Prediction of Insulin-dependent Diabetes Mellitus in Siblings of Children with Diabetes

A Population-based Study

Petri Kulmala,* Kaisa Savola,* Jacob S. Petersen,‡ Paula Vähäsalo,* Jukka Karjalainen,* Tuija Löppönen,* Thomas Dyrberg,§ Hans K. Åkerblom,‖ Mikael Knip,*¶ and the Childhood Diabetes in Finland Study Group

**Department of Pediatrics, University of Oulu, FIN-90220 Oulu, Finland;* ‡*Hagedorn Research Institute, DK 2820 Gentofte, Denmark;* §*Novo Nordisk A/S, DK-2880 Bagsvaerd, Denmark; the* ‖*Children's Hospital, University of Helsinki, FIN-00290 Helsinki, Finland; and the* ¶*Medical School, University of Tampere and Department of Pediatrics, Tampere University Hospital, FIN-33101 Tampere, Finland*

Abstract

An unselected population of 755 siblings of children with insulin-dependent diabetes mellitus (IDDM) was studied to evaluate the predictive characteristics of islet cell antibodies (ICA), antibodies to the IA-2 protein (IA-2A), antibodies to the 65-kD isoform of glutamic acid decarboxylase (GADA), insulin autoantibodies (IAA), and combinations of these markers. We also evaluated whether the histochemical ICA test could be replaced by the combined detection of other markers. 32 siblings progressed to IDDM within 7.7 yr of the initial sample taken at or close to the diagnosis of the index case (median follow-up, 9.1 yr). The positive predictive values of ICA, IA-2A, GADA, and IAA were 43, 55, 42, and 29%, and their sensitivities 81, 69, 69, and 25%, respectively. In contrast to the other three antibody specificities, GADA levels were not related to the risk for IDDM. The risk for IDDM in siblings with four, three, two, one, or no antibodies was 40, 70, 25, 2, and 0.8%, respectively. Combined screening for IA-2A and GADA identified 70% of all ICA-positive siblings, and all of the ICA-positive progressors were also positive for at least one of the three other markers. The sensitivity of the combined analysis of IA-2A and GADA was 81%, and the positive predictive value was 41%. In conclusion, combined screening for IA-2A and GADA may replace the ICA assay, giving comparable sensitivity, specificity, and positive predictive value. Accurate assessment of the risk for IDDM in siblings is complicated, as not even all those with four antibody specificities contract the disease, and some with only one or no antibodies initially will progress to IDDM. (*J. Clin. Invest.* 1998. 101: 327–336.) Key words: glutamic acid decarboxylase • IA-2 • insulin autoantibodies • insulin-dependent diabetes • islet cell antibodies

Address correspondence to Mikael Knip, M.D., Department of Pediatrics, University of Oulu, Kajaanintie 50, FIN-90220 Oulu, Finland. Phone: +358-8-315-5129; FAX: +358-8-315-5559; E-mail: llmikn@uta.fi

Received for publication 6 August 1997 and accepted in revised form 30 October 1997.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/98/01/0327/10 $2.00
Volume 101, Number 2, January 1998, 327–336
http://www.jci.org

Introduction

Insulin-dependent diabetes mellitus (IDDM),[1] one of the most serious and common metabolic disorders, is a chronic autoimmune disease with a subclinical prodromal period characterized by selective destruction of the insulin-producing β cells in the pancreatic islets (1–3). The destruction process is manifested by infiltration of the islets by mononuclear cells, and may proceed over a period of many years (4, 5). This prediabetic period offers an opportunity to identify those individuals who are likely to become insulin-dependent later and to start intervention aimed at delaying or preventing the manifestation of clinical disease.

The presence of circulating antibodies to various islet cell proteins is one of the most thoroughly characterized immune phenomena associated with IDDM (6). These autoantibodies are the first detectable markers of an ongoing destructive process in the islets, and thus provide a potential tool for identifying individuals at risk for developing the disease in the future. Humoral autoimmunity against pancreatic islets was first described by Bottazzo et al. in 1974, when they reported that antibodies to the islets could be detected in sera of patients with diabetes and polyendocrine diseases (7). Additional work has shown that most IDDM patients have these islet cell antibodies (ICA) in their sera at the clinical onset and during the preclinical phase of the disease (8–10). The association of insulin autoantibodies (IAA) with IDDM was reported in 1983 when Palmer and colleagues described their presence in 18% of untreated newly diagnosed diabetic patients (11). Antibodies to a 64-kD islet cell protein were described in IDDM sera in 1982 (12). This protein was later identified as the enzyme glutamic acid decarboxylase (GAD; 13), and was shown to be one of the major antigens for autoantibodies in sera from patients with IDDM (14–17). Antibodies to the intracellular fragment of a molecule belonging to the family of protein tyrosine-phosphatases (IA-2, ICA512) have recently been shown to be associated with IDDM (18–20), and it has been suggested that these antibodies may together with GADA replace ICA in the prediction of IDDM (21–23).

Although nearly 90% of new cases of IDDM occur sporadically (24, 25), studies in individuals with a diabetic relative are indispensable. The higher prevalence of IDDM in first-degree

1. *Abbreviations used in this paper:* CI, 95% confidence interval; GAD, glutamic acid decarboxylase; GADA, glutamic acid decarboxylase antibodies; IA-2A, antibodies to the IA-2 protein; IAA, insulin autoantibodies; ICA, islet cell antibodies; IDDM, insulin-dependent diabetes mellitus; JDF, Juvenile Diabetes Foundation; RU, relative units; TBST, Tris-buffered saline.

relatives allows enough prediabetic subjects to be identified to establish the predictive value of different disease markers and to test preventive strategies. Follow-up studies also give valuable clues to the complex and fascinating pathogenesis of IDDM. Siblings of children with IDDM form a special group of high-risk individuals who are currently being recruited for follow-up studies and intervention trials. From a practical point of view, the most urgent moment for evaluating the risk of future IDDM would be at the time when the first child in the family presents with the disease. A series of studies has been published describing the predictive characteristics of ICA, GAD antibodies (GADA), and IAA (9, 10, 21, 26–33), and more recently, IA-2 antibodies (IA-2A; 22, 34, 35), for progression to disease in the relatives of patients with IDDM. However, the series in most cases are relatively small, not population-based, and comprise both siblings and parents. Therefore, there is currently no evaluation available of the predictive value of all these markers, specifically in siblings. The time interval between the diagnosis of the index case and the first sample obtained from other family members also varied substantially in previous investigations. In addition, most previous studies did not measure all the antibodies in the whole cohort, but only in selected cases, e.g., according to positivity for ICA. We describe here the frequencies of ICA, IA-2A, GADA, IAA, and combinations of these antibodies in a well-characterized, population-based series of 755 siblings of children with recent-onset IDDM, and evaluate their utility in the prediction of IDDM. We also analyze the association among ICA, IA-2A, and GADA and consider whether detection of the other markers could replace the histochemical ICA test.

Methods

Subjects. The study population comprised the siblings from the Childhood Diabetes in Finland (DiMe) study (36), a population-based nationwide survey initiated at the beginning of September 1986, to investigate the role of genetic, immunological, and environmental factors in IDDM development. All newly diagnosed children with diabetes under 15 yr of age, their siblings under 20 yr of age, and their parents, were invited to the study. Informed consent was obtained from the subjects and/or their parents. The study design was approved by the ethical committees of all 31 participating hospitals. The recruitment of new cases terminated at the end of April 1989, by which time 801 eligible index cases had been diagnosed with a total of 977 unaffected siblings younger than 20 yr of age. The initial blood sample from each sibling was obtained at or close to the diagnosis of the index case. Subsequent blood samples were taken at intervals of 3–6 mo over the first 2 yr, and at intervals of 12 mo thereafter for up to 4 yr. Serial samples continued to be taken from the siblings having ICA on at least one occasion at intervals of 12 mo or less. All the siblings were observed up to the end of May 1997 unless IDDM was diagnosed before that date. Observation of the siblings progressing to IDDM ended at diagnosis. The diagnosis was based on clinical symptoms and an increased random blood glucose concentration (> 10 mmol/liter), elevated fasting (> 6.7 mmol/liter), or random blood glucose (> 10 mmol/liter) on two occasions in the absence of symptoms (37).

