Higher risk of severe hypoglycemia in children and adolescents with a rapid loss of C-peptide during the first 6 years after type 1 diabetes diagnosis

Annika Grönberg,1 Daniel Espes,2,3 Per-Ola Carlsson,2,3 Johnny Ludvigsson4

ABSTRACT

Introduction The progression to insulin deficiency in type 1 diabetes is heterogeneous. This study aimed to identify early characteristics associated with rapid or slow decline of beta-cell function and how it affects the clinical course.

Research design and methods Stimulated C-peptide was assessed by mixed meal tolerance test in 50 children (<18 years) during 2004–2017, at regular intervals for 6 years from type 1 diabetes diagnosis. 40% of the children had a rapid decline of stimulated C-peptide defined as no measurable C-peptide (<0.03 nmol/L) 30 months after diagnosis.

Results At diagnosis, higher frequencies of detectable glutamic acid decarboxylase antibodies (GADA) and IA-2A (p=0.027) were associated with rapid loss of beta-cell function. C-peptide was predicted positively by age at 18 months (p=0.017) and 30 months duration (p=0.038). BMI SD scores (BMISDS) at diagnosis predicted higher C-peptide at diagnosis (p=0.006), 3 months (p=0.002), 9 months (p=0.005), 30 months (p=0.022), 3 years (p=0.009), 4 years (p=0.016) and 6 years (p=0.026), whereas high HbA1c and blood glucose at diagnosis predicted a lower C-peptide at diagnosis (p=0.001) for both comparisons. Both GADA and IA-2A were negative predictors of C-peptide at 9 months (p=0.011), 18 months (p=0.008) and 30 months (p=0.001). Ten children had 22 events of severe hypoglycemia, and they had lower mean C-peptide at 18 months (p=0.025), 30 months (p=0.008) and 6 years (p=0.018) compared with others. Seven of them had a rapid decline of C-peptide (p=0.030), and the odds to experience a severe hypoglycemia were nearly fivefold increased (OR=4.846, p=0.04).

Conclusions Low age and presence of multiple autoantibodies at diagnosis predicts a rapid loss of beta-cell function in children with type 1 diabetes. Low C-peptide is associated with an increased risk of severe hypoglycemia and higher Hemoglobin A1C. A high BMISDS at diagnosis is predictive of remaining beta-cell function during the 6 years of follow-up.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Low age, ketoacidosis at onset and high HbA1c are factors associated with a rapid loss of beta-cell function after onset of type 1 diabetes, while lower HbA1c during the first years and higher frequency of the HLADR3 genotype are factors associated with a long-term preservation of C-peptide.

WHAT THIS STUDY ADDS

⇒ High BMI SD scores (BMISDS) at diagnosis predicts a slower decline of endogenous beta-cell function.
⇒ A decline of stimulated C-peptide to undetectable levels (<0.03 nmol/L) within 30 months after diagnosis increases the risk of severe hypoglycemia (five times higher OR for severe hypoglycemia), and low C-peptide is associated with higher HbA1c and insulin requirement.
⇒ Higher frequencies of detectable glutamic acid decarboxylase antibodies and islet cell autoantibodies islet antigen 2 at diagnosis were associated with rapid loss of beta-cell function.
⇒ Measurements of residual C-peptide in children and adolescents during the progression of type 1 diabetes provide valuable information about clinical course.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Repeated measurements of C-peptide during follow-up of type 1 diabetes are warranted and are important for identifying the children and adolescents at highest risk of severe hypoglycemia.

WHAT ARE THE NEW FINDINGS?

⇒ A rapid loss of C-peptide increases the risk of severe hypoglycemia.
⇒ High BMISDS at diagnosis predicts a slower decline of beta-cell function.
⇒ Higher frequencies of GADA and IA-2A at diagnosis were associated with rapid loss of beta-cell function.

INTRODUCTION

C-peptide can be detected in most children and adolescents at diagnosis of type 1 diabetes,1 2 but the disease is characterized by a progressive decline in beta-cell function.1 3 4 Several factors, including age at diagnosis,5 female gender,6 body mass index (BMI) and ketoacidosis at diagnosis1 4 7–11 as well as the occurrence of islet cell autoantibodies islet antigen 2 (IA-2A) and glutamic
Clinical care/Education/Nutrition

acid decarboxylase antibodies (GADA) are known to influence the decline of residual beta-cell function. Even though the clinical importance of residual endogenous insulin secretion is well known, further knowledge on the natural course of the disease with particular identification of influencing factors possible to modify is desirable. We have made a retrospective study of type 1 diabetes subjects, whose beta-cell functions from diagnosis and onwards, as well as clinical course, are known. The aim was to identify early characteristics associated with a rapid or slow decline of beta-cell function in these children and adolescent with newly diagnosed type 1 diabetes and to investigate how the decline of C-peptide affects the clinical course of the disease during the first 6 years.

