# Prospective Study of Predictors of β-Cell Survival in Type I Diabetes

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**We conducted a prospective study to describe the course of the pancreatic β-cell function from the time of clinical diagnosis of insulin-dependent (type I) diabetes to determine whether DR type, presence of islet cell antibodies (ICA), presence of insulin antibodies (IA), age at onset, and sex could help in the prediction of residual endogenous insulin secretion. A cohort of 68 children was followed for 18 mo after diagnosis of type I diabetes. The outcome variables selected for analysis were *1)* serum C-peptide peak concentration after a Sustacal meal, 2) time of disappearance of the serum C-peptide response, and 3) time after diagnosis at which the maximal serum C-peptide response was observed. After institution of insulin therapy, serum C-peptide peak concentrations rose temporarily for 1–6 mo and declined thereafter. Multivariate analysis of the data showed that DR type ($P$ = .2488) and presence of IA ($P$ = .1604) had no effect on serum C-peptide over time, but sex ($P$ = .0146), age at onset ($P$ = .0002), and presence of ICA ($P$ = .0147) significantly contributed to the variation of serum C-peptide over time. Furthermore, age at onset, presence of ICA, and sex were also the only significant predictors of the time of disappearance of the β-cell function. The relative risks of β-cell–function disappearance were 0.87 ($P$ = .0015), 9.43 ($P$ = .0181), and 2.25 ($P$ = .0468), respectively. In conclusion, there are distinct variations in the natural course of the β-cell function in type I diabetes. β-Cell–function survival is significantly shortened the younger the subject is at disease onset, if ICA are present at diagnosis, and if the subject is male. *Diabetes* 37:920–25, 1988**

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Spontaneous type I (insulin-dependent) diabetes results from a progressive, probably autoimmune, loss of the pancreatic β-cells (1). Indeed, the preclinical phase (2–4) and the clinical onset of the disease are associated with the presence of insulin antibodies (IA) (5–7), circulating islet cell antibodies (ICA) (8–10), and abnormalities in peripheral lymphocyte subsets and their functions (11). Moreover, specific genotypes of the class II major histocompatibility complex antigens (HLA-DR3 and -DR4) predispose to type I diabetes (12). It has been proposed that the presence of DR3 or DR4 or both loci may define different subtypes of disease (13–15), but this issue remains controversial.

The evidence that immunosuppressive therapy prevents future symptoms of type I diabetes in some animal models has led to many clinical trials of immunosuppressive therapy in the human at the onset of symptoms. Most of these trials, with the exception of those performed with cyclosporin, have not successfully prolonged the remission period in type I diabetes (16). However, cyclosporin has significant side effects, and its safety and efficacy in children, the population at highest risk of developing type I diabetes, has not been proved. In order to interpret the effects of immunosuppressive therapy and to optimize its administration with respect to timing and selection of patients who would most benefit from it, a clear understanding of the natural course of the disease is a necessary first step.

The goals of our investigation were to describe the course of β-cell function from the time of clinical diagnosis of type I diabetes and to determine which factors could predict the residual endogenous insulin secretion in a cohort of type I diabetic children enrolled in a longitudinal follow-up study.

## MATERIALS AND METHODS

Sixty-eight children with type I diabetes classified according to the National Diabetes Data Group (17) were followed for 18 mo after diagnosis. The study was approved by the in-

TABLE 1
Baseline characteristics

| | Girls | Boys | Total |
|---|---|---|---|
| *n* | 32 | 36 | 68 |
| Age (yr) | 9.6 ± 4.7 | 11.0 ± 4.7 | 10.3 ± 4.7 |
| Age distribution (%) | | | |
| <6 yr | 25.0 | 22.2 | 23.5 |
| 6–12 yr | 40.6 | 27.8 | 33.8 |
| >12 yr | 34.4 | 50.0 | 42.7 |
| Antibodies | | | |
| ICA⁺ (%) | 84.4 | 72.2 | 77.9 |
| IA⁺ (%) | 46.7 | 36.1 | 40.9 |
| HLA loci (%) | | | |
| DR3⁺ | 13.8 | 27.8 | 21.5 |
| DR4⁺ | 51.7 | 36.1 | 43.1 |
| DR3/4⁺ | 34.5 | 25.0 | 29.2 |
| DRx | 0.0 | 11.1 | 6.2 |

ICA, islet cell antibodies; IA, insulin antibodies.

stitutional review board. All patients were admitted to the hospital at the time of diagnosis and followed regularly at the clinic 1 mo after discharge and every 3 mo thereafter. Treatment consisted of one or two daily injections of a mixture of intermediate-acting (NPH) and short-acting insulin. The aims of administering the treatment were to obtain optimal growth and development, avoid severe hyperglycemia and hypoglycemia, and attain the best possible metabolic control in the patients. Insulin doses were adjusted on the basis of two to three daily self-monitored blood glucose tests.

