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HbA_{1c} Predicts Time to Diagnosis of Type 1 Diabetes in Children at Risk

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Prediction of type 1 diabetes is based on the detection of multiple islet autoantibodies in subjects who are at increased genetic risk. Prediction of the timing of diagnosis is challenging, however. We assessed the utility of HbA_{1c} levels in predicting the clinical disease in genetically predisposed children with multiple autoantibodies. Cord blood samples from 168,055 newborn infants were screened for class II HLA genotypes in Finland, and 14,876 children with increased genetic risk for type 1 diabetes were invited to participate in regular follow-ups, including screening for diabetes-associated autoantibodies. When two or more autoantibodies were detected, HbA_{1c} levels were analyzed at each visit. During follow-up, multiple (two or more) autoantibodies developed in 466 children; type 1 diabetes was diagnosed in 201 of these children (43%, progressors), while 265 children remained disease free (nonprogressors) by December 2011. A 10% increase in HbA_{1c} levels in samples obtained 3–12 months apart predicted the diagnosis of clinical disease (hazard ratio [HR] 5.7 [95% CI 4.1–7.9]) after a median time of 1.1 years (interquartile range [IQR] 0.6–3.1 years) from the observed rise of HbA_{1c}. If the HbA_{1c} level was ≥5.9% (41 mmol/mol) in two consecutive samples, the median time to diagnosis was 0.9 years (IQR 0.3–1.5, HR 11.9 [95% CI 8.8–16.0]). In conclusion, HbA_{1c} is a useful biochemical marker when predicting the time to diagnosis of type 1 diabetes in children with multiple autoantibodies.

The development of type 1 diabetes is characterized by immune-mediated destruction of the pancreatic β-cells, which eventually stop producing insulin. The incidence of type 1 diabetes has been growing worldwide, with the highest incidence rate observed in Finland, where the rate has more than doubled from 1980 to 2005 in children under the age of 15 years (1–3).

More than 50 genes conferring susceptibility to human type 1 diabetes have been identified (4). The major risk genes are located in the class II HLA region on chromosome 6p21 (5). Environmental risk factors are believed to interact with susceptibility genes and thereby contribute to the disease process (6,7). Currently, type 1 diabetes is predicted by analyzing islet autoantibodies of various specificities in an individual with increased genetic disease susceptibility. The presence of multiple (more than two) autoantibodies provides a cumulative disease risk of 50–60% over the next 5 years in children with risk-conferring HLA class II genotypes (8), and during the 15-year follow-up the risk of the development of diabetes is very high (84%) in such children (9).

Although oral glucose tolerance tests (OGTTs) have been shown to provide relatively good accuracy in the prediction of type 1 diabetes (10,11), more practical markers of glucose metabolism are needed. Glycated hemoglobin A_{1c} (HbA_{1c}) levels could be superior to OGTT results as a predictive measure, since HbA_{1c} is widely used in clinical practice as an indicator of metabolic control in patients with diabetes, and is generally not affected by

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short-term variations in food intake and physical activity (12,13). The World Health Organization and American Diabetes Association have adapted the recommendation of the International Expert Committee that an HbA_{1c} value ≥6.5% (48 mmol/mol) is diagnostic for type 2 diabetes (14), but no threshold value for the diagnosis of type 1 diabetes has been determined (15). As a laboratory test, HbA_{1c} is cost effective and easy to obtain, and it could be useful also in the prediction of type 1 diabetes (16). Although positivity for multiple islet autoantibodies has been shown to be relatively sensitive in the prediction of type 1 diabetes, there is a lack of a reliable method to predict the onset of type 1 diabetes. In this study, the goal was to monitor HbA_{1c} levels from the start of positivity for multiple (more than two) autoantibodies and to assess its potential as a predictive marker for type 1 diabetes.