Research design and methods

Study subjects

Inclusion criteria to participate in the study were children and adolescent with newly diagnosed type 1 diabetes (<18 years), with start of insulin treatment at admission, with informed consent to participate and followed regularly at Crown Princess Victoria Children’s Hospital, Linköping, Sweden. Children with secondary diabetes and transition to non-insulin treatment during the years of follow-up were excluded from the study. All subjects were followed as part of clinical routine with measurements of residual beta-cell function at regular intervals from diagnosis until residual beta-cell function was undetectable. In order to study the difference between individuals with rapid or slow loss of residual beta-cell function, respectively, we included 50 subjects, some with rapid loss of C-peptide (n=20), defined as having undetectable C-peptide (<0.03 nmol/L) within 30 months after diagnosis and others who had residual function up to 6 years after diagnosis (n=30).

The study subjects were born 1989–2007 and were diagnosed with type 1 diabetes during the years 2004–2017. They were at diagnosis of the disease at an age of 10.6±2.5 years, and 44% (n=22) of them were male. Diagnosis of type 1 diabetes was based on the American Diabetes Association criteria for diagnosis and classification of type 1 diabetes. At diagnosis, all study subjects had been hospitalized at the Children’s Hospital and started on multiple insulin injection therapy. Thereafter, they were followed by the diabetes team at regular visits. At the age of 18 years, the study subjects were transferred to a diabetes clinic for adults. In 14 of the study subjects some data were only available 4–6 years after diagnosis.

Data collection

Descriptive data were registered and collected from medical records and from the Swedish Childhood Diabetes Registry (SWEDIABKIDS), a national incidence and quality control register. Data included age, sex, HbA1c, blood glucose, blood pH and C-peptide at the time of diagnosis prior to start of insulin treatment. Weight, height, HbA1c and insulin dose (units/kg body weight/24 hours) was registered at every follow-up visit, that is, at 10 days, 1, 3, 9, 18, 24 and 30 months and 3, 4, 5 and 6 years after diagnosis. BMI (kg/m²) and BMI SD scores (BMISDS), adjusted for age and sex, were generated automatically by the SWEDIABKIDS register. The occurrence of episodes with ketoacidosis (defined as pH<7.30) or severe hypoglycemic events (SHs) were registered at every follow-up visit. SH was defined as an event of hypoglycemia (capillary blood glucose <3.5 mmol/L) with severe cognitive impairment (including coma and convulsions) requiring assistance of another person.

Procedures of study and biochemical analyses

A mixed meal tolerance test (MMTT) was performed under fasting conditions in the morning; C-peptide and glucose were sampled at baseline and at 30 min intervals during the 120 min test. Study subjects were instructed not to administer short-acting insulin within 6 hours prior to the test. An MMTT was performed at 3, 9, 18 and 30 months and at 3, 4, 5 and 6 years after diagnosis. During the first 2 years of the study (2004–2005), a standardized breakfast with 50% carbohydrates, 33% lipids and 17% proteins was ingested as the mixed meal test. From 2006, the MMTT consisted of an ingestion of a standardized liquid meal of 6 mL Sustacal/kg body weight (maximum 360 mL, 1 calorie/mL; 35% carbohydrates, 21% lipids and 24% protein). The serum samples were stored at −20°C until analysis. C-peptide concentration was measured usually within 2 weeks from sampling at the research laboratory of the Division of Pediatrics, Linköping University, using a time-resolved fluoroimmunoassay (AutoDELFIA C-peptide kit; Wallac) with a software program (1224 MultiCalc; Wallac) for automatic calculation of values. The level of detection of the assay was 0.03 nmol/L.

MMTTs were performed until the study subjects no longer had any detectable C-peptide (defined as <0.03 nmol/L). For the following time points during the study period, undetectable C-peptide levels (<0.03 nmol/L) were assigned a numeric value of 0.01 nmol/L for statistical analysis.

