A Risk Score for Type 1 Diabetes Derived from Autoantibody Positive Participants in The Diabetes Prevention Trial- Type 1

Jeffrey P. Krischer, PhD, Jerry P. Palmer, MD, Jeffrey Mahon, MD, Catherine Cowie, PhD, Carla J. Greenbaum, MD, David Cuthbertson, MS, John M. Lachin, ScD, Jay S. Skyler, MD, and the Diabetes Prevention Trial-1 Study Group

Running Title: A Risk Score for Type 1 Diabetes

Corresponding Author:
Jay M. Sosenko, MD
Division of Endocrinology
University of Miami
PO Box 016960 (D110)
Miami, FL 33101
jsosenko@med.miami.edu

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ABSTRACT

Objective: The accurate prediction of type 1 diabetes (T1D) is essential for appropriately identifying prevention trial participants. Moreover, improved prediction accuracy might ultimately result in an earlier diagnosis. Thus, we have developed a risk score for the prediction of T1D.

Research Design and Methods: Diabetes Prevention Trial-Type 1 (DPT-1) participants, islet-cell autoantibody (ICA) positive relatives of T1D patients (n=670), were randomly divided into development and validation samples. Risk score values were calculated for the validation sample from development sample model coefficients obtained through forward stepwise proportional hazards regression.

Results: A risk score based on a model including log-BMI, age, log-fasting C-peptide, and post-challenge glucose and C-peptide sums from 2-hr oral glucose tolerance tests (OGTTs) was derived from the development sample. The baseline risk score strongly predicted T1D in the validation sample (chi-square=82.3, p<0.001). Its strength of prediction was almost the same as (chi-square=83.3) a risk score additionally dependent on a decreased first-phase insulin response variable from intravenous glucose tolerance tests (IVGTTs). Neither type nor number of biochemical autoantibodies contributed significantly to the risk score model. A final T1D Risk Score was then derived from all participants with the same variables as those in the development sample model. The change in the T1D Risk Score from baseline to one year was in itself also highly predictive of T1D (p<0.001).

Conclusions: A risk score based on age, BMI and OGTT indices, without dependence on IVGTTs or additional autoantibodies, appears to accurately predict T1D in ICA-positive relatives.
The increased understanding of the immune pathogenesis of type 1 diabetes (T1D) has raised the possibility that interventions could delay or prevent its occurrence. This has led to the performance of prevention trials to test interventions in individuals with autoimmune pre-diabetes (1-3): the European Nicotinamide Diabetes Intervention Trial (ENDIT) (1) and the Diabetes Prevention Trial-Type 1 (DPT-1) (2,3). Although there was no overall treatment effect in these trials, they have shown that it is now possible to perform such studies using prediction methodologies. The recruitment of individuals with pre-specified degrees of risk was successfully achieved through the assessment of relatives of T1D patients with autoantibody determinations and metabolic testing.

Despite this measure of success, there is still a need to improve prediction methodology for T1D. Efficiency could be enhanced by refining test selection and interpreting test results more meaningfully. Procedures for assessing risk have involved oral and intravenous glucose tolerance testing; yet it isn’t known whether both tests are necessary for prediction. Also, since the magnitude of glucose levels within the normal range has been shown to be indicative of T1D risk (4), traditional definitions of hyperglycemia, such as impaired glucose tolerance, might not maximize the OGTT’s value for prediction.

Besides improving accuracy and efficiency, a quantitative, standardized approach to prediction should also be helpful. Outcomes across trials could then be interpreted in the context of target populations with clearly defined risk. Although ENDIT and DPT-1 utilized similar methods for prediction, there were still substantial differences. Such an approach might also lead to improved monitoring of the progression to T1D.

The most extensive information available for developing prediction strategies for T1D resides in the data from the two insulin intervention trials that constituted DPT-1. Using the DPT-1 data, a risk score was developed to refine the prediction of T1D in relatives of patients.