RESEARCH DESIGN AND METHODS

Study Design

The Type 1 Diabetes Prediction and Prevention (DIPP) study is a Finnish population-based study in which children with HLA-conferred susceptibility to type 1 diabetes are observed prospectively from birth (17,18). Recruitment of newborn infants for the DIPP study started in November 1994 and still continues in three university hospitals (Oulu, Tampere, and Turku) in Finland. Screening for genetic risk is performed from cord blood. Families with an infant carrying HLA genotypes associated with type 1 diabetes are invited to participate in prospective follow-up at 3- to 12-month intervals until the age of 15 years or until clinical disease is diagnosed. Islet autoantibodies are analyzed at each visit; monitoring of HbA_{1c} is initiated in children who test positive for at least two different autoantibodies in the same sample, and positivity is confirmed in the subsequent sample. The diagnosis of type 1 diabetes is based on typical symptoms and random high plasma glucose levels, or in asymptomatic patients' diagnostic plasma glucose values in two separate OGTTs, as the World Health Organization recommends (19). The study population for the current analysis comprises 466 DIPP children who tested positive for multiple autoantibodies in a minimum of two consecutive samples and had at least one HbA_{1c} sample taken between November 1994 and December 2011. The study has been approved by the ethics committees of the participating university hospital districts. All families participating in the study have provided written informed consent.

Immunological Screening

DIPP participants who seroconverted to positivity for any of the diabetes-associated autoantibodies (islet cell antibodies, insulin autoantibodies, GAD antibodies, and autoantibodies specific for the insulinoma-2–associated antigen) were scheduled for follow-up visits at 3-month intervals. The age at seroconversion was defined as the age at which at least one of the diabetes-associated autoantibodies was detected for the first time. The age at multiple autoantibody positivity was defined as the time point when at least two autoantibodies were detected in the same sample.

Autoantibodies were analyzed as described previously (8). In the Diabetes Autoantibody Standardization (DASP) workshop in 2005, the following sensitivities and specificities were reported: insulin autoantibodies 58% and 96%; GAD antibodies 82% and 96%; and insulinoma-2–associated antigen antibodies 72% and 100%.

Genetic Screening

HLA-conferred susceptibility to type 1 diabetes was analyzed centrally using cord blood, as described previously (20). According to various *HLA DRB1–DQA1–DQB1* haplotype combinations, genotypes conferring high, moderate, and low risk for the disease were determined. High risk was defined as heterozygosity for the two risk-associated haplotypes *DRB1*04:01/2/4/5/8-DQA1*03-DQB1*03:02/4* and *[DRB1*03]-DQA1*05-DQB1*02*. Moderate risk was defined as homozygosity for any of the two risk haplotypes or *DRB1*04:01/2/4/5/8-DQA1*03-DQB1*03:02/4* combined with a neutral haplotype, or the *[DRB1*03]-DQA1*05-DQB1*02/[DRB1*09]-DQA1*03-DQB1*03* genotype. Low risk was conferred by other genotypes as previously defined (21).

HbA_{1c} Assays and Measurements

All HbA_{1c} values were measured from venous blood samples in the clinical laboratories of the three university hospitals. Methods for HbA_{1c} analyses varied slightly among the centers.

In the Oulu University Hospital, an immunoassay-based method was applied throughout the study. Until 6 May 2004, Cobas Integra 700 (Hoffman-La Roche, Basel, Switzerland) was used with the reagent HbA_{1c} 2054302 (Hoffmann-La Roche). From 7 May 2004 until 20 April 2009, Advia 2400 (Siemens, Munich, Germany) was used with reagent B01–4797–01 (Bayer Healthcare, Leverkusen, Germany). From 20 April 2009 onward, Advia 1800 (Siemens) has been used with the reagent HbA_{1c} 06854744 (Siemens).

In the Tampere University Hospital, fast-protein liquid chromatography was used to measure HbA_{1c} levels until 9 June 1999 (Mono S for HbA_{1c} reagent 17–1040–01; Pharmacia, Uppsala, Sweden). From 10 June 1999 until 14 November 2007, an immunoassay-based method was used (Cobas Integra with the reagent Hemoglobin A1C; Hoffmann-La Roche). From 15 November 2007 onward, Cobas Integra has been used with the reagent Tina-quant Hemoglobin A1C Gen 2.

In the Turku University Hospital, the fast-protein liquid chromatography method was used to measure HbA_{1c} levels until 5 August 1996 (Pharmacia). Thereafter, high-performance liquid chromatography has been used. From 6 August 1996 until 13 July 2001, the Variant Classic instrumentation (Bio-Rad, Hercules, CA) was used with the reagents in VARIANT HbA_{1c} Reorder Pack #270–0003 (Bio-Rad). From 14 July 2001 until 20 November 2007, the VARIANT II instrumentation (Bio-Rad) was used with the reagents in VARIANT II HbA_{1c} Reorder Pack #270–2101 (Bio-Rad). From 21 November 2007 until 13 October 2008,

the VARIANT II instrumentation (Bio-Rad) was still used but with the reagents in VARIANT II HbA_{1c} Reorder Pack #270–2101 NU (Bio-Rad). From 14 October 2008 until 2 March 2010, the VARIANT II Turbo instrumentation (Bio-Rad) was used with the reagents in VARIANT II Turbo HbA_{1c} Reorder Pack #270–2415 (Bio-Rad), which were changed to the reagents in VARIANT II Turbo HbA_{1c} Kit 2.0 #270–2455EX (Bio-Rad) starting from 3 March 2010.