There were 755 siblings (77.3%) for whom at least one blood sample was available, and these were taken to comprise the study population. The series with a mean age 9.9 yr (range 0.8–19.7 yr) at the time of the first sample included 349 boys (46%). The median duration of follow-up for those who remained unaffected was 9.1 yr (range 7.7–10.7 yr). ICA, IA-2A, GADA, and IAA were determined in the initial samples from all subjects.

ICA and GADA were also determined in 372, and IA-2A in 374 healthy control children (age range 0–19 yr) without a family history of diabetes. IAA were analyzed in 105 healthy control children (age range 0–18 yr).

Laboratory evaluation

Islet cell antibodies. ICA were determined by a standard immunofluorescence method using sections of frozen human group O pancreas (7). All sera with detectable ICA were titrated to end-point dilution, and the results were expressed in Juvenile Diabetes Foundation (JDF) U by comparison with an international standard reference serum (38). The detection limit for ICA was 2.5 JDF U. Our laboratory had a sensitivity of 100%, a specificity of 98%, a validity of 98%, and a consistency of 98% in the fourth round of the international workshops on the standardization of the ICA assay (38).

Antibodies to the protein tyrosine–phosphatase–related IA-2 molecule. IA-2A were analyzed with a radiobinding assay modified from that described by Bonifacio et al. (20). The recombinant plasmid encoding the intracellular fragment of the full-length IA-2 protein, including amino acids 605–979, was transformed into *Escherichia coli* JM109 cells, and then purified by standard techniques. The IA-2 protein was produced with in vitro transcription and translation of the purified plasmid by the TNT® Coupled Reticulocyte Lysate System (Promega Corp., Madison, WI) in the presence of [^{35}S]methionine (Amersham International, Little Chalfont, Bucks, UK) according to the manufacturer's instructions. Unincorporated [^{35}S]methionine was removed by gel chromatography on a NAP-5 column (Pharmacia Fine Chemicals, Uppsala, Sweden). Serum samples of 2 μl were incubated at +4°C overnight in 96 deep-well plates with 10,000 cpm of labeled IA-2 protein diluted in 50 μl of TBST (50 mmol/liter Tris, 150 mmol/liter NaCl, 0.1 vol/vol Tween-20, pH 7.4). Immunocomplexes were isolated by adding 5 μl Protein-A-Sepharose CL-4B (Pharmacia Fine Chemicals) diluted in a total volume of 50 μl TBST to each well. After incubation for 1 h at +4°C with shaking, the reaction volume was transferred to a 96-well opaque filtration plate with a 0.45-μm Durapore filter at the bottom of each well (Millipore Corp., Bedford, MA). The samples were washed 10 times with 150 μl TBST using a vacuum device (Millipore Corp.). After a short drying period, 10 μl of scintillation fluid (Optiphase Supermix; Wallac, Turku, Finland) was added, and the activity of the samples was measured in a liquid scintillation counter (1450 Microbeta Trilux, Wallac). All the samples were tested in duplicate. In addition, each plate contained a dilution series of a pool of two local positive sera diluted in a pool of two local negative sera. A standard curve was constructed for each plate using the cpm results of the dilution series (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:512, and the pool of the two negative sera), the dilution being assigned arbitrary values of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, and 0.1 relative units (RU), respectively. The standard curve was a $\log_{10}/\log_{10}$ curve created separately for each plate. The cutoff limit for positivity (0.43 RU) was set at the 99th percentile for 374 nondiabetic Finnish children and adolescents. The disease sensitivity of our assay was 62%, and the disease specificity 97% based on 140 samples included in the Multiple Autoantibody Workshop (Canberra, Australia, December 8–11, 1996).

Antibodies to glutamic acid decarboxylase. GADA were quantified with a radiobinding assay described by Petersen et al. (39). Serum samples (2 μl) were incubated overnight in 96-well microtitre plates at 4°C with a total of 30,000 cpm [^{35}S]methionine-labeled in vitro–transcribed and –translated human recombinant GAD_{65} protein in a total volume of 50 μl TBST (50 mmol/liter Tris, 150 mmol/liter NaCl, 0.1 vol/vol Tween-20, pH 7.4). All the samples were analyzed in quadruplicate with and without an excess of unlabeled GAD_{65}. Competition analysis was carried out by adding 1 μg purified unlabeled GAD_{65} to the incubation mixture. Immunocomplexes were isolated by adding 7.5 mg Protein-A-Sepharose (Pharmacia, Fine Chemicals) diluted in a total volume of 100 μl TBST to each well. After incubation for 2 h at 4°C with shaking, the reaction volume was transferred to a 96-well filtration plate (Multiscreen; Millipore Corp.). The filtration plates were placed on a vacuum device (Millipore Corp.) and the immunocomplexes were washed 10 times with 150 μl of cold

(+4°C) TBST. After drying for 2 h on a tissue paper, each filter containing the immunocomplexes was punched into a vial, 2.5 ml scintillation fluid was added (Ultimagold; Packard, Groningen, Netherlands), and the radioactivity was measured in a scintillation counter (Packard Tri-Carb 4530; Packard, Downers Grove, IL).

The results are expressed in RU representing the specific binding as a percentage of that obtained with a positive standard serum: relative GAD U = 100 × [cpm (unknown sample) − cpm (unknown sample incubated with an excess of unlabeled GAD)/cpm (positive standard serum) − cpm (positive standard serum incubated with an excess of unlabeled GAD)]. The cutoff limit for GADA positivity was defined as 6.5 RU representing the 99th percentile in a series of 372 healthy control children. All samples exceeding 2.9 RU (mean + 1 SD in 372 control children) were retested in order to confirm GADA positivity or negativity. The disease sensitivity of the present assay was 80%, and the disease specificity was 94% based on the 101 samples included in the second international GAD antibody workshop (40).

Insulin autoantibodies. IAA were analyzed with a competitive radiobinding assay modified from that described by Palmer et al. (11). Endogenous insulin was removed with acid charcoal before the assay, and free and bound insulin was separated after incubation with mono-^{125}I(Tyr A 14)-labeled human insulin (Novo Research Institute, Bagsvaerd, Denmark) in the absence or presence of an excess of unlabeled insulin. IAA levels were expressed in nU/ml, where 1 nU/ml corresponds to a specific binding of 0.01% of the total counts. The cutoff limit for IAA positivity was defined as 54 nU/ml, representing the 99th percentile in a series of 105 nondiabetic children. The disease sensitivity of the IAA assay was 26%, and the disease specificity 97% based on 140 samples included in the Multiple Autoantibody Workshop.

Statistical analyses. Student's *t*-test was used to analyze normally distributed continuous variables, and Mann-Whitney U-test or Kruskall-Wallis analysis was used in the case of skewed distributions. Spearman's nonparametric correlation analysis was used to analyze the relationship among the levels of various antibodies. Differences in the distribution of individuals among groups were tested with Chi square statistics unless any expected value was less than five, when Fisher's exact test was used (41). The Kaplan-Meier method (42) was used to construct life tables for the likelihood of developing IDDM. The follow-up time for each subject was calculated from the date when the initial blood sample had been obtained to the clinical diagnosis, or for 7.7 yr, which was the minimum follow-up time in all cases. The equality of the survival distributions was tested using the log-rank test (43). Sensitivity is defined as the percentage of those who have the disease for whom the test value is positive; specificity is defined as the proportion of those without the disease correctly identified by a negative test value; and positive predictive value is defined as the likelihood that a sibling with a positive test will become diabetic. A two-tailed *P* value of 0.05 was considered to indicate statistical significance. All the statistical analyses were performed using the SPSS statistical software package (SPSS Inc., Chicago, IL).

Results

Islet cell antibodies. 60 of the 755 siblings (7.9%) had detectable levels of ICA in their initial blood sample with a median level of 40 JDF U (range 2.5–2,544 JDF U; Table I). 48 siblings (6.4%) had ICA 10 JDF U or more and 37 (4.9%) had ICA 20 JDF U or more. 6 (1.6%) of the 372 control subjects had ICA above the detection limit (median level, 6 JDF U, range, 4–34 JDF U). The siblings with ICA were significantly younger than those without these antibodies (mean age, 8.8 yr vs. 10.0 yr; $P = 0.039$, 95% confidence interval [CI] for the difference 0.06–2.4 yr). When divided into 2 age groups, ICA were slightly more frequent in siblings under 10 yr of age than in those over this age (39/401 [9.7%] vs. 21/354 [5.9%]; $\chi^2 = 3.7$, $P = 0.054$). There was no significant difference in the levels of ICA between these two age groups of antibody-positive siblings. The frequency or levels of ICA did not differ significantly between boys and girls, but the boys with ICA were significantly younger than the girls with ICA (mean age 7.6 vs. 9.8 yr; $P = 0.029$, CI for difference 0.24–4.25 yr).