Data collection at baseline and at 1, 3, 6, 9, 12, and 18 mo included gathering demographic data and sampling blood for laboratory studies. A C-peptide test was performed after the subjects fasted overnight, and the morning insulin dose was withheld until completion of the test. The outcome variables selected for analysis were *1*) the C-peptide peak response to 7 kcal/kg Sustacal (14 g/dl carbohydrate, 24 g/dl fat, and 6.1 g/dl protein, 1 kcal/ml; Mead Johnson, Belleville, Ontario); *2*) the time of disappearance of the C-peptide response, defined as the first of two consecutive negative tests (peak response ≤ 0.06 pmol/ml); and 3) the time of recovery of β-cell function, defined as the time (0, 1, 3, 6, 9, 12, or 18 mo) at which the peak C-peptide response was observed. The predictor variables selected for analysis were sex, age at diagnosis, presence or absence of ICA, and presence or absence of IA at the time of diagnosis of type I diabetes and DR type, which was identified during the 1st yr after diagnosis of diabetes. Serum C-peptide was measured by radioimmunoassay according to Heding (18), with the antiserum M1230 (Novo, Bagsvaerd, Denmark), after precipitation with polyethylene glycol. The sensitivity of the assay was 0.03 pmol/ml, and the interassay coefficient of variation was 7%. With this assay, mean ± SE C-peptide concentrations in nondiabetic children were 0.398 ± 0.08 pmol/ml at fasting and 0.98 ± 0.03 pmol/ml at the peak. HLA-DR typing was performed with the standard microlymphocytotoxicity assay in Terasaki trays (19). Subjects were classified as DRx (DR3 and DR4 not present), DR3⁺, DR4⁺, or DR3/4⁺ (DR3 and DR4 present). The presence of ICA was determined by indirect immunofluorescence (20,21). Coded serum samples were applied on 4-μm cryostat sections of monkey pancreas (Diammune ICA test, Behring Diagnostics, La Jolla, CA). Each sample was tested at 1:2 and 1:8 dilutions. After a 30-min incubation at room temperature, the sections were washed twice with phosphate-buffered saline and then exposed for 30 min to fluorescein isothiocyanate–conjugated affinity-purified goat anti-human IgG. After washing and mounting, the slides were read by two independent observers who used fluorescence microscope (Leitz Laborlux D, Wetzler, FRG). The decision for positivity or negativity was based on the appearance of ≥5 islets/section. Positive and negative control sera were run for every 20 samples. The specificity of the assay was 99%, and the sensitivity was 80%. We assayed serum IA following the technique provided by McEvoy et al. (6), using a 1-μU/assay tube of monoiodinated porcine insulin (sp act 80 μCi/μg; New England Nuclear, Boston, MA). Positive samples were those that bound >3SD above the mean of control values.

**STATISTICAL ANALYSIS**

The first outcome variable, i.e., the serum C-peptide concentration, was analyzed with analysis of variance for repeated measures over time (22). Predictive factors were first analyzed one at a time and then simultaneously.

The time to complete disappearance of the β-cell function was analyzed with statistical methods for survival data (23) with the understanding that the survival times are grouped. First, the actuarial method was used to estimate the survivor functions. Second, Cox's proportional hazards method for discrete failure times was used to model the hazard of disappearance of β-cell secretion as a function of the potential predictive factors and to estimate adjusted instantaneous-risk ratios. These ratios represent the relative risk of disappearance of β-cell secretion per year for the age factor (i.e., age vs. age − 1) and for one group with respect to another for the other factors (i.e., ICA⁺ vs. ICA⁻). The resulting estimated hazard functions were transformed to survivor functions at specified values of the covariates and plotted (23).

The analysis of the time of recovery consisted of multiple matched-pair *t* tests aimed at testing the mean changes in serum C-peptide concentration from baseline to 6 mo.