The mean HbA_{1c} level measured in the Oulu University Hospital was 5.57% (SD 0.78); in Tampere University Hospital, it was 5.56% (SD 0.46); and in Turku University Hospital, it was 5.57% (SD 0.51). The covariate adjustment was used in the linear mixed model (LMM) analysis to take into account the possible confounding effect of variable assay levels in the three hospitals.

Statistical Analyses

We established a priori three time-dependent decision rules for HbA_{1c}. First, children who had undergone at least two HbA_{1c} measurements within 3–12 months were classified into two groups according to whether they experienced an increase of at least 10% in their HbA_{1c} values or not (decision rule 1). Second, children who had undergone at least two HbA_{1c} measurements within 3–12 months and a third HbA_{1c} measurement during the next 6 months were classified into two groups according to whether they had a 10% increase in HbA_{1c} values within 3–12 months and any additional increase during the next 6 months or not (decision rule 2). Third, children were divided into two groups according to whether they had two consecutive HbA_{1c} values $\geq$5.9% (41 mmol/mol) or not (decision rule 3). The use of an increase of 10% as a decision rule was based on the data from the three laboratories involved. The interassay coefficient of variation was 3.3% in Oulu University Hospital, 2.0% in Tampere University Hospital, and 2.7% in Turku University Hospital, and the reported uncertainty of measurement values were 10%, 5.8%, and 5.9%, respectively. The calculation of the uncertainty was based on current recommendations (22). The highest reported percentage of uncertainty was selected for the analysis. The cutoff HbA_{1c} value of $\geq$5.9% (41 mmol/mol) has been suggested in previous studies of the prediction of diabetes (23–25). Two sequential measurements were required to minimize the biological and analytical variations. A time window of 3–12 months was used in the analyses according to the DIPP protocol in which follow-up visits occur every 3 months.

Cox regression with these time-dependent covariates was used to evaluate the association between HbA_{1c} and the risk of diabetes. The entry time to the analysis was the date when at least two islet autoantibodies were positive. All subjects were considered to be unexposed until the time-dependent covariate fulfilled the decision rule, and thereafter the status of the subject was exposed. Consequently, if the subject had only one (decision rules 1 and 3) or two (decision rule 2) HbA_{1c} measurements, he/she belonged to the unexposed group. Samples taken on the day of diagnosis were excluded from the analyses considering the predictive use of HbA_{1c} (decision rules), but not from the LMM analysis. The total follow-up time was partitioned into intervals according to the cut points of the decision rule. Univariate Cox regression analysis was performed to identify the risk factors of diabetes, and survival curve estimates were calculated to assess the timing and probability of diabetes after fulfilling a decision rule. Sensitivity and specificity for the three decision rules were also calculated. Multivariate Cox regression with the backward stepwise model was used to identify a set of predictors that are most effective in disease prediction. In the multivariate analysis, the explanatory variable candidates were a 10% increase in HbA_{1c} values within 3–12 months, a 10% increase in HbA_{1c} values within 3–12 months and any additional increase during the next 6 months, two consecutive HbA_{1c} values $\geq$5.9% (41 mmol/mol), age at the detection of multiple autoantibodies, type 1 diabetes in a first-degree relative (FDR), HLA risk, and sex.

LMM analysis with a random intercept and first-order autoregressive covariance structure for repeated measurements was used to analyze HbA_{1c} levels over time between the progressors (children with multiple [two or more] autoantibodies who progressed to overt type 1 diabetes) and nonprogressors (children with multiple autoantibodies in whom clinical disease did not develop during the follow-up). The random intercepts and repeated measurements were nested within subjects, and subjects were nested within the hospital. The group-by-time interaction was included in the model to test differences between group means at each time point. Sex, age at sampling, age at seroconversion, age at multiple autoantibody positivity, type 1 diabetes in an FDR, and HLA risk were included in the LMM analysis as fixed variables. Differences in proportions were tested using the standardized normal deviate test.