The difference in the proportion of siblings with detectable ICA between those who progressed to IDDM (26/32 [81.3%]) and those who remained unaffected (34/723 [4.7%]) was highly significant ($P < 0.00001$). The sensitivity decreased from 81.3 to 3.1% with higher cutoff levels for ICA positivity (Table II).

Table I. ICA, IA-2A, GADA, IAA, and Multiple Antibodies in the Initial Blood Sample from 755 Siblings of Children with IDDM

	Number of siblings positive for antibodies (total n = 755)	Median level of antibodies	Range
ICA ≥ 2.5 JDF U	60 (7.9%)	40 JDF U	2.5–2544 JDF U
ICA ≥ 10 JDF U	48 (6.4%)	40 JDF U	10–2544 JDF U
ICA ≥ 20 JDF U	37 (4.9%)	80 JDF U	20–2544 JDF U
IA-2A > 0.43 RU	40 (5.3%)	22.5 RU	0.49–277.1 RU
GADA > 6.5 RU	53 (7.0%)	58.1 RU	6.8–211.1 RU
IAA > 54 nU/ml	28 (3.7%)	79 nU/ml	55–1238 nU/ml
Multiple antibodies	47 (6.2%)		

Table II. Antibody Positivity and IDDM Development over 7.7 yr: Positive Predictive Value, Sensitivity, and Specificity of ICA and IA-2A Using Different Cutoff Limits for Positivity

Cutoff limit for positivity	Positive predictive value	Sensitivity	Specificity
	%	%	%
ICA (JDF U)			
2.5	43	81	95.3
10	52	78	96.8
20	62	72	98.1
40	58	56	98.2
80	70	50	99.0
160	75	38	99.4
320	86	19	99.9
640	67	6.3	99.9
1280	100	3.1	100.0
IA-2A (RU)			
0.43	55	69	97.5
0.70	60	66	98.1
1	63	63	98.3
2	69	63	98.8
5	71	63	98.9
10	79	59	99.3
20	75	47	99.3
50	73	34	99.4
70	80	25	99.7
90	86	19	99.9
110	86	19	99.9
120	100	13	100.0

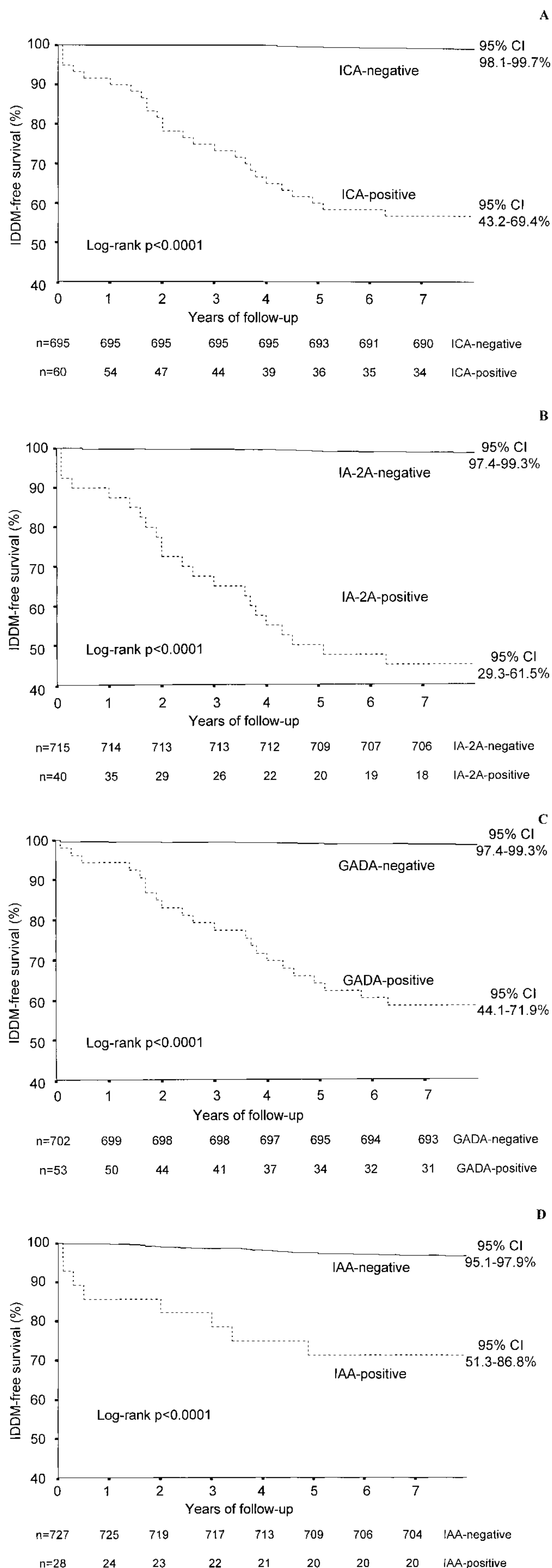

Figure 1. Probability of remaining nondiabetic in 755 siblings initially positive or negative for various antibodies. (*A*) ICA; (*B*) IA-2A; (*C*) GADA; (*D*) IAA. *Solid line,* antibody-negative siblings; *broken line,* antibody-positive siblings.

Life-table analysis showed the siblings with detectable levels of ICA in their initial blood sample to have an estimated risk of 43.3% (CI 30.6–56.8%) of developing IDDM over 7.7 yr compared with a 0.9% (CI 0.3–1.9%) risk in those without ICA (log-rank $P < 0.0001$; Fig. 1 *A*). The positive predictive value and specificity increased when the cutoff level for ICA positivity was raised (Table II). The ICA-positive individuals who contracted the disease had significantly higher levels of ICA than those who remained unaffected (median level 80 JDF U in the progressors vs. 10 JDF U in the nonprogressors; $P = 0.0001$; Fig. 2 *A*). There was no significant age or sex difference between the ICA-positive progressors and nonprogressors.

Antibodies to IA-2. 40 siblings (5.3%) tested positive for IA-2A in their initial blood sample (median level 22.5 RU, range 0.49–277.1 RU; Table I). These did not differ significantly in mean age from those testing negative, and no difference in frequency or levels of IA-2A was observed between those over and under 10 yr of age, or between the boys and girls.

As with ICA, the proportion of siblings with IA-2A was significantly higher among the progressors than among the nonprogressors (68.8% [22/32] vs. 2.5% [18/723]; $P < 0.00001$). Sensitivity decreased and specificity increased when the cutoff level for IA-2A positivity was raised (Table II). Life-table analysis revealed an estimated risk of 55.0% (CI 38.5–70.7%) for the development of IDDM over 7.7 yr in siblings with IA-2A, compared with a risk of 1.4% (CI 0.7–2.6%) for those negative for IA-2A in their initial sample (Log rank $P < 0.00001$; Fig. 1 *B*). The positive predictive value increased when the cutoff level for IA-2A positivity was increased (Table II). The IA-2A–positive progressors had significantly higher levels of IA-2A (median level, 50.9 RU vs. 1.9 RU; $P = 0.0016$, Fig. 2 *B*), and they were significantly younger (mean age, 8.6 vs. 11.4 yr; $P = 0.020$, CI for difference 0.46–5.05) than the antibody-positive nonprogressors. There was no significant sex difference between the IA-2A–positive progressors and nonprogressors.

Antibodies to GAD. 53 siblings (7.0%) had detectable GADA in their initial blood sample, with a median level of 58.1 RU (range 6.8–211.1 RU; Table I). Individuals with and without GADA did not differ significantly in the mean age. Similarly, those over 10 yr of age did not have GADA any more frequently nor at higher levels than those under 10 yr of age. No difference was found between the boys and girls in the frequency or levels of these antibodies.