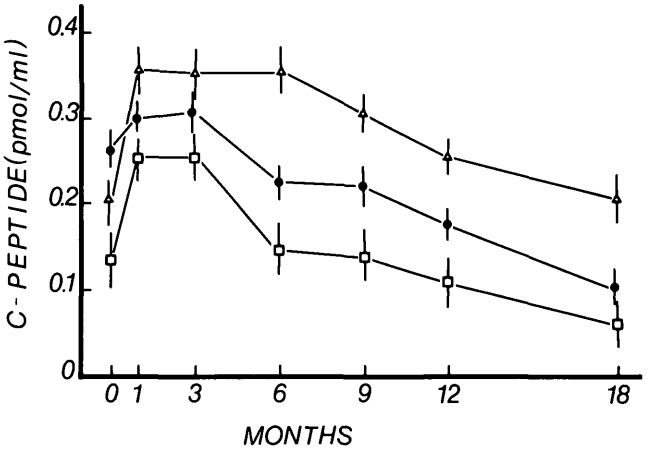


**FIG. 1. Serum C-peptide peak concentrations (means ± SE) over 18 mo after diagnosis of diabetes in children aged >12 (△), 6–12 (●), and <6 (□) yr.**

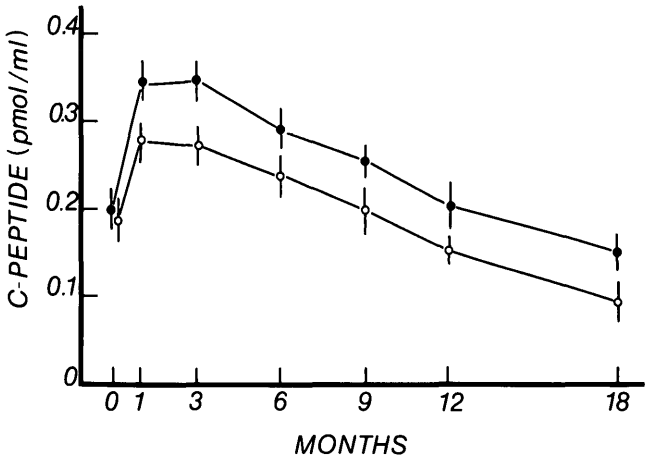


**FIG. 2. Serum C-peptide peak concentrations (means ± SE) over 18 mo after diagnosis of diabetes in boys (○) and girls (●).**

## RESULTS

Baseline characteristics of the population studied are shown in Table 1. With respect to the first outcome variable, the C-peptide peak response over time, univariate analysis of the data showed that age at onset was the only variable that significantly affected outcome of serum C-peptide concentration over time ($P$ = .007). Multivariate analysis of the data showed that DR type ($P$ = .2488) and presence of IA ($P$ = .1604) had no effect on serum C-peptide peak concentration over time, but sex ($P$ = .0146), age at onset ($P$ = .0002), and presence of ICA ($P$ = .0147) significantly contributed to the variation of mean serum C-peptide peak concentration over time. In addition, the overall changes in mean serum C-peptide peak concentration were significant ($P$ < .0001), but there was no interaction between time and each of these variables ($P$ > .26). The means ± SE serum C-peptide peak concentration throughout the study were depicted according to age at onset (Fig. 1), sex (Fig. 2), and presence of ICA (Fig. 3).

With respect to the second outcome variable, i.e., the time of disappearance of the β-cell function, Fig. 4 shows the survival curve of the whole group during the study. The estimated survival probability (mean ± SE) 18 mo after diagnosis was 59 ± 6%. Figure 5 (*middle*) shows the estimated survival functions of β-cell secretion for males and females. The 18-mo estimated survival probability was 67 ± 9% for females and 53 ± 8% for males. The estimated survival probability of β-cell secretion after 18 mo was 49 ± 7% for patients who had detectable ICA at diagnosis, and it was 93 ± 6% in those patients in whom ICA were undetectable (Fig. 5, *bottom*). When children were grouped according to age at diagnosis, the estimated β-cell survival probability after 18 mo was 21 ± 11% in children <6 yr old, 60 ± 10% in children aged between 6 and 12 yr, and 79 ± 8% in children >12 yr (Fig. 5, *top*). Using Cox's proportional hazards method for discrete failure times, we found that sex, age, and ICA were the only significant predictors of the instantaneous risk of β-cell–function disappearance and that DR type and IA had no effect. The instantaneous risk of β-cell–secretion disappearance decreased with age at onset, the risk ratio being 0.87/yr of age. The instantaneous risk ratio for the presence of ICA was 9.43 and for boys was 2.25 (Table 2). From the results of this multivariate model, three estimated survival probability curves were obtained for three combinations of the significant predictive factors (Fig. 6).