All analyses were performed using IBM SPSS version 20.0.0 for Windows, Stata/IC version 11.2 for Windows, and StatsDirect statistical software version 2.7.9. The figures were drawn using OriginPro version 8.6.0 and Stata/IC version 11.2.

RESULTS

Between November 1994 and December 2011, a total of 168,055 newborn infants were screened for HLA-conferred susceptibility to type 1 diabetes. Altogether, 14,876 children with increased genetic risk were enrolled for regular follow-up into the DIPP study. During follow-up, multiple positive autoantibodies developed in 466 children, and 201 of these children (43%) progressed to type 1 diabetes (progressors), whereas 265 children remained disease free until the end of December 2011 (nonprogressors). In the progressor group, at least two positive autoantibodies were detected in the first positive sample of 136 children (68%), whereas in the nonprogressor group, at least two positive antibodies were detected in

the first positive sample of 110 children (42%) ($P < 0.001$). The baseline characteristics of the children are presented in Table 1.

Altogether, 4,270 HbA_{1c} samples were analyzed during the prediabetic period, 1,613 from the progressors and 2,657 from the nonprogressors. An average of 8.0 measurements per child (95% CI 7.0–9.0, range 1–37) were obtained in the group of progressors and 10.0 (95% CI 8.9–11.1, range 1–44) in the group of nonprogressors.

When retrospectively comparing HbA_{1c} levels between the progressors and nonprogressors, we observed that HbA_{1c} levels started to be consistently higher in the progressors 2.0 years before the diagnosis (Fig. 1). More specifically, during the period 1.8–2.0 years before diagnosis, the adjusted mean HbA_{1c} level was 5.5% (37 mmol/mol [95% CI 5.4–5.7 (36–39)]) among the progressors compared with 5.4% (36 mmol/mol [95% CI 5.2–5.4 (33–36)]) in the nonprogressors ($P = 0.025$). During the following year, a small but significant difference was consistently observed between the two groups. Thereafter, the adjusted mean HbA_{1c} level in the progressors started to increase more steeply, as follows: 5.9% (41 mmol/mol) during the period 0.4–0.6 years before diagnosis, 6.1% (43 mmol/mol) during the period 0.2–0.4 years before diagnosis, and 6.8% (51 mmol/mol) during the period 0.01–0.2 years before diagnosis. At diagnosis, the adjusted mean HbA_{1c} level in the progressors was 7.6% (60 mmol/mol [95% CI 7.5–7.7 (58–61)]), compared with 5.5% (37 mmol/mol [95% CI 5.4–5.6 (36–38)]) among the nonprogressors in their last measurement ($P < 0.001$). The range of crude HbA_{1c} measurements at diagnosis was 4.9–12.9% (30–117 mmol/mol) in the progressors and 4.4–6.5% (25–48 mmol/mol) in the nonprogressors at the end of follow-up.

A 10% increase in the HbA_{1c} values taken 3–12 months apart increased the disease risk almost sixfold (hazard ratio [HR] 5.7 [95% CI 4.1–7.9]) (Table 2), and clinical disease developed in half of these children within 1.1 years (interquartile range [IQR] 0.6–3.1 years) (Fig. 2*B*) according to the univariate Cox regression analysis. When including any additional rise in HbA_{1c} levels in a third measurement during the subsequent 6 months, the HR was 5.1 (95% CI 3.6–7.2), and overt disease developed in 50% of the children during the next 0.7 years (IQR 0.7–1.5 years) (Table 2 and Fig. 2*C*). If a child was found to have two consecutive HbA_{1c} values ≥5.9% (41 mmol/mol), the risk of diabetes was almost 12-fold compared with the remaining children (HR 11.9 [95% CI 8.8–16.0]), and the median time to type 1 diabetes was 0.9 years (IQR 0.3–1.5) (Table 2 and Fig. 2*D*). The most effective set of predictive variables in multivariate Cox regression analysis is shown in Table 3.

