively (Department of Autoimmunology, Statens Serum Institut, Copenhagen, Denmark).

IA-2 antibodies. Antibodies to the protein tyrosine phosphatase related IA-2 molecule were analyzed with a radiobinding assay as previously described (18). The results were expressed as relative units (RU) based on a standard curve run on each plate using an automated calculation program (MULTICALC; Wallac, Turku, Finland). The limit for IA-2A positivity ($\geq$ 0.77 RU) was set at the 99th percentile in 374 non-diabetic children and adolescents. The interassay coefficient of variation was <12%. This assay had a disease sensitivity of 72% and a specificity of 100% based on the 2005 Diabetes Autoantibody Standardization Program (DASP) workshop.

Insulin antibodies. Insulin antibodies (IA) were measured by a radioligand assay in a microplate format using a modification of the method described by Williams et al. (19). The cut-off limit for positivity is 1.56 RU representing the 99th percentile in a group of 371 non-diabetic subjects. The disease sensitivity of the assay was 44% and the disease specificity 98% in the Centers for Disease Control and Prevention (CDC)-sponsored DASP Workshop in 2005.

Statistical analysis

Data were transmitted anonymously by the centers; patients were identified by center number and patient code. Data are presented as mean and range for non-normally distributed parameters and mean ± standard deviation for normally distributed parameters. Data were evaluated using cross-tabulation and chi-squared statistics and one-way analysis of variance. A p-value of <0.05 is considered significant. Data for the course over time was analyzed as a repeated measurements model. C-peptide was studied on logarithmic scale with an arbitrary effect of duration, and with an interaction with age. Prediction of C-peptide (logarithmic scale) and HbA1c at 12 months was done by multiple regression analysis including covariates for age groups (0–4.9, 5–9.9, 10+yr), gender, standard bicarbonate at

onset, initial blood glucose, BMI, HbA1c, postmeal blood glucose and C-peptide (1 month), presence of ICA, IA, IA-2A, and GADA (four variables, all evaluated at 1 month). The insignificant variables were excluded in a backward stepwise approach.

The influence of HLA risk groups on residual beta-cell function and HbA1c was investigated by regression analysis with HbA1c and the logarithm of stimulated C-peptide as dependent variables accounting for gender, age, 1, 6, and 12 months after diagnosis. The relationship between autoantibodies and HLA risk groups were analyzed by a Wilcoxon test. The analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Demographic and clinical characteristics at diagnosis

Data were obtained from 275 patients (144 females). Mean age at diagnosis was 9.1 yr (range 0.2–16.8 yr) and 84% were white Caucasian. Clinical information including anthropometric data, pubertal status, ethnic affiliation, prediagnosis symptoms, and metabolic status at diagnosis are summarized in Table 1. The gender ratio did not differ among the three age groups 0–4.9 yr, 5–9.9 yr, and >10 yr ($p = 0.15$, χ^2-test). The number of participants in the three age groups was 48, 104, and 123, respectively. The mean duration of symptoms was 3.3 wk for polydipsia and 3.1 wk for polyuria. The average reported weight loss (mean ± SD) was 3.1 ± 3.4 kg ($n = 221$) or 8% of previous weight. This compares with the mean weight gain 2.8 ± 2.4 kg from diagnosis to 1 month ($n = 259$). The duration of symptoms was shorter and the relative weight loss lower in patients with a younger age of onset (Table 1).

There were 73 non-participants not included in the analysis: 60 being unable or unwilling to participate and 13 suspected to have non-T1D. There was no significant difference with respect to gender distribution, age, anthropometric data, HbA1c at diagnosis, ethnic distribution or family history of diabetes between patients included and non-participants (data not shown). The number of subjects enrolled per center ranged from 4 to 34 and rate of participation 46–100%.

The proportions of subjects with DKA (bicarbonate levels ≤15 mmol/L) in the three age groups were 28, 22, and 19% ($p = 0.46$), respectively (Fig. 1). Blood glucose at diagnosis was significantly higher ($p < 0.001$) in the youngest age group than in the two older groups, the means (SD) being 28.0 (12.4), 23.0 (8.8), and 24.0 (8.5) mmol/L, respectively. The mean HbA1c (SD) value at diagnosis was 10.3 (1.6)% in the 0–4.9 yr age group, which was significantly lower than in the 5–9.9 yr old group [11.0 (2.0)%] and in the ≥10 yr old age group [11.7 (2.3)%] (Fig. 2).