RESEARCH DESIGN AND METHODS

Subjects. Of the 97,273 samples assessed for ICA, 3.6% were positive. Of the 711 individuals who participated in the DPT-1 insulin trials, data from 670 (n=356 for the parenteral insulin trial and n=314 for the oral insulin trial) were analyzed. Inclusion was based on data completeness for covariates used to develop predictive models. Most were excluded due to absent body mass index (BMI) determinations (n=26).

The risk algorithm in DPT-1 was described previously (2,3). The presence of islet cell autoantibodies (ICA) was required for entry into both trials. If the first-phase insulin response (FPIR) of an intravenous glucose tolerance test (IVGTT) was below a defined threshold and/or an oral glucose tolerance test (OGTT) was abnormal, individuals were estimated to be at greater than 50% 5-year risk and eligible for the parenteral insulin trial. If neither criterion was present, but insulin autoantibodies were positive, the estimated 5-year risk was 26%-50% and individuals were eligible for the oral insulin trial.

Procedures. DPT-1 participants were tested for ICA at screening. Samples were also obtained for the subsequent testing of biochemical autoantibodies. ICA positive individuals underwent IVGTs followed by OGTTs. Median intervals from the initial screen, the IVGTT and the OGTT to randomization were 1.18 years, 0.17 years and 0.04 years, respectively. The parenteral insulin trial intervention included twice daily injections of 0.125 U/kg of recombinant
human ultralente insulin (Humulin U, Eli Lilly) with adjustments for weight and hypoglycemia, along with yearly 4-day intravenous insulin infusions at 0.015 U/kg/hr with adjustments for glucose. The oral insulin trial intervention was recombinant human insulin crystals at a dose of 7.5 mg/day. OGTTs were performed at 6 month (±3 months) intervals after randomization in both trials. The oral glucose dose was 1.75 g per kg (75 g maximum). Plasma glucose and C-peptide measurements were obtained fasting, and at 30, 60, 90 and 120 minutes. Most individuals were diagnosed with T1D at routine visits. If glucose values were in the diabetic range (fasting >126 mg/dl and/or 2-hr glucose >200 mg/dl), a confirmation visit was arranged. If glucose values were not confirmed, participants continued to be followed at 6-month intervals.

**Laboratory Measures.** Methodologies for assessing autoantibody positivity have been described (5). ICA was determined by indirect immunofluorescence; titers ≥10 JDF units were considered positive. Glutamic acid decarboxylase (GAD65) and ICA512 autoantibodies were measured simultaneously by a combined radioassay. Micro insulin autoantibodies (mIAA) were also measured by radioassay.

Plasma glucose levels were measured by the glucose oxidase method. Insulin and C-peptide levels were measured by radioimmunoassay. Interassay coefficients of variation for the insulin assay were 4.5% in the high reference pool and 6.9% in the low reference pool. Those for the C-peptide assay were 6.9% and 7.8% for the respective relatively high and low reference pools.

The FPIR was defined as the sum of insulin levels at the 1st and 3rd minutes of the IVGTT. A FPIR less than the 10th percentile according to age norms (FPIR<10th percentile) was utilized for these analyses. C-peptide values in the undetectable range (<0.2 ng/ml) were assigned a value of 0.1 ng/ml. Fasting glucose measurements were obtained at −10 minutes and at time 0; the latter were utilized for the analyses except for three individuals with missing values at time 0. Initial heights and weights were considered as baseline. The homeostasis model assessment of insulin resistance (HOMA-IR) was defined as the fasting glucose (mmol/l) x fasting insulin (mU/l)/22.5 (6,7). For simplicity, the total glucose and total C-peptide, defined as the totals of OGTT post-challenge glucose and C-peptide values (computed for each as the sums at 30, 60, 90 and 120 minutes), were utilized; they correlated very well with the respective areas (trapezoidal rule) under the curve (r=0.99 for both).