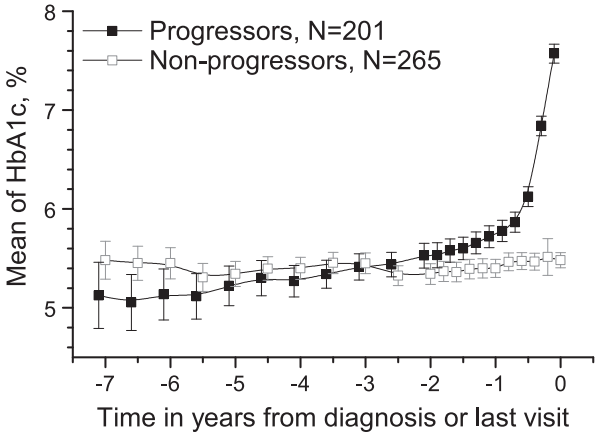
Sensitivities and specificities were calculated for the suggested decision rules. A 10% increase in HbA_{1c} levels over 3–12 months provided a sensitivity of 57% (95% CI 50–64) and a specificity of 66% (95% CI 60–72). When including an additional rise during the next 6 months, the sensitivity of the test was 22% (95% CI 17–29) and the specificity was 91% (95% CI 87–94). Two consecutive values of HbA_{1c} ≥5.9% (41 mmol/mol) presented a sensitivity of 42% (95% CI 35–49) and a specificity of 86% (95% CI 82–90).

When multiple positive autoantibodies were detected, an increase of 10% in HbA_{1c} values appeared after a mean time of 2.5 years (SD 2.0 years). For an additional rise, the

Table 1—Baseline characteristics of the study population

	Progressors (*n* = 201)	Nonprogressors (*n* = 265)	All (*N* = 466)
Sex, *n* (%)			
Boys	123 (61)	166 (63)	289 (62)
Girls	78 (39)	99 (37)	177 (38)
T1D in FDR at the time of birth, *n* (%)			
No	168 (84)	245 (92)	413 (89)
Yes	33 (16)	20 (8)	53 (11)
HLA risk,[a] *n* (%)			
Low	23 (11)	33 (13)	56 (12)
Moderate	122 (61)	178 (67)	300 (65)
High	56 (28)	53 (20)	109 (23)
Age at seroconversion, mean (SD), years	2.5 (2.0)	3.9 (2.8)	3.3 (2.6)
Age at detection of multiple (two or more) autoantibodies, mean (SD), years	3.0 (2.1)	5.2 (3.4)	4.2 (3.1)
Age at T1D diagnosis or age of nonprogressors at last measurement,[b] mean (SD), years	6.3 (3.3)	10.9 (4.1)	8.9 (4.4)
Follow-up time, mean (SD), years	3.4 (2.6)	5.6 (3.5)	4.7 (3.3)

T1D, type 1 diabetes. [a]One nonprogressor possessed a rare HLA genotype that was not possible to determine. [b]The follow-up ended on 31 December 2011.



Number of subjects

Time	-7	-6.5	-6	-5.5	-5	-4.5	-4	-3.5	-3	-2.5	-2
Progressors	8	13	14	18	25	31	40	53	61	76	63
Non-Progressors	29	35	41	51	71	75	90	99	110	122	82
Time	-1.8	-1.6	-1.4	-1.2	-1.0	-0.8	-0.6	-0.4	-0.2	0	
Progressors	52	71	81	80	80	83	101	111	108	148	
Non-Progressors	96	101	94	99	120	122	155	148	11	265	

Figure 1—Adjusted mean HbA_{1c} values during follow-up in children with multiple islet autoantibodies. In the LMM analysis, HbA_{1c} values were adjusted for sex, age at sampling, age at seroconversion, age at detection of multiple (two or more) autoantibody positivity, type 1 diabetes in an FDR, and HLA risk. The last points are from the diagnosis of type 1 diabetes (progressors, black squares) or the last follow-up visit by 31 December 2011 (nonprogressors, open squares). Whiskers show 95% CIs of the adjusted mean. HbA_{1c} levels started to be significantly and consistently higher in the progressors 2.0 years before diagnosis. The number of subjects at each time point is shown at the bottom of the figure.

time was 2.8 years (SD 2.1 years); and with two HbA_{1c} values ≥5.9% (41 mmol/mol), the mean time was 3.4 years (SD 2.4 years).

An HbA_{1c} level of ≥6.5% (48 mmol/mol) was detected in 61% of the progressors (122 of 201 progressors) and in only 2% of the nonprogressors (5 of 265 nonprogressors). However, 83% of the HbA_{1c} values ≥6.5% (48 mmol/mol) (101 of 122 values) in the group of progressors were observed at the time of diagnosis.