Since 2005, the Crown Princess Victoria Children’s Hospital participates in the nationwide cohort study ‘Better Diabetes Diagnosis’, which was started to monitor newly diagnosed children and adolescents with diabetes for genetic predisposition and clinical phenotypes.
Blood samples for C-peptide concentrations, autoantibodies and HLADQ genotypes were collected and analyzed at diagnosis. Fasting C-peptide concentrations were also collected and analyzed 10 and 30 days after diagnosis. C-peptide was analyzed in Linköping. Autoantibodies glutamic acid decarboxylase antibodies (GADA; detection limit 5 IU/mL) and islet antigen-2 antibodies (Islet antigen-2 antibodies; detection limit 7.5 kU/L) were analyzed using two-sided ELISA test. Samples negative for ELISA IA-2A were further analyzed with a high sensitivity IA-2A radio binding assay.6 HLA DQA1-DQB1 genotypes were determined with PCR.7 Analyses of autoantibodies and HLA genotypes were performed at the Department of Clinical Chemistry, Skåne University Hospital, Malmö, Sweden.

The study subjects were based on the characteristics of almost half of the study subjects having undetectable C-peptide concentrations at 30-month follow-up, divided into two groups based on rate of C-peptide decline, rapid progressors (n=20), which were then defined as having undetectable C-peptide within 30 months after diagnosis and slow progressors (n=30) for which C-peptide was still detectable 3–6 years after diagnosis.

Analyses of HbA1c, pH and blood glucose were performed at the Department of Clinical Chemistry, Linköping University Hospital. The laboratory is certified by a Swedish government authority (Swedac).

From October 2010, HbA1c was analyzed according to the International Federation of Clinical Chemistry and Laboratory Medicine reference method and expressed as mmol/mol. Prior to October 2010, analyses were according to the Mono S standard expressed in per cent. In SWEDIABKIDS, analyses performed with the Mono S standard were recalculated using the expression HbA1c (IFCC; mmol/mol) = 10.45 × HbA1c (Mono S; %) – 10.62 (http://www.ngsp.org/convert1.asp).

**Statistics**

Statistical analyses were performed using SPSS V.28.0.0. Values are given as mean±SD (range). Student’s t-test for independent samples was used to compare differences between two groups of normal distributed continuous data, and χ² test and Fisher’s exact test were used for analyses of categorical data. Fisher’s test was used when expected cell count was less than 5. Predictors of residual C-peptide secretion as a binary outcome were compared using univariate and multivariate logistic regression analyses and analysis of variance for the main effects, expressed with ORs and 95% CI. P values <0.05 were considered statistically significant.

<table>
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<th>Time</th>
<th>All subjects</th>
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<th>Rapid progressor</th>
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<td>n=50</td>
<td>n=30</td>
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<td>Day 0</td>
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<td>3 months</td>
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<td>(0.04–0.78)</td>
<td>(0.08–0.75)</td>
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<td>9 months</td>
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<td>(0.04–0.29)</td>
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<td>18 months</td>
<td>0.15±0.14</td>
<td>0.22±0.15</td>
<td>0.07±0.05</td>
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<td>24 months</td>
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<td>30 months</td>
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<td>3 years</td>
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<td>4 years</td>
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<td>5 years</td>
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<td>6 years</td>
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</table>

P values <0.05 were considered statistically significant.
RESULTS

The C-peptide concentration was highest at diagnosis (0.37±0.36 nmol/L (mean±SD) (n=42)) (table 1).

Thirty months after diagnosis, C-peptide was not measurable in the rapid progressor group (n=20) (C-peptide <0.03 nmol/L) (table 1). There was no difference between sexes but a difference in C-peptide at every time point, except for 3 months after diagnosis, between the slow and rapid progressor groups (figure 1A) (table 1). In a linear regression analysis, C-peptide was...
predicted positively by age at diagnosis at 18 months (r=0.125, p=0.017) and 30 months (r=0.087, p=0.038) and also positively predicted by BMISDS at diagnosis (r=0.195, p=0.006), 3 months (r=0.203, p=0.002) (online supplemental figure S1), 9 months (r=0.174, p=0.005), 30 months (r=0.118, p=0.022), 3 years (r=0.125, p=0.009), 4 years (r=0.136, p=0.016) and 6 years (r=0.134, p=0.026). High HbA1c at diagnosis predicted a lower C-peptide at diagnosis (r=-0.300, p<0.001) and at 10 days (r=0.126, p=0.015). Blood glucose, but not sex, pH or ketoacidosis, at diagnosis was also negatively associated with C-peptide at diagnosis (r=-0.301, p=0.001). The average calculated HbA1c during the first and second year was negatively associated with C-peptide at 30 months (r=-0.088, p=0.039) and (r=-0.094, p=0.030) for first and second year, respectively. Detectable GADA at diagnosis was a negative predictor for C-peptide, with lower C-peptide at 18 months (p=0.025) and 30 months (p=0.042). Detectable IA-2A was also a negative predictor for C-peptide at 9 months (p=0.005), 18 months (p=0.003) and 30 months (p=0.001). Both GADA and IA-2A were negative predictors of C-peptide at 9 months (p=0.011), 18 months (p=0.008) and 30 months (p=0.001).

