Use of an Islet Cell Antibody Assay to Identify Type 1 Diabetic Patients With Rapid Decrease in C-Peptide Levels After Clinical Onset

KATELIJN DECOCHEZ, MD
BART KEYMEULEN, MD, PHD
GUIDO SOMERS†, MD, PHD
HARRY DORCHY, MD, PHD
IVO H. DE LEEUW, MD, PHD
CHANTAL MATHIEU, MD, PHD
RAOUL ROTTIERS, MD
FREDERIC WINNOCK, MPHARM
KRISTIEN VER ELST, MD
ILSE WEETS, MD
LEONARD KAUFMAN, PHD
DANIEL G. PIPELEERS, MD, PHD
FRANS K. GORUS, MD, PHD
THE BELGIAN DIABETES REGISTRY

OBJECTIVE — To investigate whether the presence of antibody markers at diagnosis could help predict the rapid decrease in residual β-cell function noted in some, but not all, patients with recent-onset type 1 diabetes.

RESEARCH DESIGN AND METHODS — We measured random C-peptide levels (radioimmunoassay); islet cell cytoplasmic antibodies (ICA) (indirect immunofluorescence); and antibodies against IA-2 protein, 65-kDa glutamate decarboxylase, and insulin (liquid-phase radiobinding assays) in 172 patients <40 years of age with type 1 diabetes. The patients had been consecutively recruited at diagnosis by the Belgian Diabetes Registry and were followed for 2 years.

RESULTS — Two years after diagnosis, random C-peptide levels had decreased significantly ($P < 0.001$) in ICA^+ patients but not in ICA^- patients. C-peptide values <50 pmol/l were noted in 88% of patients diagnosed before 7 years of age, in 45% of patients diagnosed between ages 7 and 15 years, and in 29% of patients diagnosed after 15 years of age ($P < 0.001$). In cases of clinical onset before age 15 years, a rapid decline in random C-peptide values was observed almost exclusively in patients with high-titer ICA (≥50 Juvenile Diabetes Foundation [JDF] units) at diagnosis (69 vs. 17% in patients with lower ICA titers, $P < 0.001$). In patients diagnosed after 15 years of age, 36% of patients with ICA titers ≥12 JDF units developed low C-peptide levels compared with 14% of patients with ICA titers <12 JDF units ($P < 0.03$). Multivariate analysis confirmed that C-peptide levels after 2 years were inversely correlated with ICA levels ($P < 0.001$) and to a lesser degree positively correlated with age at diagnosis ($P < 0.02$), regardless of the levels or number of molecular autoantibodies.

CONCLUSIONS — Young age at diagnosis and high-titer ICA identify a group of type 1 diabetic patients at high risk of rapidly losing residual β-cell function. Using these selection criteria, it is possible to better target β-cell–preserving interventions to patients with or without such rapid progression, depending on the nature of the tested substance. The ICA assay measures clinically relevant antibodies not detected in antibody assays that use recombinant human autoantigens for substrate.

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Type 1 diabetes is caused by an immune-mediated destruction of pancreatic insulin-producing β-cells (1). The histopathological hallmarks of the disease comprise a markedly reduced β-cell mass and a focal infiltration of the islets by mononuclear cells termed *insulitis* (2). Very little is known about the nature, intensity, and kinetics of the underlying histopathological lesions during the allegedly long preclinical disease phase (3,4). Serendipitous anatomopathological observations in diabetic patients who died days to years after diagnosis have revealed a marked heterogeneity in the intensity of these histopathological changes at clinical onset and with disease duration (3). Residual β-cells were a constant finding at clinical onset, but the β-cell mass tended to decrease with time after diagnosis. β-Cells invariably disappeared after clinical onset in patients diagnosed before 7 years of age (3) but could still be demonstrated up to 30 years after diagnosis in ~50% of the patients with clinical onset after 7 years of age (3). It is thus worthwhile to consider interventions at diagnosis that attempt to avoid the losses in β-cells after clinical onset, which might ultimately lead to better long-term metabolic control and thus a reduced risk of chronic diabetic complications (5,6). This strategy would benefit from the identification of biologic markers that can be used to select and monitor those patients who are more susceptible to lose their β-cells after onset.