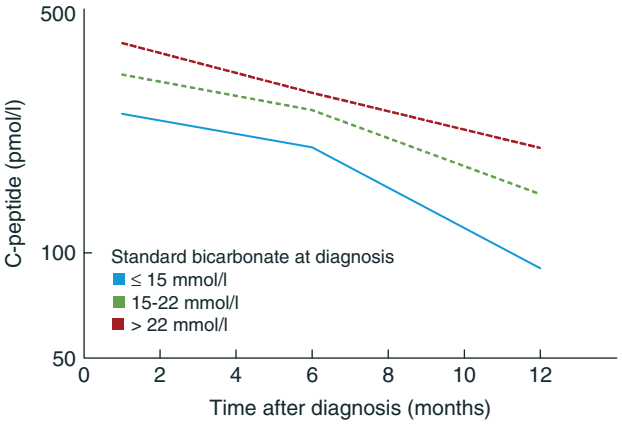

Fig. 1. The influence of standard bicarbonate at diagnosis on stimulated C-peptide during 12 months of follow-up. DKA groups: ≤15 mmol/L (solid), 15–22 mmol/L (dashed), and >22 (dot-dashed) mmol/L. Children without ketoacidosis at diagnosis (standard bicarbonate above 22 mmol/L) had significantly higher stimulated C-peptide at 1 ($p = 0.004$) and 12 months ($p = 0.0003$) after diagnosis compared to those with standard bicarbonate below 15 mmol/L.

Table 1. Clinical and demographic data at onset by age groups

	Age <5 yr	5 ≥ age <10 yr	age ≥10 yr
Number (male/female)	19/29	57/47	55/68
Mean age (Range) (yr)	3.1 (0.21–4.9)	7.8 (5.1–9.9)	12.4 (10.0–16.8)
Mean height (Range) (cm)	99.6 (74–136)	129 (104–158)	155 (128–191)
Mean weight (Range) (kg)	15.0 (9.0–26.0)	25.9 (14–45)	43.1 (23–90)
Mean BMI (Range) (kg/m^2)	15.2 (11.3–20.7)	15.4 (10.1–21.3)	17.9 (12.1–31.6)
Prepubertal (%)	48 (100)	101 (97.1)	47 (38.2)
Postmenarcheal (%)	0	0	25 (36.8)
White Caucasian (%)	41 (85.4)	89 (85.6)	101 (82.1)
Mean duration polyuria (wk)	2.5 (0–8)	2.5 (0–10)	4.0 (0–52)
Mean duration polydipsia (wk)	2.5 (0–8)	2.6 (0–10)	4.1 (0–52)
Mean weight loss (Range) (% of previous weight)	5.5 (0–25)	7.9 (0–29.6)	9.1 (0–31.8)
Mean HbA1c (SD) (%)	10.3 (1.6)	11.0 (2.0)	11.7 (2.3)
Range	7.6–15.3	6.3–15.3	7.3–17.4

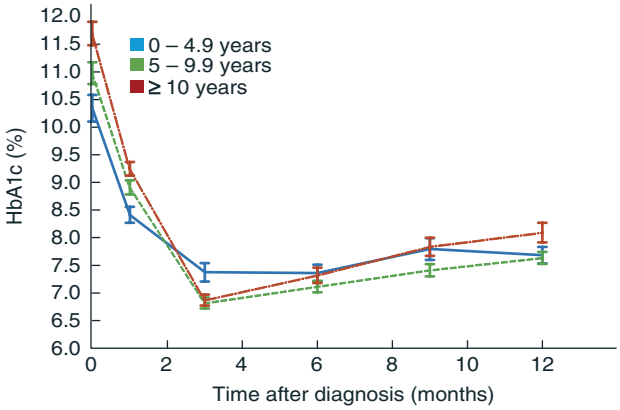

Fig. 2. HbA1c percentage during the 12 months follow-up. After 3 months duration HbA1c reaches the lowest level and subsequently increases in all age groups. The HbA1c level in the very young children are significantly lower at onset ($p < 0.0001$) and at 1 month ($p < 0.0002$) compared with older age groups and very few of the young children enter partial remission at 3 months. The 5–9.9 yr old children have significantly lower HbA1c values at 9 ($p < 0.04$) and 12 months ($p < 0.03$) compared to the older children.