When comparing clinical features in the slow versus rapid progressor groups during the study period (figure 1B–D), there was no difference at diabetes diagnosis in sex, age, HbA1c, blood glucose, BMISDS, pH, ketoacidosis or HLA risk profile (HLADQ genotypes DRB1*04-DQA103-DQB10302) but a higher frequency of both detectable GADA and IA-2A in rapid progressors (p=0.027) (table 2).

We observed no difference between slow and rapid progressors in mean HbA1c for each individual year or in BMISDS (figure 1B,C). Daily insulin doses were lower in slow progressors at 24-month follow-up (0.82±0.32 vs 1.01±0.31 U/kg/24 hours, p=0.044) (figure 1D). IDAA1C was lower in slow progressors at 9-month follow-up (8.8±1.2 vs 9.9±1.7, p=0.019), but there was no difference in number of study subjects in partial remission. There were no episodes of diabetic ketoacidosis during the study period.

Ten children (six girls and four boys) experienced 22 events of SH during the study period: 3/30 (10%) from the slow progressor group and 7/20 (35%) from the rapid progressor group (p=0.030). During the first 3 years after diagnosis, six study subjects experienced an event of SH, and all of these subjects were rapid progressors (p=0.002). The study subjects in the rapid progressor group had altogether 18 events of SH during the study period. There was a lower mean C-peptide concentration in those with SH (n=10) versus not any SH (n=40) at 18 months (p=0.025), 30 months (p=0.008) and 6 years (p=0.018) after the diagnosis (figure 2). When comparing slow and rapid progressors, low C-peptide concentrations at 18 months (0.22±0.15 nmol/L) versus (0.07±0.05 nmol/L) (p<0.001) and 30 months (0.19±0.17 nmol/L) versus (0.01±0.00 nmol/L) (p<0.001) (table 1) were associated with SH.

When applying a binary logistic regression analysis, expressed with ORs, there was no difference for any of clinical characteristics between the progressor groups at diagnosis. However, there was a difference in OR in the rapid progressor group for detectable GADA and IA-2A (OR: 5.893, p=0.038) and for lower C-peptide at diagnosis (OR=0.001, p=0.037). The study subjects with rapid progress had also nearly five times higher odds to experience a SH during the study period (OR=4.846, p=0.04).

**DISCUSSION**

The present study depicts the natural course of beta-cell function and clinical outcome in 50 children and adolescents newly diagnosed with type 1 diabetes during the first 6 years of follow-up. By comparing early clinical characteristics associated with a rapid or slow decline of stimulated C-peptide, we have investigated the impact of beta-cell function for the clinical course of type 1 diabetes.

Based on stimulated C-peptide levels and clinical descriptive data, we have identified that low age at diagnosis and presence of multiple autoantibodies can predict a rapid decline of beta-cell function, which affects the clinical course by increasing the risk and frequency of severe hypoglycemia. A high BMISDS at diagnosis is the most important predictor for maintaining beta-cell function over time. Rapid progressors, that is, C-peptide was undetectable (<0.03 nmol/L) within 30 months after diagnosis, had a higher frequency of detectable autoantibodies GADA and IA-2A at diagnosis, but there was no difference in other clinical characteristics. Occurrence of multiple autoantibodies indicate an autoimmune progressive destruction of beta-cells and is a well-known predictor of rapid decline in C-peptide secretion.4 8 12 19 The present results show that autoantibodies at diagnosis also are a predictor of rapid beta-cell functional loss in all subjects from the ninth month after diagnosis.
HbA1c did not differ between rapid and slow progressors, but study subjects with less C-peptide had higher average calculated HbA1c first 2 years and slow progressors needed less insulin. Thus, even if it seems to be possible to achieve good glycemic control with use of intensive insulin treatment in spite of low residual C-peptide, even low own residual beta cell function improves metabolic control.