The proportion of siblings with GADA was significantly higher among the progressors than among the nonprogressors (68.8% [22/32] vs. 4.3% [31/723]; $P < 0.00001$). The sensitivity decreased and specificity increased when the cutoff level for GADA positivity was increased (Table III). As with ICA and IA-2A, life-table analysis revealed a significantly higher risk for IDDM development over 7.7 yr in siblings with GADA in their initial blood sample than in those testing negative for GADA (41.5% [CI 28.1–55.9%] vs. 1.4% [CI 0.7–2.6%]; log-rank $P < 0.0001$; Fig. 1 *C*). Unlike ICA and IA-2A, the positive predictive value did not increase when the cutoff level for

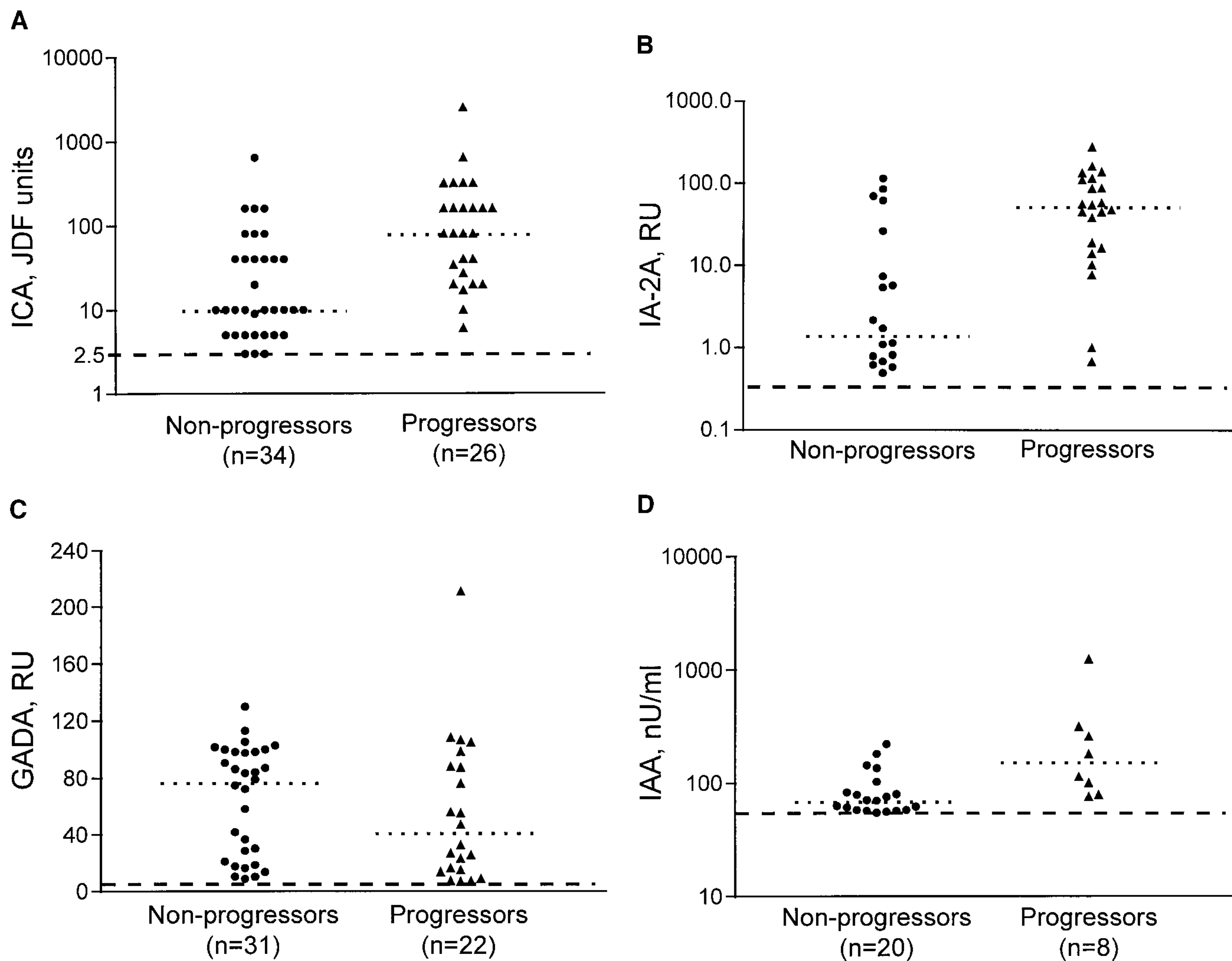

Figure 2. Antibody levels in antibody-positive progressors and nonprogressors. (*A*) ICA in JDF units; (*B*) IA-2A in relative units; (*C*) GADA in relative units; (*D*) IAA in nU/ml. *Dashed line,* cutoff limit for positivity; *dotted line,* median level of antibodies.

GADA positivity was raised (Table III). The levels of GADA did not differ significantly between the antibody-positive siblings who contracted IDDM and those who remained unaffected (median level, 39.8 RU in the progressors vs. 79.0 RU in the nonprogressors; $P = 0.248$) (Fig. 2 *C*). There were no significant age or sex differences between the GADA-positive progressors and nonprogressors.

Insulin autoantibodies. IAA were detected in 28 siblings (3.7%), with a median level of 79 nU/ml (range, 55–1,238 nU/ml; Table I). Those testing positive for IAA did not differ significantly in mean age from those testing negative. There was no difference in the proportions of siblings positive for IAA between those under 10 yr of age and those over 10 yr, although the median level of IAA was significantly higher in the younger age group (109 nU/ml vs. 62 nU/ml; $P = 0.0034$). Neither the frequency of IAA nor their levels differed significantly between the boys and girls.

As with the other antibody specificities, the proportion of siblings testing positive for IAA was significantly higher among the progressors than among the nonprogressors (25.0 [8/32] vs. 2.8% [20/723]; $P = 0.00001$). Also, the sensitivity decreased and specificity increased when higher cutoff levels for IAA positivity were used (Table III). Life-table analysis revealed an estimated risk of 28.6% (CI 13.2–48.7%) for IDDM development over 7.7 yr in the siblings with IAA in their initial blood sample, compared with a risk of 3.3% (CI 2.1–4.9%) among those without IAA (Log rank $P < 0.0001$; Fig. 1 *D*). The positive predictive value increased when the cutoff level for IAA positivity was raised (Table III). IAA levels were significantly higher in those with the antibodies who developed IDDM than in those who remained unaffected (median level 148 nU/ml vs. 71 nU/ml; $P = 0.007$, Fig. 2 *D*), and the progressors were significantly younger (mean age 5.0 vs. 11.4 yr in nonprogressors; $P < 0.001$, CI for difference 3.8–8.9 yr). There was no significant sex difference between the IAA-positive progressors and nonprogressors.

Multiple antibodies. Multiple antibodies, i.e., at least two antibodies, were found in the initial blood sample of 47 siblings (6.2%; Table I). Five of these (10.6%) tested positive for all four antibodies, 30 (63.8%) for three antibodies, and 12 (25.5%) for two antibodies (Table IV). 47 siblings had only one antibody, and 661 did not have any detectable antibodies

Table III. Antibody Positivity and IDDM Development over 7.7 yr: Positive Predictive Value, Sensitivity, and Specificity of GADA and IAA Using Different Cutoff Limits for Positivity

Cutoff limit for positivity	Positive predictive value	Sensitivity	Specificity
	%	%	%
GADA (RU)			
6.5	42	69	95.7
10	38	56	95.8
20	39	47	96.7
30	35	38	96.9
50	35	31	97.4
70	31	25	97.5
90	31	16	98.5
110	33	3.1	99.7
130	100	3.1	100.0
IAA (nU/ml)			
54	29	25	97.2
60	36	25	98.1
80	46	19	99.0
100	55	19	99.3
120	50	13	99.4
140	57	13	99.6
150	67	13	99.7
200	75	9	99.9
300	100	6.3	100.0

(Table IV). No significant age or sex differences were found between those with multiple antibodies and those with one or no antibodies.

The progressors had multiple antibodies more often than the nonprogressors (81.3% [26/32] vs. 2.9% [21/723]; $P <$ 0.00001). Life-table analysis showed the estimated risk for progression to IDDM in siblings with four, three, two, one, or no antibodies in their initial blood sample to be 40% (CI 5.3–85.3%), 70% (CI 50.6–85.3%), 25% (CI 5.5–57.2%), 2% (CI 0.05–11.3%) and 0.8% (0.2–1.8%), respectively, over 7.7 yr. When the siblings with three and four antibodies were combined, their risk for progression to IDDM was 66% (CI 47.8–80.9%; Fig. 3). Accordingly, when combining all of these groups, the siblings with multiple antibodies had an estimated risk of 55% (CI 40.1–69.8%) within 7.7 yr compared with a risk of only 0.8% (CI 0.3–1.8%) in those with one or no antibodies (log-rank $P < 0.0001$). The siblings with multiple antibodies who progressed to clinical disease were significantly younger than those who remained unaffected (mean age 7.8 vs. 10.6 yr; $P = 0.012$, CI for difference 0.6–4.9 yr). There were no significant sex differences between the progressors and the nonprogressors among the siblings with multiple antibodies.