With respect to the third outcome variable, the time of recovery of the β-cell function, we found that after the diagnosis there was a significant increase of peak serum C-peptide concentration at 1 and 6 mo ($P$ = .0193).

## DISCUSSION

The objective of this study was to identify independent predictors of β-cell function in a cohort of type I diabetic children and to distinguish markers of the progression to complete β-cell loss over a given observation period. To our knowledge, this is the first prospective study in diabetic children to report factors that predict the course of the β-cell function from the time of diagnosis. In the final regression model, three factors were found to be significant predictors of

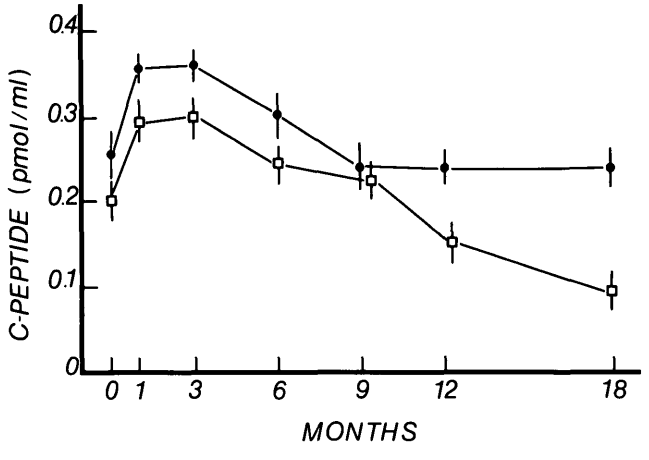


**FIG. 3. Serum C-peptide peak concentrations (means ± SE) over 18 mo after diagnosis of diabetes in children who tested positive (□) or negative (●) for islet cell antibodies.**

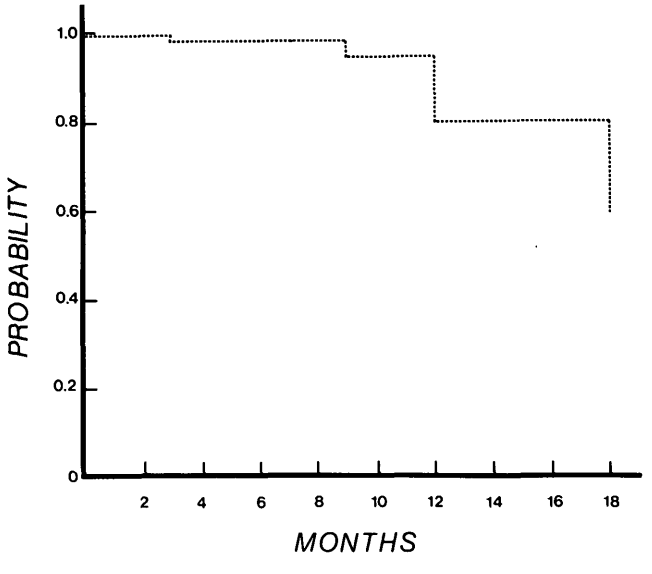


**FIG. 4. Survival of β-cell function (as measured by serum C-peptide peak concentration) for whole group ($n$ = 68) over 18 mo after diagnosis of diabetes.**