**Data Analysis.** The t-test and chi-square test were utilized for simple comparisons, and the log-rank test was used to compare the distributions of event-times between groups. Linear regression and Pearson correlations were used to describe linear relations among variables. The Cox Proportional Hazards regression model was employed to examine covariate effects on the T1D risk over time from randomization. The Kaplan-Meier estimate of the survival function was used to obtain an estimate of the cumulative incidence of T1D over time. These results were used to estimate the 5-year T1D risk for each participant using the methods described in the Appendix. Prediction accuracy was assessed by computation of the cumulative incidence within subgroups defined according to the estimated 5-year risk. The accuracy of prediction was also assessed with receiver-operator curves.

A split-sample cross-validation procedure was utilized to develop and assess a risk score. A randomly selected development sample of half the participants was used for
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RESULTS

The mean±SD age and BMI for the 670 participants (56% male) were 13.8±9.6 years (range: 3.0-46.0 years) and 19.8±5.0 kg/m² (range: 12.5-52.1 kg/m²). On average, T1D occurred after 2.5±1.5 years of follow-up (n=241). Those who did not develop T1D were followed for 3.6±1.7 years (n=429).

By a random process the full cohort was divided equally into development and validation samples (n=335 for both). There were no significant differences between the two samples with regard to age, BMI, gender or the occurrence of T1D (37% in the development sample and 35% in the validation sample). As shown in Table 1, there were also no significant differences for any of the metabolic or autoantibody variables examined.

In univariate analyses that utilized proportional hazards models, there were statistically significant, positive associations of T1D with the 2-hr glucose and total glucose (p<0.001 for both), but not with the fasting glucose or log-HOMA-IR. T1D was inversely related to the C-peptide indices (p=0.003 for log-fasting C-peptide; p<0.001 for total C-peptide). T1D was also significantly associated with ICA512 (p=0.003), but not with GAD65 or mIAA (n=257 for the latter due to missing values). There were also positive associations of T1D with FPIR<10th percentile (p=0.034), log-(HOMA-IR/FPIR) (p<0.001), and the number of biochemical autoantibodies (p=0.029; n=257); there were inverse associations with height (p<0.001), log-BMI (p=0.049), and age (p<0.001).

To obtain a risk score from the development cohort, forward stepwise modeling was performed in which all variables with univariate p-values <0.05 (and n=335) were included. T1D remained significantly associated with the total glucose (p<0.001), total C-peptide (p<0.001) and log-fasting C-peptide (now positively associated at p=0.039), FPIR<10th percentile (p=0.021), log-BMI (now positively associated at p<0.001) and age (p<0.001). When each of the autoantibody variables was added back to the model, none was significantly associated with T1D. (The backward elimination procedure yielded the same subset of predictors.)

Risk scores were determined from a model that included all variables remaining from the stepwise regression and a model that excluded FPIR<10th percentile (Table 2). The performance of each risk score was examined in the validation sample. The degree of association of T1D with the risk scores was almost the same (proportional hazards model chi-square=83.3 with and 82.3 without the inclusion of FPIR<10th percentile, both p<0.001 on 1 df). The area under the receiver-operator curve for the risk score with FPIR<10th percentile excluded was almost identical to the risk score with it included (0.81 for both, p<0.001). In the subsequent analyses shown, only the model without FPIR<10th percentile is utilized.

The model derived from the development sample was applied to the independent validation sample cohort. Using the development sample coefficients, a risk score was calculated for each validation sample participant that was then used to obtain an estimate of that participant’s 5-year risk of T1D (see Appendix). Validation sample participants were then divided into risk score development. The other half of the participants constituted the validation sample. Risk scores from the development sample were formulated according to coefficients of variables remaining from forward stepwise proportional hazards modeling. A univariate p-value <0.05 was required for entry into the stepwise modeling and a p-value <0.05 was required for final selection into the model.

The SAS 9.1.3 version was used for the analyses. All p-values are 2-sided.
subgroups according to the estimated 5-year risk, <25%, 26-49%, 50-74% and ≥75%. Figure 1 shows the cumulative incidence of T1D within these 5-year risk subgroups. The curves were progressively steeper as the predicted risk increased (log-rank: p<0.001). Within these subgroups the respective estimated 5-year risks were 13%, 29%, 55%, and 87%.