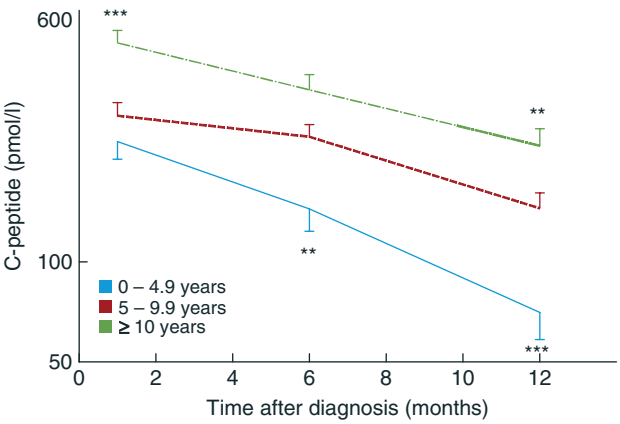

Fig. 3. Effect of age and diabetes duration on residual beta-cell function. Stimulated C-peptide level at 1 ($p < 0.0001$) and 12 months ($p = 0.02$) after diagnosis was significantly greater in the older age group than in the 5–9.9 yr while stimulated C-peptide at 6 ($p < 0.005$) and 12 months ($p = 0.0007$) was significantly lower in the youngest age group compared to the 5–9.9 yr. The difference between age 0–4.9 and 10–16 yr is significant at all time points ($p < 0.001$).

Disease progression during the first 12 months after diagnosis

Residual beta-cell function as assessed by stimulated C-peptide concentrations was related to the initial standard bicarbonate concentration. Those with a standard bicarbonate below 15 mmol/L had significantly lower C-peptide levels 1 ($p = 0.004$, estimate for log C-peptide –0.41 suggesting 34% lower residual beta-cell function) and 12 months after diagnosis ($p = 0.0003$, estimate for log C-peptide –0.71 suggesting 51% lower residual beta-cell function) compared to those with standard bicarbonate above 22 mmol/L (Fig. 1).

Overall mean HbA1c (SD) declined from 11.2 (2.1)% at diagnosis to 9.0 (1.3)% at 1 month and reached a nadir at approximately 3 months in all age groups [6.9 (1.1)%]. Subsequently, HbA1c increased to a level of 7.9 (1.5)% at 12 months (Fig. 2**).**

Throughout the study period the very young children (<5 yr) had significantly poorer residual beta-cell function with about half the stimulated C-peptide level as in children 10 yr or older ($p < 0.0001$) (Fig. 3). The youngest children had also shown a faster loss of residual beta-cell function compared to the older age groups during the first 12 months of disease. The decline in beta-cell function for a child below 5 yr from 1 to 12 months was 69%; for a child 10 yr or older, the decrease was 50% ($p < 0.001$) for the difference in decline. Furthermore, the youngest children had significantly higher IA 1 month after diagnosis ($p = 0.04$) compared to the older age groups.

After 1 month 47% of the patients tested positive for ICA and this decreased to 34% at 12 months, while the frequency of GADA remained relatively stable at 67 and 61% during the observation period as did IA-2 antibodies at 73 and 69%. By contrast, the proportion of patients with IA increased from 69 to 98% during the same time period. Those with the presence of IA at 1 month required higher daily insulin dose at 9 (0.13 U/kg, $p = 0.006$) and 12 months (0.13 U/kg, $p = 0.008$) (Fig. 4). Interestingly, IA at 6 and 12 months were not associated with stimulated C-peptide at 12 months. There was no significant relationship of HbA1c to IA during the observation period.

Prediction of stimulated C-peptide and HbA1c after 12 months of clinical disease

The best predictors at diagnosis for stimulated C-peptide concentrations at 12 months were age group ($p = 0.009$) (estimate for log C-peptide –0.43 for 0–4.9 yr suggesting 35% lower C-peptide; estimate for ≥10 yr 0.16 suggesting 17% higher C-peptide) and standard bicarbonate level (estimate 0.028/mmol/L suggesting 2.8% higher C-peptide per 1 mmol/L increase in bicarbonate, $p = 0.005$). The 1 month measurements contributing were C-peptide (estimate 0.59, $p < 0.0001$), presence of IA (estimate –0.38, suggesting 32% lower C-peptide, $p = 0.02$), GADA (estimate –0.48, suggesting 39% lower C-peptide, $p < 0.0004$), and postmeal blood glucose (estimate –0.06, suggesting 6% lower C-peptide by 1 mmol/L increase $p < 0.0004$) (Fig. 4). BMI, HbA1c, or HLA risk groups were not significantly related to stimulated C-peptide levels at 12 months. The best predictors for HbA1c at 12 months were HbA1c at diagnosis (estimate 0.23, $p < 0.0001$), GADA at 1 month (estimate 0.48% higher HbA1c, $p = 0.012$), and non-white Caucasian ethnicity (estimate 0.86% higher HbA1c, $p = 0.002$).