When ICA was excluded from these analyses, 38 siblings (5.0%) had multiple antibodies, i.e., at least two of the three antibodies to biochemically defined islet antigens (IA-2A, GADA, IAA). Six siblings (0.8%) tested positive for all three antibodies, 32 (4.2%) had two antibodies, 39 (5.2%) had one antibody, and 678 were negative for all of them. The positive predictive values for three, two, one, or no antibodies were 33% (CI 4.3–77.7%), 66% (CI 46.8–81.4%), 10% (CI 2.9–24.2%), and 0.7% (CI 0.2–1.7%), respectively, and that for multiple antibodies was 61% (CI 43.4–76.0%) compared with 1.3% (CI 0.6–2.4%) for one or no antibodies (log-rank $P <$ 0.0001). The risk of developing IDDM over 7.7 yr in siblings with any of these three antibodies in their initial blood sample ($n = 76$) was 35% (CI 24.9–47.3%; sensitivity 84%, specificity 93%).

IDDM progressors. 32 siblings (4.2%) progressed to clinical IDDM during the follow-up, 15 (46.9%) of whom were boys. The median follow-up time for the progressors was 3.1 yr (range, 0.01–7.5 yr). The progressors were significantly younger than the nonprogressors (mean age 7.5 yr vs. 10.0 yr, $P = 0.002$, CI for difference 0.9–4.1). When divided into two age groups, the proportion becoming diabetic was significantly

Table IV. Combinations of Antibodies in the Initial Sample of 755 Siblings

Antibody status	*n*	Progression to IDDM *n* (%)
No antibodies	661	5 (0.8)
One antibody	47	1 (2.1)
ICA	17	0
IA-2A	5	0
GADA	12	1 (8.3)
IAA	13	0
Two antibodies	12	3 (25.0)
ICA and IA-2A	2	1 (50.0)
ICA and GADA	6	1 (16.7)
ICA and IAA	1	1 (100.0)
IA-2A and GADA	1	0
GADA and IAA	2	0
Three antibodies	30	21 (70.0)
ICA, IA-2A and GADA	23	16 (69.6)
ICA, IA-2A and IAA	3	3 (100.0)
ICA, GADA and IAA	3	2 (66.7)
IA-2A, GADA and IAA	1	0
Four antibodies	5	2 (40.0)

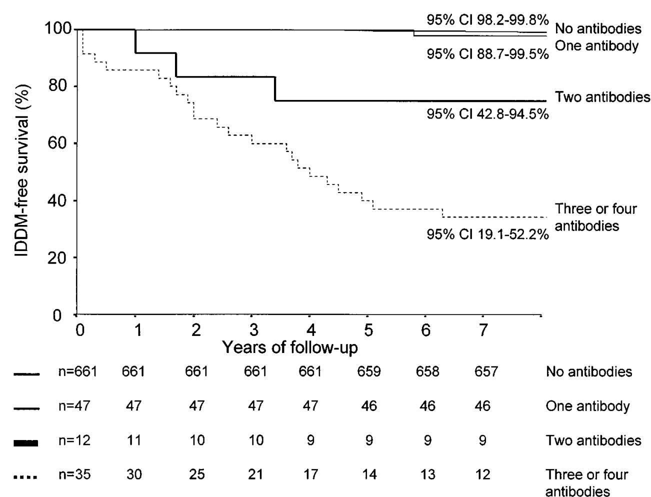

Figure 3. Probability of remaining nondiabetic in relation to the number of antibodies in the initial sample of 755 siblings; comparison of siblings with four and three, two, one, or no antibodies.

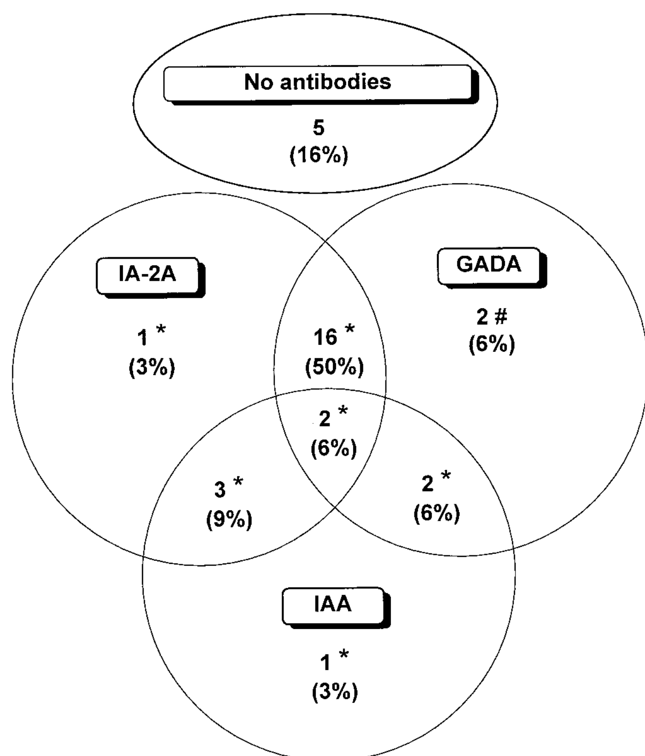

Figure 4. Prevalences of antibodies among siblings who progressed to IDDM (n = 32). The asterisk (*) indicates those who tested positive also for ICA. # indicates two siblings out of whom one tested positive and the other negative for ICA.

higher among the siblings under 10 yr of age than in those over 10 yr of age (5.7% [23/401] vs. 2.5% [9/354]; χ^2 = 4.7, P = 0.030).

Two progressors (6.3%) had all four antibodies, 21 (65.6%) had three antibodies, three (9.4%) had two antibodies, one (3.1%) had one antibody, and five (15.6%) did not have any of these antibodies detectable in their initial blood sample. Of those with three antibodies, 16 had ICA, IA-2A, and GADA, three had ICA, IA-2A., and IAA, and two had ICA, GADA, and IAA. Of those with two antibody specificities, one had ICA and IA-2A, one had ICA and GADA., and one had ICA and IAA. The sibling with only one antibody had GADA. The prevalences of the antibodies among the IDDM progressors are shown in Fig. 4.

Five of the six progressors who did not have ICA initially converted to ICA positivity in their prediabetic follow-up samples. IA-2A were detected in follow-up samples from six of the ten initially IA-2A–negative progressors, GADA were detected in six of the ten who were initially GADA-negative, and IAA were detected in 18 of 24 who were initially IAA-negative. Accordingly, 31 progressors (97%) had ICA, 28 (87.5%) had IA-2A, 28 (87.5%) had GADA, and 26 (81%) had IAA on one or more occasions during the follow-up before the diagnosis of IDDM. All 32 progressors had one or more of these antibodies during their prediabetic follow-up.

Association among ICA, IA-2A, and GADA. IA-2A were detected in 33 (55.0%) of 60 ICA-positive siblings, and GADA were detected in 37 (61.7%). The levels of ICA were significantly higher in those with IA-2A than in those testing negative for IA-2A (median level, 80 JDF U [range, 6–254] vs. 10 JDF U [range 3–32]; P < 0.0001) and higher in those with GADA than in those without GADA (median level, 80 JDF U [range 6–2,544] vs. 10 JDF U [range 3–64]; P = 0.0001). Of those with ICA 10 JDF U or more, 66.7%, (32/48) had IA-2A, and 75.0% (36/48) tested positive for GADA, while of those with ICA 20 JDF U or more, 78.4%, (29/37) had IA-2A, and 83.8% (31/37) had GADA. Combined screening for the presence of IA-2A and/or GADA identified 70% (42/60) of all the ICA-positive siblings, 85% (41/48) of those with ICA 10 JDF U or more, and 95% (35/37) of those with ICA 20 JDF U or more. The levels of ICA were significantly higher in the siblings with IA-2A and/or GADA than in those without these antibodies (median level, 80 JDF U [range 6–2544] vs. 5 JDF U [range 3–16]; P < 0.0001).

A relatively strong positive correlation was seen between the levels of IA-2A and ICA (r = 0.46, P < 0.001), but no correlation was seen between the levels of GADA and ICA (r = 0.17, P = 0.150) or the levels of IA-2A and GADA (r = −0.12, P = 0.33).