DISCUSSION

The prediction of type 1 diabetes has so far been based mainly on the presence of islet autoantibodies in subjects who are at increased genetic risk (8,26,27). Young age at seroconversion also has a clear impact on the risk of progression to clinical disease (17). In addition to autoantibodies, consecutive OGTT results have been of interest in the prediction of type 1 diabetes, giving at best almost 90% accuracy during a 2-year follow-up among the relatives of patients affected by type 1 diabetes (10,11,28–31). When evaluating the potential of HbA_{1c} level as an additional marker in the prediction of type 1 diabetes, we looked for new and practical ways to predict the timing of the diagnosis. The following three new criteria were established: a 10% rise in HbA_{1c} values taken 3–12 months apart, an additional rise during the subsequent 6 months, and two consecutive values of ≥5.9% (41 mmol/mol) that could be used to predict the clinical disease in a child with multiple autoantibodies. These results are important since families with a child positive for multiple islet autoantibodies are concerned about the time remaining until the development of clinical disease. They are well aware of the high disease risk and need expert counseling. Stable HbA_{1c} values in consecutive measurements suggest that the child is not going to present with overt diabetes in the near future. In contrast, an increase in HbA_{1c} level gives a warning of incipient disease, which may help in early diagnosis and thereby reduce the risk of acute complications, such as diabetic ketoacidosis (32).

Age at multiple (two or more) autoantibody positivity, the time from seroconversion to multiple autoantibodies, the presence of affected FDRs, and a high-risk HLA class II genotype have previously been identified as risk factors for type 1 diabetes (8,16,17,33). In our analyses, age at multiple autoantibody positivity was a significant predictive factor in both univariate and multivariate analyses. Children with an affected FDR had more than a twofold disease risk, demonstrating that genetic factors also have an important effect on the progression from β-cell autoimmunity to clinical disease. The HLA class II genotype appeared to play a role in disease progression, although with a lower HR than the HbA_{1c} variables.

Our prediabetic cohort of 201 Finnish children with multiple autoantibodies represents the largest series of young children who have participated in a long-term intensive follow-up from birth to diagnosis of type 1 diabetes. Only one study (16) on the evolution of HbA_{1c} levels during prediabetes has been published earlier. Although that study included a relatively small number of 28 islet autoantibody–positive children who progressed to clinical diabetes, the results were similar to our study's, showing that HbA_{1c} levels start to increase within the normal reference range and that the rise is steepest during the last 6 months before diagnosis. The strength of our study is the fact that with a considerably larger sample size and higher number of HbA_{1c} measurements per child we were able to get more accurate and reliable estimates when analyzing the predictive characteristics of HbA_{1c}. Our study population came from three clinical centers that used different methods to analyze HbA_{1c}, which might have produced slightly different absolute values. This was taken into account using covariate adjustment in the analyses. Furthermore, we used a relative increase in HbA_{1c} values rather than absolute values in our predictive model to make the results of our study more generalizable. Currently, there are no exact threshold values for a significant change in HbA_{1c} levels. Our decision rules were based on the available literature and

Table 2—Univariate Cox regression HRs for the contribution of clinical factors to the progression of type 1 diabetes

	Progressors (n = 201)	Nonprogressors (n = 265)	HR	95% CI	P value
10% increase in HbA_{1c} values within 3–12 months, n (%)					
No	87 (43)	175 (66)	1		
Yes	114 (57)	90 (34)	5.7	4.1–7.9	<0.001
10% increase in HbA_{1c} values within 3–12 months and any additional increase during the next 6 months, n (%)					
No	156 (78)	242 (91)	1		
Yes	45 (22)	23 (9)	5.1	3.6–7.2	<0.001
Two consecutive HbA_{1c} values ≥5.9% (41 mmol/mol), n (%)					
No	116 (58)	229 (86)	1		
Yes	85 (42)	36 (14)	11.9	8.8–16.0	<0.001
Age at detection of multiple (two or more) autoantibodies, mean (SD), years	3.0 (2.1)	5.2 (3.4)	0.8	0.8–0.9	<0.001
Time from seroconversion to detection of multiple (two or more) autoantibodies, mean (SD), years	0.4 (1.1)	1.3 (2.1)	0.7	0.6–0.8	<0.001
T1D in FDR at the time of birth, n (%)					
No	168 (84)	245 (92)	1		
Yes	33 (16)	20 (8)	1.9	1.3–2.7	0.001
HLA risk,[a] n (%)					
Low	23 (11)	33 (13)	1		
Moderate	122 (61)	178 (67)	0.9	0.6–1.5	0.816
High	56 (28)	53 (20)	1.3	0.8–2.1	0.313
Sex, n (%)					
Male	123 (61)	166 (63)	1		
Female	78 (39)	99 (37)	1.1	0.8–1.5	0.534

T1D, type 1 diabetes. [a]One nonprogressor possessed a rare HLA genotype that was not possible to determine.

variances reported by the university hospital laboratories involved, and they are described in more detail in the RESEARCH DESIGN AND METHODS section.