Clinical onset of type 1 diabetes is almost always preceded and accompanied by the presence of insulin autoantibodies (IAA) or autoantibodies against as yet incompletely identified cytoplasmic islet cell antigens, known as islet cell

From the Diabetes Research Center (K.D., B.K., G.S., F.W., K.v.E., I.W., D.G.P., F.K.G.) and the Department of Biostatistics (L.K.), Vrije Universiteit Brussel, Brussels; the Department of Diabetology (H.D.), Hôpital Universitaire des Enfants Reine Fabiola, Brussels; the Department of Endocrinology (I.H.D.L.), Universitair Ziekenhuis Antwerpen, Antwerp; the Department of Endocrinology (C.M.), Universitair Ziekenhuizen Gasthuisberg, Leuven; the Department of Endocrinology (R.R.), Universitair Ziekenhuis Gent, Ghent; and the Belgian Diabetes Registry, Brussels, Belgium.

Address correspondence and reprint requests to Frans K. Gorus, MD, PhD, Diabetes Research Center, Vrije Universiteit Brussel, Laarbeeklaan, 101, B-1090, Brussels, Belgium. E-mail: bdr@vub.ac.be.

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Abbreviations: ANOVA, analysis of variance; BDR, Belgian Diabetes Registry; GADA, GAD antibodies; GLIMA, glycosylated islet cell membrane antigen; IA-2A, IA-2 (protein tyrosine phosphatase) antibodies; IAA, insulin autoantibodies; ICA, islet cell cytoplasmic antibodies; JDF, Juvenile Diabetes Foundation.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of type 1 diabetic patients at clinical onset of diabetes (0–39 years of age)*

Characteristics	Antibody-positive*	Antibody-negative†
n (%)	160 (93)	12 (7)
Age (years)	19 (0–38)	22 (6–31)
Sex (M/F)	85/75	9/3
Ketonuria	122/150 (81)	11/12 (92)
BMI (kg/m^2)‡	16.5 (12.6–19.2)	19.1 (14.7–22.9)
Random C-peptide (pmol/l)‡§	176 (88–254)	128 (51–249)
Random plasma glucose (mmol/l)‡	13.6 (9.8–18.3)	13.8 (11.1–19.1)
Insulin dose (U/kg)‡	0.55 (0.37–0.77)	0.49 (0.34–0.61)
Fructosamine (μmol/l)‡‖	390 (334–465)	409 (343–546)

Data are *n* (%), *n/n* (%), medians (range), *n*, and medians (interquartile ranges). *ICA, GADA, IA-2-A, or IAA; †ICA, GADA, IA-2A, and IAA; ‡interquartile range; §reference range for random C-peptide: 410–2,140 pmol/l; ‖2 years after diagnosis.

cytoplasmic antibodies (ICA) (4,7–14). Antibodies against GAD (GADA) and IA-2 protein tyrosine phosphatase (IA-2A) have been proposed as major constituents of ICA (15). Detection of these different antibodies constitutes the cornerstone of early diagnosis and classification of type 1 diabetes (4,7–14). However, it remains largely unknown how the presence of these antibodies relates to the intensity and evolution of the underlying disease process in the pancreas (such as insulitis and β-cell aggression or death), to associated defense mechanisms (such as cellular repair and regeneration), or to the effects of insulin treatment (3,4,16,17).

Several studies have investigated the association of autoantibodies, mainly of ICA, with the temporal changes in residual functional β-cell mass (as expressed by C-peptide levels) after diagnosis in insulin-requiring patients. Conflicting observations have been published in various studies, showing either no correlation (18–20), a positive correlation (21–23), or a negative correlation (24–27). These conflicting results may be due to differences in patient selection criteria (e.g., age at diagnosis) and in the type and methodology of the biologic markers used. In the present study, diabetes-associated antibodies (ICA, IA-2A, GADA, and IAA) were measured at clinical diagnosis in a representative group of insulin-requiring type 1 diabetic patients from the Belgian Diabetes Registry (BDR). The antibodies were related to random C-peptide levels during the first 2 years after diagnosis, as well as to other biologic, clinical, and demographic data, to identify biologic predictors of changes in β-cell function.