A T1D Risk Score (mean±SD: 6.64±1.00) was derived from all 670 participants with the same variables as those in the development sample model in order to maximize available information (see Appendix). As expected, T1D was significantly associated with all variables that were included in the development sample risk score model (p<0.001), and the T1D Risk Score was highly correlated with the development sample risk score (r=0.98). When FPIR<10th percentile and each of the autoantibody variables were added back individually to the model, none was significantly associated with T1D. The remaining analyses utilize the T1D Risk Score.

Prediction by the T1D Risk Score was assessed separately in the parenteral and oral insulin trial cohorts. Although these cohorts differed in that the parenteral trial cohort had a much higher occurrence rate [47% vs. 27%; hazard ratio and 95% confidence limits: 2.06 (1.59-2.67), p<0.001], the T1D Risk Score was strongly predictive of T1D in each cohort (proportional hazards model chi-squares for the parenteral and oral trial cohorts were 119.2 and 55.8 respectively, both p<0.001 on 1 df).

We assessed whether a change in the risk score was in itself predictive of T1D. There was an overall increase (0.16±0.92, p<0.001, n=490) in the T1D Risk Score from baseline to the one-year visit that was greater (0.37±1.20 vs. 0.07±0.78, p=0.004) in those who developed T1D (n=157) than in those who remained non-diabetic (n=333). In proportional hazards regression, change in the T1D Risk Score from baseline to the one-year visit (adjusted for the baseline risk score) was highly predictive of T1D (chi-square: 62.9, p<0.001). The impact of change in the T1D Risk Score is evident among participants in the 50-74% 5-year risk category at baseline who were seen at the one-year visit. T1D occurred more frequently in those progressing to the ≥75% 5-year risk category at the one-year visit than in those remaining in the 50-74% risk category (28/40 vs. 17/41, p=0.002).

CONCLUSIONS

The comprehensive baseline data from DPT-1 has provided the opportunity to develop and validate a risk score for T1D among relatives with autoimmune “pre-diabetes”. Using a split-sample validation procedure, a model was fit in a development sample and then its performance was examined in a validation sample. The development sample risk score was highly predictive of T1D in the validation sample.

The change in the T1D Risk Score from baseline to the one-year visit was also strongly predictive of T1D. This finding further verifies the T1D Risk Score and suggests that it can be used to monitor T1D progression. With such monitoring individuals initially deemed ineligible for prevention studies could subsequently be enrolled. Also, monitoring with a risk score could have clinical utility as interventions for pre-diabetes become available. The increase in the risk score from baseline to the one-year visit in those who subsequently developed T1D is consistent with DPT-1 findings (8) of a gradual progression to T1D.

The T1D Risk Score was strongly predictive of T1D in both the parenteral and oral insulin trial cohorts, despite substantially different T1D occurrence rates. Although these findings should not be viewed as part of the validation procedure, they suggest that the
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T1D Risk Score could have applicability over a range of risk levels and occurrence rates. Since the findings are contingent upon the presence of ICA autoantibodies in a selected cohort, one cannot say to what extent the T1D Risk Score and its derived risk estimates apply to relatives identified through autoantibodies other than ICA or to ICA negative relatives. Also, other selection criteria for the DPT-1 trials besides ICA could affect the T1D Risk Score’s applicability. Moreover, if methodologies for glucose and C-peptide measurements differ from those utilized in DPT-1, the risk estimates derived from the T1D Risk Score might not be fully applicable. Finally, the T1D Risk Score is not a definitive disease indicator; rather it provides a probability.

The biochemical autoantibodies added little to the strength of the T1D Risk Score model. Those autoantibodies are known predictors of T1D (9-12). However, it is possible that once autoantibody positivity is established and other information is available, the presence or absence of other autoantibodies adds relatively little to prediction. Even though FPIR<10\textsuperscript{th} percentile was predictive of T1D, it contributed little additional prediction accuracy when it was included in the risk score model. This was evident in the similar chi-square values for the risk scores and their almost identical areas under the receiver-operator curves with and without FPIR<10\textsuperscript{th} percentile included as a variable in the risk score model. Thus, IVGTTs may not contribute much prediction information beyond that of OGTTs.