Life-table analysis showed the 63 siblings with IA-2A and/or GADA in their initial blood sample to have a 41.3% (CI 29.0–54.4%) estimated risk of developing IDDM over 7.7 yr compared with 0.9% (CI 0.3–1.9%) for those testing negative for both antibodies in their initial sample (Log rank P < 0.00001). The sensitivity of the combined analysis of IA-2A and GADA was 81.3%, and the specificity was 94.7%. Accordingly, the predictive characteristics of a combined test for IA-2A and GADA were similar to those of the ICA assay (Table II, Fig. 5).

Discussion

This study allows a nonbiased view on the value of ICA, IA-2A, GADA, IAA, and combinations of these antibodies for predicting IDDM in siblings over a period of 7.7 yr subsequent to the diagnosis of the index case in the family (7.7 yr was the minimum follow-up time among all siblings). We also evaluated whether combined testing for other antibodies could replace the histochemical ICA test. The study population was unselected and well-defined, comprising 755 siblings under 20 yr of age. All four antibodies were analyzed in all cases,

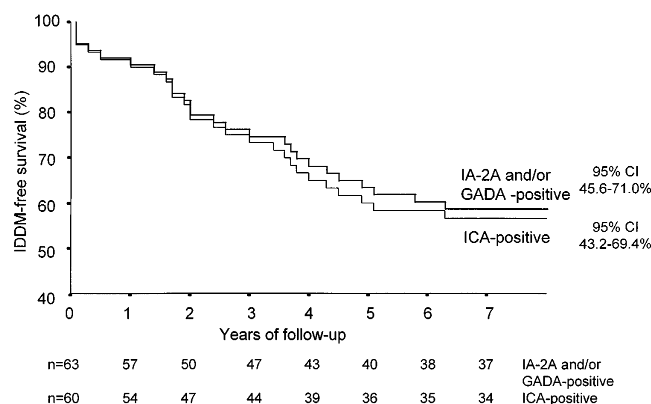

Figure 5. Probability of remaining nondiabetic in siblings positive for ICA and in those positive for IA-2A and/or GADA.

which has not been done in most previous studies (e.g. 31, 32, 44). Also, the majority of the published reports (9, 10, 29, 45, 46) include all first-degree relatives (siblings, parents and offspring), whereas we evaluate the predictive value of these humoral immune markers specifically in siblings, the follow-up starting at or close to the diagnosis of the index case.

Some variation exists in reported frequencies of ICA, IA-2A, GADA, and IAA in siblings. In this series, the frequency of ICA above the detection limit (2.5 JDF U) was 7.9%, and ICA of 10 JDF U or more were present in 6.4% of the siblings. This is somewhat higher than the frequencies reported elsewhere (9, 10, 28), although the varying detection thresholds for positivity make it difficult to compare the results directly. The frequency of IA-2A was 5.3%, and that of GADA 7.0%, which are comparable with the frequency of ICA. The frequency of IAA (3.7%) corresponds to that reported in Florida (30). The presence of IA-2A, GADA, and IAA was not dependent on the age of the sibling, but ICA were more frequently found in younger siblings. The levels of ICA, IA-2A, and GADA were not related to age, but those of IAA were significantly higher in younger siblings.

All four antibodies are shown here to be useful markers for the prediction of IDDM in siblings initially tested at, or close to the diagnosis of the first affected child in the family. ICA were the most sensitive single marker, since 81% of those who contracted the disease had ICA in their initial blood sample. The sensitivity of IA-2A was equal to that of GADA (69%), while that of IAA was the lowest (25%). IA-2A had the highest positive predictive value (55%) for the development of IDDM over 7.7 yr, the values for ICA, GADA, and IAA being 43, 42, and 29%, respectively.

The performance characteristics of the autoantibody assays have a critical impact on their predictive value, and therefore continuous quality control of the assays is essential. According to the present results, IAA had the lowest disease sensitivity and positive predictive value for IDDM among the autoantibodies tested. This could partly be a consequence of a lower disease sensitivity of our IAA assay than that of the most sensitive IAA assays using 600 μl of serum. Based on the results from the recent Multiple Autoantibody Workshop, the average disease sensitivity of the large-volume IAA assays was 39% (range 24–49%), while that of the present assay is 26% (C. Verge and D. Stenger, personal communication). Hence, an IAA assay with optimal sensitivity would probably result in somewhat better predictive characteristics than those observed for IAA in this survey.

The use of different thresholds for antibody positivity has been shown to alter the positive predictive value and sensitivity of ICA (9). As expected, those progressing to IDDM in our series had higher levels of ICA than those remaining unaffected, so that the positive predictive value of ICA increased as the cutoff limit for antibody positivity was raised. Use of $\geq$ 10 JDF U as a cutoff limit gave a positive predictive value of 52%, but this resulted in a decrease in sensitivity since one prediabetic sibling with an ICA level of 6 JDF U in his initial blood sample was missed. Even higher cutoff limits increased the positive predictive value still further, but the sensitivity also decreased substantially. Similarly, the positive predictive values of IA-2A and IAA increased when higher cutoff limits were used, but the sensitivities decreased. Unlike the other autoantibodies, the levels of GADA did not differ significantly between the progressors and the nonprogressors, and thus, the positive predictive value did not increase when the threshold for positivity was raised, but the sensitivity decreased markedly.

The presence of several antibodies has recently been shown to result in higher predictive values for IDDM than does single antibody positivity (21, 34, 44, 47). However, the populations evaluated in those studies were relatively small and selected (21, 44, 47), or the follow-up of the unselected relatives was short (34). Thus, these findings have not been confirmed in any large population of unselected subjects with an adequate length of follow-up. We found ~ 6% of the siblings to have multiple antibodies (i.e., at least two out of four examined here) in their initial blood sample, and they had a 55% estimated risk to contract IDDM within 7.7 yr (sensitivity 81%, specificity 97.0%) compared with a risk of only 0.8% in those with one or no antibodies. Excluding ICA from these analyses, 5% of the siblings carried multiple antibodies, i.e., at least two out of three antibodies to biochemically defined antigens. Their risk for progression to IDDM was 61%, which is higher than the positive predictive value for any single antibody. The exclusion of ICA also reduced the sensitivity of multiple antibodies to 72%, however, because three progressors who tested positive for both ICA and one of the antibodies to biochemically defined antigens (one for IA-2A, one for GADA, and one for IAA) were now classified as positive for a single antibody specificity.

Verge et al. (34) reported that the risk for IDDM increases as the number of autoantibodies increases, and that relatives with three autoantibodies (IA-2A, GADA, and IAA) had a 100% estimated risk of contracting IDDM within 5 yr. Interestingly, only two of our six siblings with antibodies to all three biochemically characterized antigens have presented with IDDM to date (the time period between initial testing and clinical disease was 0.2 and 2.9 yr, whereas the follow-up for the unaffected cases ranged from 8.9 to 10.5 yr). Thus, the positive predictive value was highest for double-positive siblings, although the wide confidence intervals because of the small number of siblings with three antibodies have to be noticed. Similarly, although the positive predictive value for the presence of all four antibodies after including ICA was lower than that for three antibodies, one has to take into account that the number of siblings in the former group was small, resulting in wide confidence intervals. After combining the siblings with three and four antibodies there was still some overlap in the confidence intervals between this group and siblings with two antibodies (Fig. 3). On the other hand, the presence of only one of these four antibodies did not rule out progression to IDDM, since four progressors had initially only one antibody (two had GADA, one IA-2A, and one IAA). Of those three nonprogressors who initially tested positive for all four antibodies analyzed, two have remained positive for all of them, while one has turned negative for IA-2A, GADA, and IAA, but remained positive for ICA.

32 siblings progressed to IDDM during the follow-up, 27 (84%) of whom were identified on the basis of antibodies in their initial blood sample. Most of these were positive for more than one antibody. Five progressors did not have any of these four antibodies in their initial sample. However, it is worthwhile to observe that antibodies (one or more) emerged later during the follow-up in all of these cases. Thus, all 32 siblings who progressed to IDDM could be identified on the basis of antibodies before the manifestation of clinical disease.