RESEARCH DESIGN AND METHODS

Subjects

A group of 172 type 1 diabetic patients was consecutively recruited by the BDR according to the following criteria: patients must have *1*) been Belgian residents (≥6 months before diagnosis) of Caucasian ethnicity; *2*) had clinical diabetes onset before 40 years of age; *3*) received insulin treatment at diagnosis and ≥2 years afterward; and *4*) had available blood samples taken at the start of insulin treatment (0–7 days on insulin) and after 24 ± 4 months. The data collected by the BDR have been shown to be representative of the Belgian population of type 1 diabetic patients (4,28). The study group comprised 160 (93%) patients who were antibody-positive (for ICA, IA-2A, GADA, and/or IAA) and 12 (7%) who were antibody-negative at the time of diagnosis. At that time, antibody-positive and antibody-negative patients did not differ significantly in age, sex ratio, BMI, prevalence of ketonuria, random levels of C-peptide and glucose, or daily insulin intake (Table 1). These patient characteristics are similar to those in age-matched type 1 diabetic patient groups, as recruited by the BDR in previous studies (4). Eleven of the 12 initially antibody-negative patients presented ketonuria at diagnosis; 2 developed IA-2A, GADA, and/or ICA after diagnosis; and all developed insulin antibodies during insulin treatment. Two years after diagnosis, antibody-positive and antibody-negative patients did not differ in fructosamine concentrations (Table 1). The study was approved by the ethics committees of the BDR and of participating universities. We obtained informed consent from the patients.

Assays

ICA levels were determined by indirect immunofluorescence and end-point titers expressed as Juvenile Diabetes Foundation (JDF) units (29). IA-2A, GADA, and IAA were measured by liquid-phase radiobinding assays and expressed as percent tracer-bound (30). Cutoff values for antibody positivity were determined by receiver operating characteristics curve analysis (31) of antibody levels obtained in 608 type 1 diabetic patients and 789 nondiabetic control subjects. The values amounted to ≥12 JDF units for ICA, ≥0.6% for IAA, ≥2.6% for GADA, and ≥0.4% for IA-2A (32). The various autoantibody assays were validated by repeated participation in immunology of diabetes workshops and proficiency testing programs of the University of Florida (Gainesville, FL) and the Louisiana State University (New Orleans, LA). In the latter programs, our assays achieved 100% sensitivity, specificity, consistency, and validity. The C-peptide assay was performed with a commercial kit (^{125}I-human C-peptide, guinea pig anti-human C-peptide serum; Linco), which had its lower detection limit at 20 pmol/l (33). Plasma glucose levels were determined on a Vitros 950 IC analyzer using Vitros glucose slides (Ortho Clinical Diagnostics, Beerse, Belgium). Fructosamine levels were determined using a colorimetric assay adapted to a Cobas Mira *S* analyzer (29). Plasma C-peptide, glucose, and fructosamine levels were determined on the same blood samples collected at random postprandially. DNA polymorphisms at the HLA-DQA1 and HLA-DQB1 gene loci were determined as previously described (34).

Statistical analysis

Statistical differences between prevalences were assessed by means of the Fisher's exact test. Differences in median values were determined by the Mann-Whitney *U* test for unpaired values and by the Wilcoxon's test for paired values. All tests were performed 2-tailed and considered significant whenever $P < 0.05$ or, in case of k comparisons, whenever $P < 0.05/k$ (Bonferroni correction). Pearson's correlation coefficients were calculated to estimate linear correlation between continuous variables. Multivariate analysis was performed by stepwise multiple linear regression, forward logistic regression analysis, and analysis of variance (ANOVA). C-peptide and autoantibody levels were log-transformed before Pearson's correlation or multivariate analysis by multiple linear regression. Normality

of the residuals was verified by a 1-sample Kolmogorov-Smirnov test. All statistical tests were calculated by the SPSS for Windows 8.0 statistical package for personal computers (SPSS, Chicago).