Furthermore, loss of residual C-peptide is known to be an important predictor for short-term complications such as severe hypoglycemia, which we confirm in this study, and ketoacidosis as well as for long-term diabetes complications.

We observed no episodes of ketoacidosis, but 22 events of SH during the 6 years of follow-up. SH was only experienced by study subjects with rapid loss of C-peptide during the first 3 years after diagnosis. These findings are well in line with previous evidence of a protective effect even of low levels of residual C-peptide on the frequency of SH. We found that the slow progressors even with low C-peptide (<0.03 μmol/L) still had reduced risk of SH during the follow-up. The study subjects that experienced an SH during the 6 years of follow-up had lower levels of C-peptide at 18 and 30 months after diagnosis.

Subjects with a rapid decline of C-peptide presented with lower levels of C-peptide already at diagnosis and for every time point during the follow-up period, except during the remission period 3 months after diagnosis. These results are indicative of a lower beta-cell function in rapid progressors already at diagnosis, which could be related to a more aggressive immunological processes or a delayed diagnosis. At 3 months, the glycemic control was improved in both groups with the lowest average HbA1c and insulin requirements during the whole study period. The remaining beta-cells might temporarily recover, a clinical partial remission phase (honeymoon) starting after exogenous insulin treatment is initiated at diagnosis with improvement of the metabolic disturbance and peripheral insulin sensitivity. The duration of the remission period depends at least partly on the recovery of the beta-cell function, with a shorter period of C-peptide secretion in the rapid progressor, with a maximum stimulated C-peptide 3 months after diagnosis versus 9 months in slow progressor.

In this study, BMI SDS was an important predictor of higher C-peptide levels at diagnosis and during the 6 years of follow-up. BMI SDS at baseline has previously been reported as an important predictor of residual C-peptide and to be associated with a decline of C-peptide during the first years after diagnosis. Increased linear growth and overweight, as well as progression of insulin resistance, before diagnosis may be an accelerator for symptoms at an early stage of type 1 diabetes, with higher BMI SDS and lower HbA1c then associated with greater remaining beta-cell function. Lower average calculated HbA1c in study subjects during the first and second year predicted higher residual C-peptide 30 months after diagnosis. The residual C-peptide had a clinically beneficial effect on the frequency of SH, reflecting remaining endogenous insulin production during the first 30 months following diagnosis.

Sex did not emerge as predictor for residual C-peptide as previously shown.

There was no age-dependent differences in C-peptide at diagnosis, but younger age at diagnosis predicted lower C-peptide levels 18 and 30 months after diagnosis. This is likely explained by a more aggressive form of the disease or an overall lower beta-cell mass in young children. Age at diagnosis was one of the most important predictors of residual C-peptide, as also shown previously by most but not all studies. HLA risk profile was not associated with the progression of the C-peptide decline during the study period, which suggests that the progression of beta-cell destruction are less affected by genetic factors. The HLA risk profile may be more important as a risk for developing type 1 diabetes.

A strength of this study is the use of an MMTT analyzed with the same C-peptide assay during a longitudinal follow-up in children and adolescent, treated at a single pediatric diabetes center with a uniform treatment program and guidelines for pediatric diabetes with an ambitious treatment target. There is a limitation in that some analyses were not available when the study subjects were transferred to a diabetic clinic for adults at the age of 18 years. The registered report of the frequency of hypoglycemia and ketoacidosis might have been under-reported.

CONCLUSION

We conclude that low age at diagnosis and the presence of multiple autoantibodies predict a rapid decline of beta-cell function. Loss of C-peptide is associated with an increased risk and higher frequency of severe hypoglycemic events and less good blood glucose control. A higher BMI SDS at diagnosis is predictive of a slow decline of beta-cell function.

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Contributors JL initiated and designed the study. JL coordinated and recruited the study subjects. JL, AG and DE collected the data, and AG created the database, analyzed the data and contributed with statistical support and is also responsible for the overall content as the guarantor. AG drafted the first manuscript, and all authors edited and approved the final version of the manuscript for publication.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The Research Ethics Committee of the Faculty of Health Sciences, Linköping University approved the study with reference number 03-244. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.
Supplemental material

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