The suggested decision rules of a 10% rise in HbA_{1c} level during 3–12 months and two consecutive HbA_{1c} values ≥5.9% (41 mmol/mol) both provide high HRs with relatively short median time to diagnosis of type 1 diabetes (1.1 and 0.9 years, respectively). However, a 10% rise provides superior sensitivity (57% vs. 42%), whereas two consecutive results of ≥5.9% (41 mmol/mol) has a higher specificity (86% vs. 66%). Since children with multiple (two or more) autoantibodies have a high risk of the development of type 1 diabetes over a variable period of time, it is probably more practical from the clinical point of view to use two consecutive HbA_{1c} results ≥5.9% in the prediction of the timing of diagnosis. It is still noteworthy that a relative increase of 10% in HbA_{1c} level is more independent from methodological differences and reference values, therefore remaining important alongside the absolute values.

Our retrospective analysis showed that HbA_{1c} values start to increase ~2 years before the diagnosis, reflecting the gradual deterioration in endogenous insulin secretion and increasing fluctuation in plasma glucose levels. There were some differences in the demographic characteristics between the nonprogressors and the progressors, however. The nonprogressors were older both at initial seroconversion and when multiple autoantibodies developed. The mean age of the nonprogressors was 10.9 years at the end of follow-up, whereas the age of the progressors at diagnosis was 6.3 years. The nonprogressors had affected family members less often. On the other hand, our data show that the mean HbA_{1c} level remained very stable in the children in the nonprogressor group during the follow-up, varying from 5.3% (34 mmol/mol) to 5.6% (38 mmol/mol), and therefore the differences in age and follow-up time are probably not interfering with this analysis.

Our results provide important data on the natural evolution of HbA_{1c} levels during the prediabetic period in young children, which can be used in future prevention trials. In secondary prevention trials aimed at slowing down or reversing the progression of β-cell dysfunction in subjects with islet autoantibodies, there is a clear need for markers that can be used to monitor the disease process. HbA_{1c} level is an indirect measure of glucose control and cannot be used as a direct estimate of β-cell function. It is noteworthy that the time interval between the HbA_{1c} measurements used to calculate the presence or absence of a 10% increase varied between 3 and 12 months, and may represent a potential confounder, given that a 10% increase occurring during a 3-month interval might have a different weight in terms of prediction compared with a similar increase in samples derived from a 12-month interval. As HbA_{1c} level represents a variation in plasma glucose levels during the lifetime of erythrocytes (120 days), with the weight on the preceding 6–8