RESULTS — At clinical onset, random C-peptide levels did not differ according to the presence or absence of ICA, IA-2A, GADA, or IAA (Fig. 1). Two years after diagnosis, C-peptide levels had significantly decreased in initially ICA$^+$ patients but not in initially ICA$^-$ patients (Fig. 1). The lower C-peptide levels in ICA$^+$ patients were associated with significantly higher insulin requirements after 2 years (median [interquartile range], 0.61 U/kg [0.43–0.85] vs. 0.43 U/kg [0.27–0.60] in ICA$^-$ patients; $P < 0.001$ by Mann-Whitney *U* test) despite similar levels of glucose (10.1 mmol/l [6.3–14.3] vs. 8.6 mmol/l [5.0–10.8] in ICA$^-$ patients) and fructosamine (391 µmol/l [337–464] vs. 389 µmol/l [333–473] in ICA$^-$ patients). The lower C-peptide levels in ICA$^+$ patients were also detected when the comparison was restricted to samples with blood glucose values ≥6 mmol/l, the glucose concentration at which virtually all human β-cells are activated (35) (median [interquartile range] after 2 years, 60 pmol/l [20–187] vs. 217 pmol/l [127–370] at diagnosis; $P < 0.001$ by Wilcoxon's test). When a similar comparison was made between patients with and without IA-2A, GADA, or IAA at clinical onset, the C-peptide levels after 2 years tended to decrease in both antibody-positive and antibody-negative patients (Fig. 1). Insulin requirements and levels of glucose and fructosamine after 2 years were similar regardless of initial GADA or IAA status (results not shown). There was a tendency toward higher insulin requirements in initially IA-2A$^+$ patients (median [interquartile range], 0.62 U/kg [0.43–0.85] vs. 0.49 U/kg [0.34–0.74] in IA-2A$^-$ patients, $P < 0.02$ by Mann-Whitney *U* test), despite similar levels of glucose and fructosamine in both conditions (not shown).

We further analyzed our data in terms of the occurrence of low random C-peptide values 2 years after diagnosis in patients stratified according to age at clinical onset and ICA titer (Table 2). To examine this effect, we defined several factors beforehand. For age at diagnosis, we chose 7 years, which corresponded with the age below which β-cells tend to disappear more rapidly after diagnosis, and 15 years, above which insulitis was no longer a constant finding in anatomopathological investigations performed soon after disease onset (3). For ICA, 12 JDF units (positivity at 1/20 serum dilution in our assay) represent the 99th percentile of a reference population (n = 789) (32), whereas 50 JDF units represent high-titer positivity corresponding to 2 titer steps (2-fold dilution) above