Risk factors for T1D have been examined, but little information is available regarding risk scores for T1D. A “prognostic risk index” was developed in a recent study (11). In a sub-cohort that was followed in that study, HOMA-IR/FPIR was predictive of T1D. This finding is consistent with that of an earlier study (10) and another study from DPT-1 (13). Although T1D was related to HOMA-IR/FPIR in the present study, it was not among the variables retained for the risk score model.

T1D occurrence has been found to be associated with autoantibodies (9-17), FPIR (10-13, 16,18), glucose intolerance (19,20), and an elevated proinsulin:C-peptide ratio (17). In addition, we have recently shown that glucose and C-peptide data from OGTTs can be strongly predictive (4), even in relatives with normal glucose tolerance. BMI has been reported to be predictive of T1D in DPT-1 (18). Also, indirect evidence suggests an association between T1D occurrence and BMI (21,22).

Since the analyses were performed solely for developing a predictive model, associations in the multivariate analyses should be interpreted cautiously. Certain variables that added relatively little to prediction, such as biochemical autoantibodies and IVGTT indices, are certainly still important for understanding the development of T1D.

DPT-1 used a two-stage approach for assessing risk in relatives of T1D patients. The first stage identified autoantibody positive relatives through screening, whereas the second stage further characterized risk according to the presence of abnormal glucose tolerance and IVGTT abnormalities. It appears that for future prevention trials, a first stage that includes autoantibody testing should be maintained. However, with the judicious selection of available information and with more quantitative methodology, risk characterization in the second stage can now be accomplished more efficiently and accurately.

In conclusion, a risk score that utilizes age and adiposity together with measures of oral glucose tolerance and beta-cell function from OGTTs appears to accurately quantify the risk of developing T1D among relatives.
with autoimmunity as characterized by ICA positivity.

ACKNOWLEDGEMENTS
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REFERENCES


APPENDIX. Calculation of 5-Year Risk from a Proportional Hazards Model

The Cox Proportional Hazards model expresses the hazard function and corresponding survival probability for a subject with a covariate vector X as a linear function of a set of regression coefficients β using the linear predictor X'β. Let X designate the mean of the covariates in the sample and let S(t) designate the Kaplan-Meier estimate of the survival function in the complete cohort as of time t. Then from the proportional hazards model, this implies that the survival function from an individual with covariate vector x is obtained as

\[ S(t|x) = S(t)^{\exp[(x-x_0)' \beta]} = S(t)^{\exp[x'\beta - x_0' \beta]} \]

The corresponding t-year risk of the event is then obtained as 1-S(t|x).

From the final model based on all DPT-1 subjects, the T1D Risk score (linear predictor X'β) was calculated according to the formula

\[
T1D \ Risk \ Score = x' \beta = 1.569 \times [\text{log-BMI (kg/m}^2\text{)}] \\
-0.056 \times [\text{Age (years)}] \\
+0.813 \times [\text{Total Glucose (mg/dl)/100}] \\
+0.476 \times [\text{log-Fasting C-peptide (ng/ml)}] \\
-0.848 \times [\text{Total C-peptide (ng/ml)/10}] 
\]

This T1D Risk Score is then converted to the estimated 5-year risk according to the formula

\[ 5\text{-year Risk} = 1 - 0.543^{\exp(x'\beta-6.638)} \]