We found here that combined screening of IA-2A and GADA identified 70% of all ICA-positive siblings, 85% of those with ICA of 10 JDF U or more, and as many as 95% of those with ICA of 20 JDF U or more. Besides this, all but one of the 26 IDDM progressors with ICA were also positive for IA-2A and/or GADA, the one exception testing positive for IAA. Thus, none of the initially antibody-positive progressors would have been missed if ICA had not been analyzed. The positive predictive value for the presence of IA-2A and/or GADA was 41%, with a sensitivity of 81% and a specificity of 95%. These data, together with the additional observation that ICA in the absence of other islet antibody specificities did not predict future IDDM, supports the view that combined screening for IA-2A and GADA can replace the histochemical ICA test. Such a screening procedure provides predictive characteristics comparable to those of a well-standardized ICA assay in terms of sensitivity, specificity, and positive predictive value, although neither alternative is capable of identifying all future cases with IDDM. From a practical point of view detection of IA-2A and GADA offers a feasible and effective strategy due to the high throughput capacity and the small sample volume requirements of these two assays. The positive predictive value of such a screening program could be enhanced further by testing individuals positive for IA-2A and/or GADA for ICA. This fact is illustrated by the observation in the present series that a second-step screening for ICA in those 63 siblings testing positive for IA-2A and/or GADA would have increased the positive predictive value from 41 to 60%.

In conclusion, all four islet antibody specificities are useful predictive markers for IDDM development in siblings initially tested at or close to the diagnosis of the first affected child in the family, ICA having the highest sensitivity, and IA-2A having the highest positive predictive value. The higher the levels of ICA, IA-2A, or IAA, the higher was the risk for IDDM. In contrast, although the risk for IDDM was strongly associated with the presence of GADA, it was not related to the levels of these antibodies. Combined analysis of autoantibodies seems to provide a useful approach to screening for subjects at risk of developing IDDM, since most of the prediabetic siblings were identified on the basis of antibodies initially present in their sera, and all of them developed at least one antibody specificity later during the follow-up. The presence of multiple antibodies in the sera of siblings at or close to the diagnosis of the first affected child in the family is highly predictive of future IDDM development. Accurate assessment of the risk for IDDM in siblings is a complicated matter, however, as not all of those with even four antibody specificities contract the disease after a follow-up of more than 8 yr, whereas some of those with one or no antibodies initially will progress to IDDM.

Acknowledgments

We thank Else Jost Jensen, Susanna Heikkilä, Päivi Koramo, Riitta Päkkilä and Sirpa Anttila for their skillful technical assistance. The members of the Childhood Diabetes in Finland (DiMe) Study Group are as follows: principal investigators: H.K. Åkerblom and J. Tuomilehto; coordinators: R. Lounamaa and L. Toivanen; data management: E. Virtala and J. Pitkäniemi; local investigators: A. Fagerlund, M. Flittner, B. Gustafsson, C. Häggquist, A. Hakulinen, L. Herva, P. Hiltunen, T. Huhtamäki, N.-P. Huttunen, T. Huupponen, M. Hyttinen, T. Joki, R. Jokisalo, M.-L. Käär, S. Kallio, E.A. Kaprio, U. Kaski, M. Knip, L. Laine, J. Lappalainen, J. Mäenpää, A.-L. Mäkelä, K. Niemi, A. Niiranen, A. Nuuja, P. Ojajärvi, T. Otonkoski, K. Pihlajamäki, S. Pöntynen, J. Rajantie, J. Sankala, J. Schumacher, M. Sillanpää, M.-R. Ståhlberg, C.-H. Stråhlman, T. Uotila, M. Väre, P. Varimo, and G. Wetterstr; and special investigators: A. Aro, H. Hurme, M. Hiltunen, H. Hyöty, J. Ilonen, J. Karjalainen, M. Knip, P. Leinikki, A. Miettinen, T. Petäys, L. Räsänen, H. Reijonen, A. Reunanen, T. Saukkonen, E. Savilahti, E. Tuomilehto-Wolf, P. Vähäsalo, and S.M. Virtanen.

This study was supported by grants from the Juvenile Diabetes Foundation International (grant 188517), the Finnish Diabetes Research Foundation, the Medical Research Council, Academy of Finland (grant 26109), and the Alma and K.A. Snellman Foundation, Oulu, Finland. The Childhood Diabetes in Finland Study has in addition been supported by grants from the Association of Finnish Life Insurance Companies, the Sigrid Jusélius Foundation, the National Institutes of Health (grant DK 37957), the University of Helsinki, and the Novo Nordisk Foundation A/S, Denmark. J.S. Petersen was the recipient of a postdoctoral fellowship from the Juvenile Diabetes Foundation International.

References

1. Cooke, A. 1990. An overview on possible mechanisms of destruction of the insulin-producing beta cell. *Curr. Top. Microbiol. Immunol.* 164:125–142.

2. Castano, L.E., and G.S. Eisenbarth. 1990. Type 1 diabetes: a chronic autoimmune disease of man, mouse and rat. *Annu. Rev. Immunol.* 8:647–679.

3. Atkinson, M.A., and N.K. Maclaren. 1990. What causes diabetes? *Sci. Am.* 7:62–67.

4. Gepts, W. 1965. Pathology and anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes*. 14:619–633.

5. Gorsuch, A.N., K.M. Spencer, J. Lister, J.M. McNally, B.M. Dean, G.F. Bottazzo, and A.G. Cudworth. 1981. Evidence for a long prediabetic period in type 1 (insulin-dependent) diabetes mellitus. *Lancet*. 2:1363–1365.

6. Palmer, J.P. 1993. Predicting IDDM. Use of humoral immune markers. *Diabetes Reviews*. 1:104–115.

7. Bottazzo, G.F., A. Florin-Christensen, and D. Doniach. 1974. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet*. 2:1279–1282.

8. Lendrum, R., G. Walker, and D.R. Gamble. 1975. Islet-cell antibodies in juvenile diabetes mellitus of recent onset. *Lancet*. 1:880–883.

9. Bonifacio, E., P.J. Bingley, M. Shattock, BM. Dean, D. Dunger, E.A.M. Gale, and G.F. Bottazzo. 1990. Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. *Lancet*. 335:147–149.

10. Riley, W.J., N.K. Maclaren, J. Krischer, R.P. Spillar, J.H. Silverstein, D.A. Schatz, S. Schwartz, J. Malone, S. Shah, C. Vadheim, and J.I. Rotter. 1990. A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. *N. Engl. J. Med*. 323:1167–1172.

11. Palmer, J., P. Clemons, K. Lyen, O. Tatpati, P.K. Raghu, and T.L. Paquette. 1983. Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science*. 222:1337–1339.

12. Baekkeskov, S., J.H. Nielsen, B. Marner, T. Bilde, J. Ludvigsson, and Å. Lernmark. 1982. Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. *Nature*. 298:167–169.

13. Baekkeskov, S., H.-J. Aanstoot, S. Christgau, A. Reetz, M. Solimena, M. Cascalho, F. Folli, H. Richter-Olesen, and P. deCamilli. 1990. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature*. 347:151–156.

14. Baekkeskov, S., M. Landin, J.K. Kristensen, S. Srikanta, G.J. Bruining, T. Mandrup-Poulsen, C. deBeaufort, J.S. Soeldner, G. Eisenbarth, F. Lindgren, et al. 1987. Antibodies to a 64,000 M_r human islet cell antigen precede the clinical onset of insulin-dependent diabetes. *J. Clin. Invest*. 79:926–934.

15. Atkinson, M.A., N.K. Maclaren, D.W. Scharp, P.E. Lacy, and W.J. Riley. 1990. 64,000 M_r autoantibodies as predictors of insulin-dependent diabetes. *Lancet*. 335:1357–1360.

16. Christie, M., M. Landin-Olsson, G. Sundkvist, G. Dahlquist, Å. Lernmark, and S. Baekkeskov. 1988. Antibodies to a M_r 64,000 islet cell protein in Swedish children with newly diagnosed type 1 (insulin-dependent) diabetes. *Diabetologia*. 31:597–602.

17. Sabbah, E., P. Kulmala, R. Veijola, P. Vähäsalo, J. Karjalainen, E. Tuomilehto-Wolf, H.K. Åkerblom, M. Knip, and the Childhood Diabetes in Finland Study Group. 1996. GAD_{65} antibodies in relation to other antibodies and genetic risk markers in children with newly diagnosed IDDM. *J. Clin. Endocrinol. Metab*. 81:2455–2459.

18. Payton, M.A., C.J. Hawkes, and M.R. Christie. 1995. Relationship of the 37,000- and 40,000-M_r tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA512). *J. Clin. Invest*. 96:1506–1511.