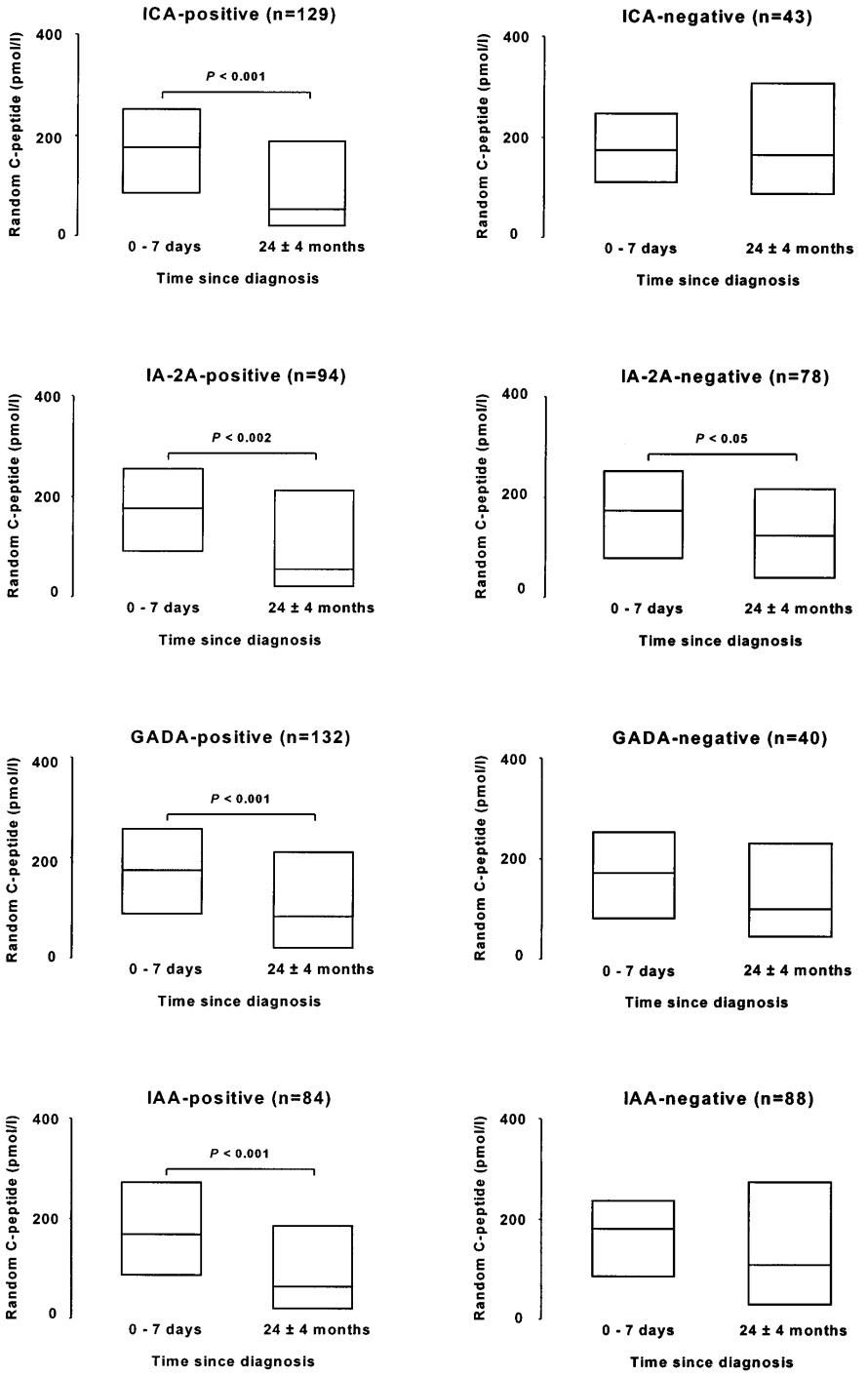

Figure 1—*Evolution of random C-peptide levels during the first 2 years after diagnosis in type 1 diabetic patients stratified according to the presence or absence of (from top to bottom) ICA, IA-2A, GADA, or IAA. In each panel, the lower and upper edges of the boxes represent the interquartile range, and the horizontal line within the boxes represents the median value of random C-peptide values at diagnosis and 2 years later.* P *values were computed by the Wilcoxon's test for paired values and considered significant whenever* P $< 0.05/8$*, i.e.,* P < 0.006 *(Bonferroni correction).*

Table 2—*Prevalence of low random C-peptide levels 24 ± 4 months after diagnosis of type 1 diabetes in patients stratified according to age at diagnosis and ICA titer*

Age at diagnosis (years)	Prevalence of rapid decline in C-peptide (<50 pmol/l after 2 years)				
		ICA in JDF units			
	All patients	≥12	<12	≥50	<50
0–6	14/16 (88)*†	14/14 (100)§	0/2 (0)	12/12 (100)	2/4 (50)
7–14	23/51 (45)‡	22/45 (49)	1/6 (17)	22/37 (59)¶	1/14 (7)
15–39	30/105 (29)	25/70 (36)‖	5/35 (14)	18/51 (35)	12/54 (22)

Data are *n*/*n* (%). *$P < 0.004$ vs. age-group 7–14 years and †$P < 0.001$ and ‡$P < 0.05$ vs. age-group 15–39 years by Fisher's exact test; §$P < 0.008$ and ‖$P < 0.03$ vs. patients of same age-group with ICA <12 JDF units and ¶$P < 0.002$ vs. patients of same age-group with ICA <50 JDF units (Fisher's exact test). *P* values were considered significant whenever $P < 0.05/21$, i.e., $P < 0.002$ (Bonferroni correction).