REFERENCE

### TABLE 1. Characteristics* of the Development and Validation Samples.

<table>
<thead>
<tr>
<th></th>
<th>Development (n=335)</th>
<th>Validation+ (n=335)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>86.2 ± 9.3</td>
<td>86.2 ± 9.1</td>
</tr>
<tr>
<td>2-hr Glucose (mg/dl)</td>
<td>114 ± 28</td>
<td>113 ± 27</td>
</tr>
<tr>
<td>Total Glucose (mg/dl)/100</td>
<td>5.26 ± 1.06</td>
<td>5.25 ± 1.07</td>
</tr>
<tr>
<td>Fasting C-peptide (ng/ml)</td>
<td>1.03 ± 0.66</td>
<td>1.06 ± 0.70</td>
</tr>
<tr>
<td>Total C-peptide (ng/ml)/10</td>
<td>1.70 ± 0.75</td>
<td>1.65 ± 0.68</td>
</tr>
<tr>
<td>Biochemical Autoantibodies**</td>
<td>1.36 ± 0.99</td>
<td>1.45 ± 0.99</td>
</tr>
<tr>
<td>ICA512 (%)</td>
<td>43</td>
<td>47</td>
</tr>
<tr>
<td>GAD65 (%)</td>
<td>67</td>
<td>69</td>
</tr>
<tr>
<td>mIAA (%)**</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>FPIR&lt;10th Percentile (%)</td>
<td>42</td>
<td>43</td>
</tr>
</tbody>
</table>

* Mean±SD shown if not designated as (%)
** n=257 for development sample and n=258 for validation sample
+ p>0.05 for all comparisons
TABLE 2. Models for Prediction Scores from Stepwise Proportional Hazards Regression in the Development Sample (n=335)

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Coefficient ± SE</th>
<th>Chi-Square</th>
<th>Hazard Ratio ++</th>
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<tr>
<td>Log-BMI (kg/m^2)</td>
<td>2.85 ± 0.58</td>
<td>24.2</td>
<td>17.2 (5.5-53.5)**</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.11 ± 0.02</td>
<td>22.5</td>
<td>0.89 (0.85-0.94)**</td>
</tr>
<tr>
<td>Total Glucose (mg/dl)/100</td>
<td>0.84 ± 0.08</td>
<td>103</td>
<td>2.33 (1.98-2.74)**</td>
</tr>
<tr>
<td>Log-Fasting C-peptide (ng/ml)</td>
<td>0.39 ± 0.19</td>
<td>4.3</td>
<td>1.48 (1.02-2.13)*</td>
</tr>
<tr>
<td>Total C-peptide (ng/ml)/10</td>
<td>-0.79 ± 0.22</td>
<td>12.8</td>
<td>0.45 (0.29-0.70)**</td>
</tr>
<tr>
<td>FPIR&lt; 10th Percentile</td>
<td>0.49 ± 0.21</td>
<td>5.4</td>
<td>1.63 (1.08-2.47)*</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Model 2</th>
<th>Coefficient ± SE</th>
<th>Chi-Square</th>
<th>Hazard Ratio ++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log-BMI (kg/m^2)</td>
<td>2.41 ± 0.55</td>
<td>19.5</td>
<td>11.2 (3.8-32.8)**</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.09 ± 0.02</td>
<td>19.1</td>
<td>0.91 (0.88-0.95)**</td>
</tr>
<tr>
<td>Total Glucose (mg/dl)/100</td>
<td>0.85 ± 0.08</td>
<td>111</td>
<td>2.34 (2.00-2.74)**</td>
</tr>
<tr>
<td>Log-Fasting C-peptide (ng/ml)</td>
<td>0.41 ± 0.19</td>
<td>4.8</td>
<td>1.51 (1.05-2.19)*</td>
</tr>
<tr>
<td>Total C-peptide (ng/ml)/10</td>
<td>-0.93 ± 0.22</td>
<td>17.9</td>
<td>0.40 (0.26-0.61)**</td>
</tr>
</tbody>
</table>

++ 124 of the participants developed T1D
++ 95% confidence intervals are in parentheses
* p<0.05; ** p< 0.001
FIGURE LEGEND

**Figure 1.** This shows cumulative incidence curves in the validation sample (n=335) according to estimated 5-year T1D risk categories (25% intervals) from the development sample risk score. The curves were progressively steeper as the predicted risk category increased (log-rank: p<0.001).
FIGURE 1