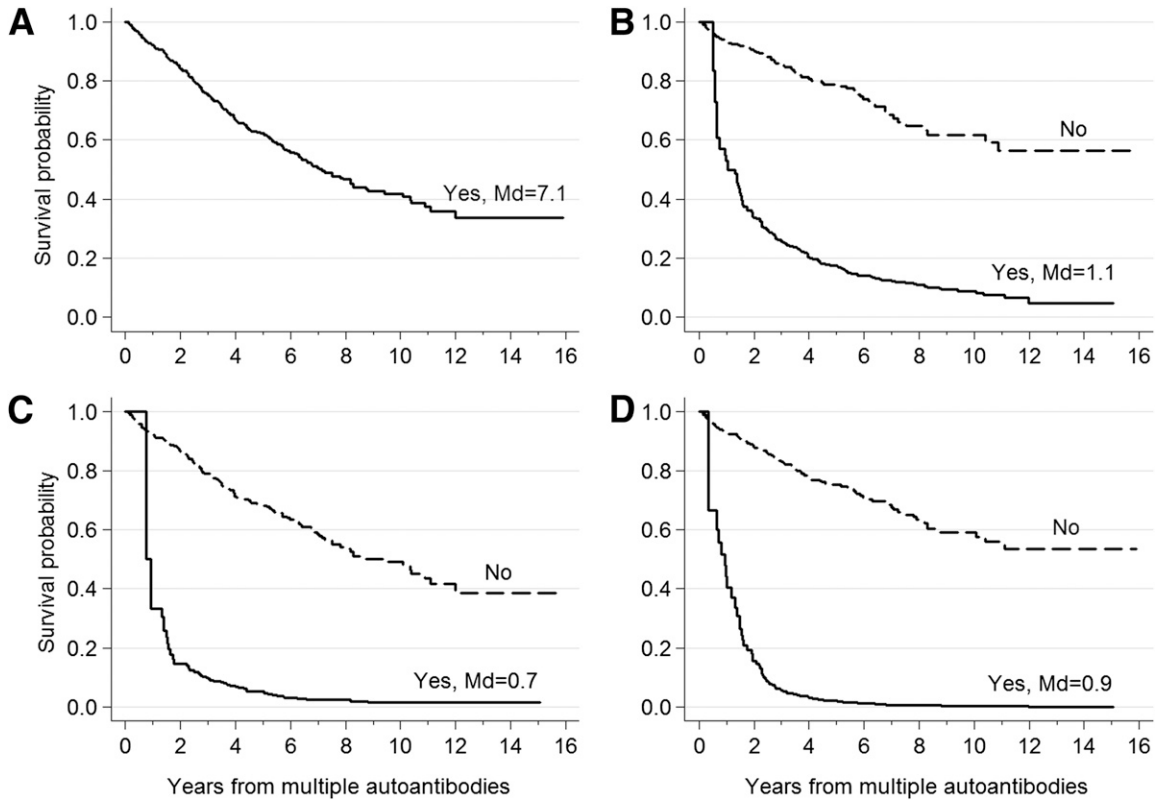


Figure 2—Cox regression estimates showing the time to the diagnosis of type 1 diabetes from the time when multiple autoantibodies were detected among children with multiple islet autoantibodies. Median survival time is indicated (Md, in years). The four panels show survival curves for all children with multiple (two or more) islet autoantibodies (*A*), children with/without a 10% increase in HbA_{1c} levels during 3–12 months (*B*), children with/without a 10% increase in HbA_{1c} levels during 3–12 months and any additional increase in HbA_{1c} level during the next 6 months or not (*C*), and children with/without two consecutive HbA_{1c} values ≥5.9% (41 mmol/mol) (*D*).

weeks, it seems unnecessary to measure HbA_{1c} levels more often than every 3 months. Since the three laboratories involved reported rather high uncertainty of measurements varying from 5.8% to 10% and the methodology changed over the study period, one has to consider the possibility that some changes in HbA_{1c} levels are due to variations in methodology. However, this possible source of error affects the progressor and nonprogressor groups equally. The HbA_{1c} values suggested on the basis of the current analyses for the prediction of clinical type 1 diabetes are from a single study, and validation of the results is needed in additional populations.

In conclusion, we have shown that HbA_{1c} level is a useful biochemical marker for the estimation of the time to the diagnosis of type 1 diabetes in genetically susceptible children with multiple islet autoantibodies.

Table 3—Multivariate Cox regression adjusted HRs for the contribution of clinical factors to progression of type 1 diabetes after controlling for other variables

	Progressors (*n* = 201)	Nonprogressors (*n* = 265)	Adjusted HR	95% CI	*P* value
10% increase in HbA_{1c} values within 3–12 months, *n* (%)	114 (57)	90 (34)	2.8	2.0–4.1	<0.001
Two consecutive HbA_{1c} values ≥5.9% (41 mmol/mol), *n* (%)	85 (42)	36 (14)	8.5	6.1–11.9	<0.001
Age at detection of multiple (two or more) autoantibodies, mean (SD), years	3.0 (2.1)	5.2 (3.4)	0.8	0.7–0.9	<0.001
High HLA risk, *n* (%)	56 (28)	53 (20)	1.7	1.2–2.2	0.001
T1D in FDR at the time of birth, *n* (%)	33 (16)	20 (8)	1.5	1.0–2.3	0.043

Explanatory variable candidates were a 10% increase in HbA_{1c} values within 3–12 months, a 10% increase in HbA_{1c} values within 3–12 months and any additional increase during the next 6 months, two consecutive HbA_{1c} values ≥5.9% (41 mmol/mol), age at detection of multiple (two or more) autoantibodies, T1D in an FDR, HLA risk, and sex. T1D, type 1 diabetes.

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