cutoff (positivity at 1/80 serum dilution) (29). For C-peptide, 50 pmol/l represents a value below the interquartile range of random values observed at diagnosis (Table 1). The fraction of patients with rapidly declining C-peptide levels (<50 pmol/l) 2 years after clinical onset decreased as age at diagnosis increased, ranging from 88% under age 7 years to 29% after age 14 years (Table 2). In clinical onset before 15 years of age, the presence of high-titer ICA (≥50 JDF units) at diagnosis is associated with a rapid decrease in random C-peptide levels in 34 of 49 patients (69%) compared with 3 of 18 (17%) patients with ICA <50 JDF units ($P < 0.001$) (Table 2). In patients with diagnosis after 15 years of age, the prevalence and titers of ICA are overall lower than in younger patients (29); also in this group, however, the presence of ICA ≥12 JDF units tended to be associated with more frequent progression to low random C-peptide levels (25 of 70 patients [36%] vs. 5 of 35 patients [14%] with ICA <12 JDF units, $P < 0.03$) (Table 2).

Because IA-2A and GADA may account for an important part of the ICA activity (15) and are more sensitive analytical tools than ICA (30) and because the male-to-female ratio of type 1 diabetic patients and the number and type of their biologic markers (autoantibodies and HLA-DQ risk haplotypes) tend to vary with age at clinical onset (4), we reanalyzed the reduction in C-peptide levels according to these baseline variables at diagnosis (Table 3). In univariate analysis, C-peptide levels 2 years after diagnosis were correlated with age at diagnosis ($P = 0.001$) and inversely related to ICA levels ($P < 0.001$; negative Pearson's correlation coefficient [Table 3]). After Bonferroni correction, there was a nonsignificant trend toward association of low C-peptide levels with the presence of at least 2 different types of molecular autoantibodies (IAA, IA-2A, or GADA) and with IAA levels. No associations were observed between C-peptide levels after 2 years and the levels of GADA or IA-2A at diagnosis, sex, or the presence of HLA-DQ risk haplotypes or genotypes (Table 3).

To further exclude the possibility that the observed association of young age and ICA positivity with a rapid decline in C-peptide levels after diagnosis may be due to the confounding effect of other variables, we submitted our data to stepwise linear regression analysis (Table 3). We used ICA, IAA, GADA, and IA-2A levels at diagnosis; sex; age at diagnosis; and presence of HLA-DQA1*-DQB1* risk haplotypes (0301-0302 or 0501-0201) or genotype (0301-0302/0501-0201) as possible independent predictors of decreased 2-year C-peptide levels. Random C-peptide levels 2 years after diagnosis were very significantly and inversely correlated ($\beta = -0.253$) with ICA levels ($P < 0.001$) and, to a lesser degree, positively correlated ($\beta = 0.187$) with age at diagnosis ($P = 0.012$), with β representing the partial regression coefficient when all independent variables are expressed in standardized form (*Z* score). We noted a nonsignificant trend toward lower C-peptide levels after 2 years in male patients ($\beta = -0.136$, $P = 0.067$) (Table 3). These results were confirmed with forward logistic regression (where we used C-peptide status [above or below 50 pmol/l] as the dependent variable) and with ANOVA. None of these methods found an association of C-peptide values after 2 years with fructosamine levels after 2 years, with persistence of ICA as expressed by ICA titers after 2 years, or by the presence or absence of decreasing ICA titers during the first 2 years after diagnosis (results not shown).