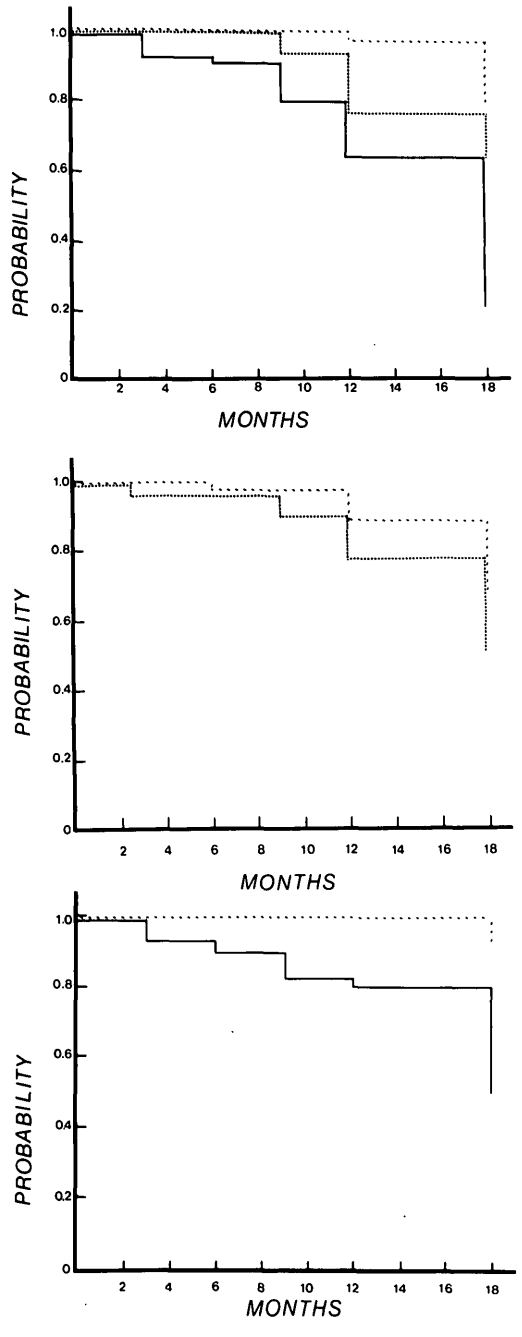


**FIG. 5. Survival of β-cell function as measured by serum C-peptide peak concentration over 18 mo after diagnosis of diabetes. *Top*: estimated survival for children aged >12 (*light dotted line*), 6–12 (*dark dotted line*), and <6 (*solid line*) yr. *Middle*: estimated survival for males (*dark dotted line*) and females (*light dotted line*). *Bottom*: estimated survival for children who tested positive (*solid line*) or negative (*dotted line*) for islet cell antibodies.**

β-cell function after diagnosis: the presence of ICA, age at diagnosis, and sex. The finding that three variables independently have a significant effect is striking and suggests strong associations. One of the three variables, the presence of ICA at diagnosis, is almost certainly a marker of disease rather than a determinant of the time of disappearance of β-cell function and may simply indicate that an individual affected with diabetes has a progressive autoimmune destruction of the β-cell mass. As others have suggested (2,3), ICA

TABLE 2
Predictors of instantaneous risk of β-cell disappearance

| | Relative risk | 95% Confidence interval | *P* |
|---|---|---|---|
| Age (yr) | 0.87 | 0.80–0.94 | .0015 |
| Islet cell antibodies ($^+$ vs. $^-$) | 9.43 | 1.53–58.09 | .0181 |
| Sex (male vs. female) | 2.25 | 1.03–4.94 | .0468 |

Multivariate analysis with Cox's proportional hazards method for discrete failure times was used for the calculations.

may be an early marker of β-cell destruction during the presymptomatic phase. The degree of association observed between the presence of ICA and subsequent course of serum C-peptide concentration raises the question of whether ICA titers during the prediabetic phase or at diagnosis may correlate with β-cell function, whereas the two other factors, age of onset and sex, may indicate susceptibility to a more rapid destruction of β-cell mass. The effect of the presence of ICA on β-cell function found in our study contrasts with the data of Madsbad et al. (24), who reported a lack of association between ICA and β-cell function. In that study, however, the data were not collected prospectively, and there were few children with newly diagnosed diabetes. Marner et al. (25), however, found that in a cohort of 82 type I diabetic patients (most of whom were adults), the presence of ICA in the first 2 yr after diagnosis was associated with a more rapid loss of endogenous insulin secretion and increased insulin requirements. Kobayashi et al. (26) reported heterogenous time courses of ICA persistence and β-cell function in a prospective study of non-insulin-dependent diabetic patients. The authors showed that β-cell function deteriorated progressively in patients with persistent ICA but improved in patients in whom ICA became undetectable. In addition, in the latter group there were more