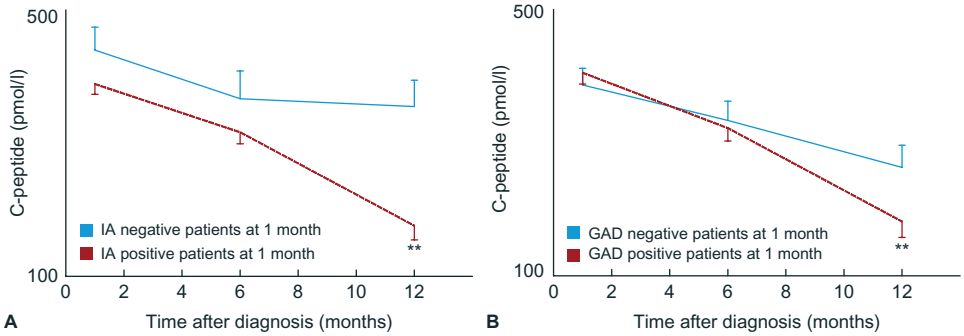

Fig. 4. (A) Insulin antibody (IA) negative patients (31% of the total) and (B) glutamic acid decarboxylase antibody (GADA) negative patients (33%) at 1 month had significantly better residual beta-cell function (IA, p = 0.003, GADA, p = 0.005) 12 months after diagnosis compared to IA and GADA positive patients.

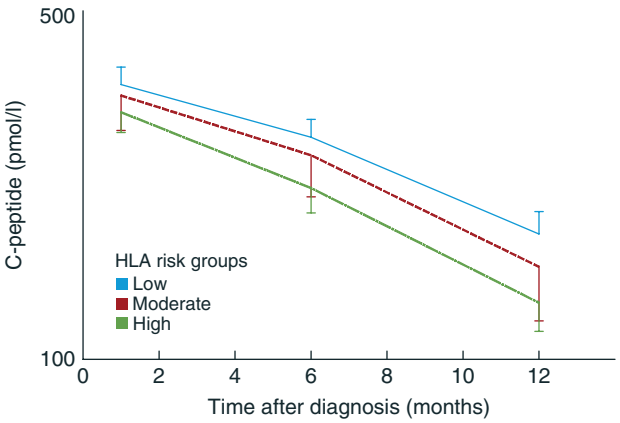

Fig. 5. Stimulated C-peptide according to HLA risk. The C-peptide level progressively decreased in all HLA risk groups in the first 12 months after diagnosis. However, there were tendencies to suggest a higher stimulated C-peptide level for patients of low HLA risk, but this was not statistically significant at any of the time points.

Age, gender, standard bicarbonate, BMI, initial blood glucose at diagnosis and ICA, IA, GADA, IA-2A, C-peptide, and postmeal blood glucose at 1 month had no significant effect.

The influence of HLA risk groups on residual beta-cell function, HbA1c and autoantibodies

There was no statistically significant association between stimulated C-peptide and HLA risk groups (low, moderate, and high risk) at 1 (p = 0.60), 6 (p = 0.29), and 12 (p = 0.15) months after diagnosis (Fig. 5). In addition HLA risk groups were not statistically significantly associated with HbA1c and autoantibodies (ICA, IA, GADA, IA-2A) at any of the time points (data not shown).

Discussion

This multicenter prospective study was designed to investigate, in children and adolescents with T1D, interacting factors which might influence the decline of beta-cell function and glycemic control during the first 12 months after diagnosis. The main findings at diagnosis are that the youngest children have higher BG, lower HbA1c, more severe ketoacidosis, and higher levels of IA. This may represent a shorter but more aggressive prediabetes phase corresponding to a rapid and more extensive destruction of beta-cells, relatively smaller beta-cell mass or less regenerative beta-cell capacity, in the very young children (Figs 1–3). Several studies have reported an association between greater loss of beta-cell function and younger age (10, 13, 14, 20), and more severe ketoacidosis at onset (21).