CONCLUSIONS

— A marked reduction in β-cell mass is the most constant histopathological finding in patients who died soon after clinical diagnosis of type 1 diabetes (2,3). Large interindividual differ-

Table 3—*Association between characteristics of type 1 diabetic patients at diagnosis and C-peptide levels 2 years later*

Characteristics	Univariate analysis*		Multivariate analysis†	
	r‡	*P*	β§	*P*
Age‖	0.242	0.001	0.187	0.012
More than 2 types of molecular autoantibodies	NA	0.014#	−0.070	0.390
ICA level‖	−0.293¶	<0.001	−0.253¶	0.001
IAA level	−0.173	0.023	−0.057	0.467
GADA level	−0.083	0.280	0.009	0.910
IA-2A level	−0.080	0.298	0.075	0.357
HLA DQA1*-DQB1*				
0301-0302/0501-0201	NA	0.302	0.067	0.355
0301-0302	NA	0.496	0.071	0.327
0501-0201	NA	0.501	0.036	0.624
Male sex	NA	0.539	−0.136	0.067

*Pearson's correlation for continuous variables after log-transformation of C-peptide and antibody levels and Mann-Whitney *U* test for categorical variables, with a threshold for significance of $P < 0.05/10$, i.e., $P < 0.005$ (Bonferroni correction); †independent predictor ability of the variables studied by stepwise linear regression analysis after log-transformation of antibody levels and C-peptide levels; ‡Pearson's correlation coefficient; §partial regression coefficient when all independent variables are expressed in standardized form; ‖variables selected in the stepwise linear regression. For each of the other variables, the β coefficient is that for the model in which the corresponding variable has been included in the case of stepwise linear regression analysis; ¶negative *r* and β-coefficients indicate inverse correlation; #lower in the case of the presence of at least 2 types of molecular antibodies (IAA, GADA, or IA-2A). NA, not applicable.

ences have, however, been noted in the further disappearance of β-cells after diagnosis. In pancreases procured 1–30 years after diagnosis, no β-cells could be seen in patients younger than7 years of age at clinical onset; however, residual β-cells were still detectable—although in variable amounts—in ~50% of patients with older age at presentation (3). The persistence of β-cell function after diagnosis is reflected by a less pronounced decrease in circulating C-peptide levels; it facilitates optimal metabolic control of diabetic patients under insulin treatment, thereby reducing the risk of developing the devastating chronic complications of the disease (5,6).

In line with these histopathological observations, the present study confirms the age-dependent heterogeneity in loss of β-cell function as expressed by C-peptide levels after diagnosis (22,25,36,37). In the present study, we analyzed randomly collected postprandial blood samples, which have been shown to be reliable measures of remnant insulin secretion (38). Rapidly (i.e., within 2 years) decreasing random C-peptide concentrations were observed in ~90% of patients younger than age 7 years at diagnosis but only in ~30% of patients 15 years of age or older at diagnosis. Both univariate and multivariate analyses have documented that ICA levels and, to a lesser degree, young age at diagnosis are more closely associated with declining C-peptide levels than the levels of IAA, GADA, or IA-2A, despite the fact that the latter 2 antibody types previously have been proposed as major components of ICA (15). Our results indicate that the use of ICA levels, instead of ICA positivity, adds to the association found. Differences in antibody status were not associated with differences in metabolic control expressed as fructosamine levels. This finding indicates that the lower C-peptide levels were not caused by poorer metabolic control in ICA^+ patients. However, to achieve similar fructosamine levels, ICA^+ patients required larger doses of insulin than ICA^- patients.

ICA levels are determined by indirect immunofluorescence using cryosections of human blood group O pancreas for substrate. The assay depends on the quality of the donor organ used, is difficult to standardize, and yields, at best, semiquantitive results (4,39). Interlaboratory differences in ICA technology and the use of small or selected patient groups are factors that may help explain previous conflicting reports on the relationship between ICA and C-peptide levels (18–27). ICA are made up of a mix of autoantibodies with different specificities, including IA-2A and GADA. These molecular autoantibodies are more easily quantified than ICA and facilitate between-laboratory comparisons (39). The combined use of IA-2A and GADA assays has been claimed to advantageously replace ICA testing in the prediction of clinical type 1 diabetes in risk groups such as siblings of known patients (14,40). However, through multivariate analysis, the present report indicates that ICA levels are strongly associated with a higher risk for rapid decrease in C-peptide levels after clinical onset, although this is not the case for IAA, IA-2A, or GADA levels or for multiple-antibody positivity. Our results are in partial contradiction to a recent report of Sabbah et al. (27), who found IA-2A positivity and multiple-antibody positivity to be associated with a marked reduction in residual β-cell function in Finnish diabetic children. Those authors, however, did not analyze their data in terms of the possible confounding effect of ICA positivity.