19. Passini, N., J.D. Larigan, S. Genovese, E. Appella, F. Sinigaglia, and L.

Rogge. 1995. The 37/40-kilodalton autoantigen in insulin-dependent diabetes mellitus is the putative tyrosine phosphatase IA-2. *Proc. Natl. Acad. Sci. USA.* 92:9412–9416.

20. Bonifacio, E., V. Lampasona, S. Genovese, M. Ferrari, and E. Bosi. 1995. Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. *J. Immunol*. 155:5419–5426.

21. Bonifacio, E., S. Genovese, S. Braghi, E. Bazzigaluppi, V. Lampasona, P.J. Bingley, L. Rogge, M.R. Pastore, E. Bognetti, G.F. Bottazzo, E.M. Gale, and E. Bosi. 1995. Islet autoantibody markers in IDDM: risk assessment strategies yeilding high sensitivity. *Diabetologia*. 38:816–822.

22. Gorus, F.K., P. Goubert, C. Semakula, C.L. Vandewalle, J. DeSchepper, M.R. Christie, D.G. Pipeleers, and the Belgian Diabetes Registry. 1997. IA-2- autoantibodies complement GAD_{65}-autoantibodies in new-onset IDDM patients and help predict impending diabetes in their siblings. *Diabetologia*. 40:95–99.

23. Wiest-Ladenburger, U., R. Hartmann, U. Hartmann, K. Berling, B.O. Böhm, and W. Richter. 1997. Combined analysis and single-step detection of GAD65 and IA2 autoantibodies in IDDM can replace the histochemical islet cell antibody test. *Diabetes*. 46:565–571.

24. Dahlquist, G., I. Blom, G. Holmgren, B. Hagglof, Y. Larsson, G. Sterky, and S. Wall. 1985. The epidemiology of diabetes in Swedish children 0–14 years: a six-year prospective study. *Diabetologia*. 28:802–808.

25. Veijola, R., H. Reijonen, P. Vähäsalo, E. Sabbah, P. Kulmala, J. Ilonen, H.K. Åkerblom, M. Knip, and the Childhood Diabetes in Finland (DiMe) Study Group. 1996. HLA-DQB1 defined genetic susceptibility, beta-cell autoimmunity and metabolic characteristics in familial and non-familial insulin-dependent diabetes mellitus. *J. Clin. Invest*. 98:2489–2495.

26. Ziegler, A.G., R. Ziegler, P. Vardi, R.A. Jackson, J.S. Soeldner, and G.S. Eisenbarth. 1989. Life-table analysis of progression to diabetes of anti-insulin autoantibody positive relatives of individuals with type I diabetes. *Diabetes*. 38:1320–1325.

27. Karjalainen, J.K. 1990. Islet cell antibodies as predictive markers for IDDM in children with high background incidence of the disease. *Diabetes*. 39: 1144–1150.

28. Deschamps, I., C. Boitard, J. Hors, M. Busson, A. Marcelli-Barge, A. Mogenet, and J.-J. Robert. 1992. Life table analysis of the risk of type 1 (insulin-dependent) diabetes mellitus in siblings according to islet cell antibodies and HLA markers. An 8-year prospective study. *Diabetologia*. 35:951–957.

29. Thivolet, C.H., M. Tappaz, A. Durand, J. Petersen, A. Stefanutti, P. Chatelain, B. Vialettes, W. Scherbaum, and J. Orgiazzi. 1992. Glutamic acid decarboxylase (GAD) autoantibodies are additional predictive markers of type 1 (insulin-dependent) diabetes mellitus in high risk individuals. *Diabetologia*. 35: 570–576.

30. Krischer, J.P., D. Schatz, W.J. Riley, R.P. Spillar, J.H. Silverstein, S. Schwartz, J. Malone, S. Shah, C.M. Vadheim, J.I. Rotter, et al. 1993. Insulin and islet cell autoantibodies as time-dependent covariates in the development of insulin-dependent diabetes: a prospective study in relatives. *J. Clin.Endocrinol. Metab*. 77:743–749.

31. Schott, M., D. Schatz, M.A. Atkinson, J. Krischer, H. Mehta, B. Vold, and N. Maclaren. 1994. GAD65 autoantibodies increase the predictability but not the sensitivity of islet cell and insulin autoantibodies for developing insulin dependent diabetes mellitus. *J. Autoimmunity*. 7:865–872.

32. Schmidli, R.S., P.G. Colman, and L.C. Harrison. 1994. Do glutamic acid decarboxylase antibodies improve the prediction of IDDM in first-degree relatives at risk for IDDM? *J. Autoimmunity*. 7:873–879.

33. Tuomilehto, J., P. Zimmet, I.R. Mackay, P. Koskela, G. Vidgren, L. Toivanen, E. Tuomilehto-Wolf, K. Kohtamäki, J. Stengård, and M.J. Rowley. 1994. Antibodies to glutamic acid decarboxylase as predictors of insulin-dependent diabetes mellitus before clinical onset of disease. *Lancet*. 343:1383–1385.

34. Verge, C.F., R. Gianani, E. Kawasaki, L. Yu, M. Pietropaolo, R.A. Jackson, H.P. Chase, and G.S. Eisenbarth. 1996. Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes*. 45:926–933.

35. Seissler, J., N.G. Morgenthaler, P. Achenbach, E.F. Lampeter, D. Glawe, M. Payton, M. Christie, W.A. Scherbaum, and the DENIS Study Group. 1996. Combined screening for autoantibodies to IA-2 and antibodies to glutamic acid decarboxylase in first degree relatives of patients with IDDM. *Diabetologia*. 39:1351–1356.

36. Tuomilehto, J., R. Lounamaa, E. Tuomilehto-Wolf, A. Reunanen, E. Virtala, E.A. Kaprio, H.K. Åkerblom, and the Childhood Diabetes in Finland Study Group. 1992. Epidemiology of childhood diabetes in Finland: background of a nationwide study of type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*. 35:70–76.

37. WHO Study Group. 1985. Diabetes Mellitus. *Technical Report Series 727*. Geneva:10–12.

38. Greenbaum, C.J., J.P. Palmer, S. Nagataki, Y. Yamaguchi, J.L. Molenaar, W.A.M. VanBeers, N.K. Maclaren, Å. Lernmark, and participating laboratories. 1992. Improved specificity of ICA assays in the fourth international immunology of diabetes serum exchange workshop. *Diabetes*. 41:1570–1574.

39. Petersen, J.S., K.R. Hejnaes, A. Moody, A.E. Karlsen, M.O. Marshall, M. Hoier-Madsen, E. Boel, B.K. Michelsen, and T. Dyrberg. 1994. Detection of GAD_{65} antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. *Diabetes*. 43:459–467.

40. Schmidli, R.S., P.G. Colman, and E. Bonifacio. 1995. Disease sensitivity and specificity of 52 assays for glutamic acid decarboxylase antibodies. The second international GADAb workshop. *Diabetes*. 44:636–640.

41. Schuster, J. 1988. EXACTB and CONF: exact unconditional procedures for binominal data. *Am. Stat*. 42:234.

42. Kaplan, E., and P. Meier. 1958. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* 53:457–481.

43. Mantal, N. 1966. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother*. 50:163–170.

44. Bingley, P.J., M.R. Christie, E. Bonifacio, R. Bonfanti, M. Shattock, M.-T. Fonte, G.F. Bottazzo, and E.A.M. Gale. 1994. Combined analysis of autoantibodies improves prediction of IDDM in islet cell autoantibody-positive relatives. *Diabetes*. 43:1304–1310.

45. Roll, U., M.R. Christie, S. Eberhard, and A.-G. Ziegler. 1994. Associations of anti-GAD antibodies with islet cell antibodies in first-degree relatives of type 1 diabetic patients. *Diabetes*. 43:154–160.

46. Bärmeier, H., D.K. McCulloch, J.L. Neifing, G. Warnock, R.V. Rajotte, J.P. Palmer, and Å. Lernmark. 1991. Risk for developing type 1 (insulin-dependent) diabetes mellitus and the presence of islet 64K antibodies. *Diabetologia*. 34:727–733.

47. Christie, M.R., U. Roll, M.A. Payton, E.C.I. Hatfield, and A.-G. Ziegler. 1997. Validity of screening for individuals at risk for type I diabetes by combined analysis of antibodies to recombinant proteins. *Diabetes Care*. 20: 965–970.