The best predictors for stimulated C-peptide levels at 12 months are age and bicarbonate level at diagnosis, and the presence of IA and GADA levels 1 month after diagnosis, which seem to be associated with a more rapid loss of residual beta-cell function. There was no association with high-risk HLA status. The best predictors of glycemic control (HbA1c) at 12 months were HbA1c at diagnosis, non-white Caucasian ethnicity, and presence of GADA at 1 month but not C-peptide levels. Most studies have been either retrospective or with small numbers of young people, but in one of the larger prospective studies from a single center, younger age, male gender, and initial ICA positivity made a significant contribution to the variation in C-peptide levels and in loss of beta-cell function (20). That study also reported that shorter symptom duration at onset and ketoacidosis significantly predicted the rate of loss of C-peptide secretion but showed no significant differences in glycated hemoglobin values between patients who secreted C-peptide and those who did not (22). We have not been able to find a correlation between duration of symptoms prior to diagnosis and subsequent beta-cell function, perhaps because our study is multicenter based and the questionnaire lacked sensitivity in this area. Moreover, we have not found a predictive association between beta-cell function and HbA1c and this is in agreement with

other studies (10, 23). Our results show a relationship between two immunological markers (GADA, IA) and stimulated C-peptide concentration indicating that patients with these antibodies have a more rapid decline in residual beta-cell function (Fig. 4). Others have shown a significant independent effect of GADA with decreased C-peptide concentration at 6 months after diagnosis (10, 24), and GADA negative patients were more likely to exhibit a remission phase (10, 25). Some studies have also indicated that ICA-negative patients have higher C-peptide levels (14, 26) but that neither IA nor metabolic state at onset affects C-peptide secretion (14, 26).

In the prediabetic phase among family members at high risk of developing diabetes it has been argued that the measurement of cytoplasmic ICA's remains a better predictor of beta-cell destruction than GADA and IA-2A (27). Although this is not necessarily applicable to the decline in residual beta-cell function after diagnosis, our data indicate that ICA does not predict the decline in endogenous insulin secretion 12 months after diagnosis, whereas GADA and IA do. Our finding of IA in 69% of the patients 1 month after diagnosis is not surprising, as they represent a mixture of insulin autoantibodies and antibodies induced by exogenous insulin. However, the finding that IA after 1 month can predict the C-peptide level after 12 months but not when measured at 6 and 12 months indicates that IA at 1 month mainly consist of autoantibodies. At 6 and 12 months the antibodies are influenced by exogenous insulin treatment. At diagnosis IA can be detected in more than 50% of children (25) and IA levels increase soon after the initiation of insulin therapy but may remain relatively stable thereafter (26). Most studies have not shown an association between IA and C-peptide secretion (14, 26), although it has been argued that the finding of immune positivity (ICA, IA, etc.) indicates a wider level of autoimmunity to as yet unidentified autoantigens which may be important in the initiation and progression of beta-cell loss (28). This same HSG cohort has been studied by Nielsen et al. (29) who reported that the IA titers at 1 and 6 months were significantly lower in the INS VNTR (*IDDM2*) class III/III and class I/III genotype groups compared with the class I/I genotype. In addition stimulated serum C-peptide concentrations were twice as high among carriers of the class III/III genotype as compared with class I/I and class I/III genotypes implicating a direct connection *in vivo* between the INS VNTR class III alleles, a reduced humoral immune response to insulin, and preservation of beta-cell function in recent onset T1D. This is consistent with our finding that high IA levels 1 month after diagnosis is associated with a more rapid decline in residual beta-cell function. Our observation indicates that the type of antibodies (such as IA and GADA) is a better predictor of the endogenous insulin secretion at 12 months than the number of positive antibodies.

Glycated hemoglobin is the important clinical outcome measured during the remission phase, and indeed when the remission phase is defined as a low insulin dose, near normal glycemia with minimal glycosuria, then glycated hemoglobin has been observed to be similarly reduced (Fig. 2). Some studies have found a significant inverse relationship between C-peptide and HbA1c at onset (30), and at 6 and 12 months we observed a similar relationship, whereas other studies have failed to find such an association (10, 26). In another report based on this HSG cohort we have explored the importance of the inverse relationship between HbA1c, insulin dose, and C-peptide to create a surrogate measure of stimulated C-peptide which is convenient and easy to use in the daily routine (31). In adults it has been shown that prolonged secretion of C-peptide may contribute to better metabolic control (32).