Our results may suggest the existence of at least a third diabetes-associated autoantigen, which, together with IA-2 and GAD, contributes to the cytoplasmic islet cell antigens recognized by sera from type 1 diabetic patients. This putative third antigen is unlikely to be the recently described 38-kDa glycosylated islet cell membrane antigen (Glima), in view of its relatively low prevalence in recent-onset diabetes (~20%) (41). Alternatively, our observation could indicate recognition of different antigenic epitopes or antibody affinities in the ICA assay compared with molecular assays. Unlike liquid-phase radiobinding assays for GADA or IA-2A, which use recombinant human antigens, the ICA assay is in essence a solid-phase assay, whereby relevant autoantigens such as IA-2 and GAD are anchored in cellular membranes (42,43). Moreover, the preparation of pancreatic cryosections may unmask hidden antigenic epitopes, which, in vivo, may only be presented to the immune system during β-cell destruction. If so, these epitopes could then react with antibodies from diabetic patients in the ICA assay but not in the liquid-phase assays using soluble recombinant antigens. The biologic significance of this type of ICA reactivity is currently unknown but might relate to rapidly progressive β-cell destruction or to ineffective β-cell regeneration or metabolic protection by insulin treatment. Our data also warrant a reevaluation of the predictive value of ICA versus molecular antibody assays in nondiabetic family members for the development of diabetes and for the relatives' outcome in prophylactic intervention studies with insulin administration. Indeed, we observed a rapid decline in C-peptide levels in many ICA^+ patients despite insulin treatment.

Nevertheless, approximately half of the ICA^+ subjects failed to display a rapid decrease in C-peptide levels, whereas ~1 in 7 ICA^- patients still progressed to low C-peptide concentrations. Multivariate analysis indicated that apart from ICA levels and young age at diagnosis, none of the other parameters under study could serve as additional independent predictors of low C-peptide concentrations 2 years later. These noninformative variables comprised the presence of molecular autoantibodies, alone or in combination, and the presence of the HLA DQA1*0301-DQB1*0302 risk haplotype (associated with high-titer ICA in childhood-onset diabetes [29]) or of HLA DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201, the highest-risk genotype claimed to be associated with rapidly decreasing C-peptide levels (44) and young age at diagnosis (29). In multivariate analysis, there was a nonsignificant tendency toward lower C-peptide values after 2 years in male subjects, which is in line with previous findings (25,36).

Although rapid progressors to low C-peptide values should in theory benefit most from preventive interventions, they may be unresponsive to any type of intervention if they already have reached a "point of no return." At any rate, determination of ICA allows better discrimination between rapid and slow progressors than molecular assays. Doing so thus provides a more objective criterion for the outcome of recent-onset type 1 diabetic patients and for their enrollment in intervention trials that attempt to preserve residual β-cell mass through metabolic or immunological modulation at diagnosis. So far, β-cell–preserving strategies based on nicotinamide or insulin administration have been largely ineffective in very young patients, but these strategies may offer better results in older patients with slower decline of C-peptide (45–47). However, it is conceivable that other, more aggressive types of intervention may be beneficial to patients at high risk of rapidly losing their residual β-cells. This possibility is illustrated by the capacity of various immune interventions to transiently arrest immune-mediated β-cell destruction around the time of clinical manifestation in juvenile diabetes (48) or in animal models of type 1 diabetes (49,50).

ICA levels and, to a lesser degree, young age at diagnosis identify a group of patients at high risk to develop a rapid decrease in C-peptide levels. The ICA assay is therefore not only important for the classification and prognosis of recent-onset patients but also provides an objective selection criterion for intervention trials aiming at preserving residual β-cell mass. Depending on the nature of these interventions, patients with slow or rapid progression can be enrolled on basis of age and ICA status.

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