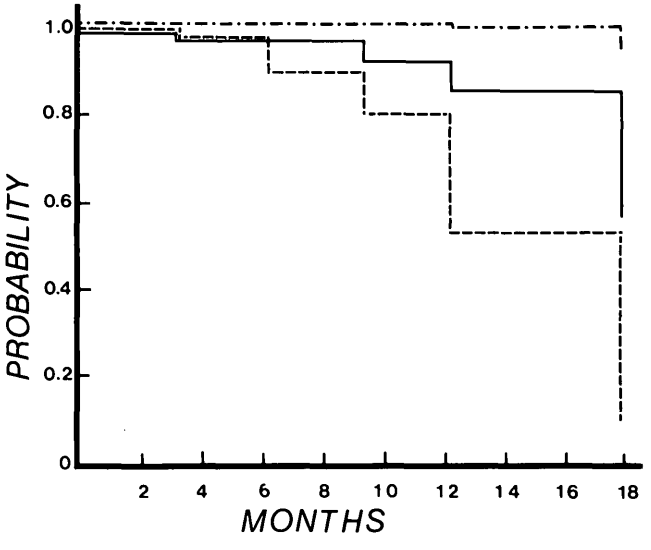


**FIG. 6. Estimated β-cell survival probability curves derived from Cox's proportional hazards method for discrete failure times for 15-yr-old girl who tested negative for islet cell antibodies (*dotted dashed line*), average diabetic patient (*solid line*), and 5-yr-old boy who tested positive for islet cell antibodies (*dashed line*).**

females. These findings support our data on type I diabetic patients in whom the progression to disappearance of β-cell function appeared to be shortened if ICA were detectable at diagnosis.

With respect to the predictive effect of the age of onset on β-cell function, Crossley et al. (27), in a study of 21 type I diabetic children, showed a significant positive correlation between 24-h urinary C-peptide excretion at 1 and 2 yr after diagnosis and age of onset. The findings of Hoogwerf et al. (14), in a large cross-sectional study of insulin-dependent diabetes mellitus families, and other studies (25) also support an association between age of onset and duration of β-cell function. In addition, the report of Deschamps et al. (28) showing that the empirical risk estimates of developing type I diabetes in the HLA-identical and -haploidentical siblings were almost fivefold greater in siblings <10 yr old further suggests an age-related effect in the onset and progression of type I diabetes. It is possible that the β-cell mass or the capacity for cell regeneration is smaller at a younger age, and therefore the same diabetogenic insult would result in greater damage in younger children who are susceptible to developing type I diabetes.

Our observation that males have a more rapid destruction of their β-cell mass has not been reported before. A possible explanation is that in the other studies published the number of patients was too small, the effect of sex was not specifically examined, or the remission phase was defined as an insulin requirement of $<0.5\ U \cdot kg^{-1} \cdot day^{-1}$ and not by serum C-peptide concentrations. Ludvigsson et al. (15) reported an association between HLA-DR3 and a slowly progressive form of the disease. In their study, however, the severity of progression was assessed by clinical impression and not by measurement of serum C-peptide concentrations, which more accurately define remission. On the other hand, Hoogwerf et al. (14) showed better preservation of the β-cell function in $DR4^+$ individuals. In neither of these two studies was β-cell function assessed prospectively. In our study, we did not find DR type to be a predictive factor of β-cell function by single-factor or multifactor analysis. A longer follow-up period and a larger sample size may yield different results.

In conclusion, our report provides evidence for the existence of distinct variations in the natural history of the β-cell function in onset of type I diabetes in children. The survival of the β-cell function is significantly shortened by younger age of onset, presence of ICA, and male gender, all of these variables contributing independently to augment the risk of accelerated destruction of the β-cell mass. Our results may be helpful in the selection of patients who would most likely benefit from immunosuppression or other therapies. Because of the known side effects of some of these interventions, our findings could help prevent the exposure of those patients who, on the basis of an accelerated destruction of their β-cell mass, have less chance of responding to the treatment than others. Furthermore, the information derived from the analysis of the recovery phase may be helpful in identifying the optimal timing for the intervention strategies. Our data suggest that in family studies intended to identify prediabetes through the measurement of ICA titers, immune changes, and glucose intolerance, individuals at risk for accelerated destruction of the β-cell mass (i.e., young $ICA^+$ males) should be followed up at short intervals, when adequate β-cell function could still be preserved by early therapeutic intervention and thus increase the effectiveness of future immunosuppressive therapy.

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