We found that HbA1c at 12 months was predicted only by HbA1c at diagnosis and GADA titers at 1 month. It is of interest that there may already be factors operating before diagnosis determining glycemic control later in the first year, including ethnicity. When comparisons have been made between children with new onset T1D in different parts of Europe, ethnic differences were suggested as a cause for a more aggressive disease process (33). Furthermore a study between Canada and Finland showed that the Finish children at diagnosis had lower blood glucose and HbA1c values and higher C-peptide levels (34). These intriguing differences are the subject of further investigation by our multinational study group.

In Finland with a higher prevalence of multiplex families with T1D, evidence has been generated that the rate of onset and severity of symptoms of T1D is increased in patients who are Dw3/Dw4 heterozygotes (35) and that C-peptide secretion is affected by certain high-risk HLA genotypes. Our results do not show a statistically significant association between the HLA status and the progression of the disease as assessed by the C-peptide levels (Fig. 5), and this is consistent with other studies (10, 22). It suggests that HLA genotypes are predisposing to the onset of the disease but the partial recovery of the injured beta-cell depends on other factors, including the cytokine attack (36), improvement of peripheral insulin sensitivity (7), and a regenerating beta-cell mass (8).

However, as suggested above with respect to the INS VNTR genes, there are other biological markers which may well influence the beta-cell function, the immunological processes and the rate of beta-cell destruction (36). Our results suggest that C-peptide and HbA1c at 12 months are predicted by the same

variables at diagnosis or 1 month later. This may illustrate the different dimensions of the disease severity. Other variables that seem important are age, severity of DKA, and ethnic factors. Both IA and GAD autoantibodies seem to influence the process, suggesting that, if predictions of beta-cell decline or potential responses to interventional treatment are required, it will be of value to measure these early in the course of the disease. In our study antibodies were measured at the same time (1 month) as C-peptide stimulation, which could not take place earlier for ethical reasons. In future studies, it might be preferable to measure these and other antibodies at the time of diagnosis, as they have a high predictive value for the future course of the disease.

Conclusions

The study shows that residual beta-cell function and glycemic control after 1 yr of clinical disease can be predicted by objective factors present at diagnosis or soon after diagnosis such as age, degree of metabolic decompensation, and humoral immune responses.

The HSG is continuing to explore more complex mechanisms, which may influence the decline in beta-cell function such as recently described genetic factors, levels of cytochemical attack, other factors which mediate kemokines from the innate immune system and beta-cell growth factors like the incretin hormones.

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Appendix

Members of the Hvidøre Study Group on Childhood Diabetes who have contributed samples on immunologic and genetic markers in the Remission Phase Study.

Henk-Jan Aanstoot, MD, PhD, Department of Pediatrics, Ijsselland Hospital, Capelle, the Netherlands

Carine de Beaufort, MD, Clinique Pédiatrique, Luxembourg

Francesco Chiarelli, MD, Professor, Clinica Pediatrica, Chieti, Italy

Knut Dahl-Jørgensen, MD, Dr Med. SCI, Professor, and Hilde Bjørndalen Göthner, MD, Department of Paediatrics, Ullevål University Hospital, Oslo, Norge

Thomas Danne, MD, Charité, Campus Virchow-Klinikum, Berlin, Germany

Patrick Garandeau, MD, Unité D'endocrinologie Diabetologie Infantile, Institut Saint Pierre, Montpellier, France

Stephen A. Greene, MD, DC, University of Dundee, Dundee, Scotland

Reinhard W. Holl, MD, University of Ulm, Ulm, Germany

Mirjana Kocova, MD, Professor, Pediatric Clinic-Skopje, Skopje, Republic of Macedonia

Pedro Martul, MD, PhD, Endocrinologia Pediatrica Hospital De Cruces, Baracaldo, Spain

Nobuo Matsuura, MD, Professor, Kitasato, University School of Medicine, Kanagawa, Japan

Henrik B. Mortensen, MD, Dr Med. SCI, Department of Pediatrics, Glostrup University Hospital, Glostrup, Denmark

Kenneth J. Robertson, MD, Royal Hospital for Sick Children, Yorkhill, Glasgow, Scotland

Eugen J. Schoenle, MD, University Children's Hospital, Zurich, Switzerland

Peter Swift, MD, Leicester Royal Infirmary Childrens Hospital, Leicester, UK

Rosa Maria Tsou, MD, Paediatric Department, Oporto, Portugal

Maurizio Vanelli, MD, Paediatrics, University of Parma, Parma, Italy

Jan Åman, MD, PhD, Department of Paediatrics, Örebro Medical Centre Hospital, Örebro, Sweden

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