Optimized Autoantibody-Based Risk Assessment in Family Members

Implications for future intervention trials

POLLY J. BINGLEY, MD, FRCP
ALISTAIR J.K. WILLIAMS, BSC
EDWIN A.M. GALE, FRCP

OBJECTIVE — To determine the best autoantibody-based testing strategy for recruiting relatives for future intervention trials and to establish the role of islet cell antibodies (ICAs) within this strategy.

RESEARCH DESIGN AND METHODS — ICAs, insulin autoantibodies (IAAs), GAD antibodies, and IA-2 antibodies were determined in serum samples at study entry of 3,655 nondiabetic first-degree relatives of patients with type 1 diabetes who were followed for a median of 5.5 years. The cumulative risk of diabetes associated with single and combined antibody marker levels of ≥97.5th percentile in schoolchildren was calculated by using life-table analysis.

RESULTS — Of the 26 relatives who developed insulin-requiring diabetes during follow-up, 16 were aged <20 years and 7 were aged 20–39 years at study entry. Of the 23 cases aged <40 years, 83% had IA-2 and/or GAD antibodies, and 87% had IAA and/or GAD antibodies ≥97.5th percentile compared with 61% who had ICAs of ≥5 Juvenile Diabetes Foundation units (JDF U). A two-step strategy with parallel testing for IA-2/GAD antibodies followed by IAA testing identified 50% of cases aged <20 years and was associated with a 71% risk within 10 years. In subjects aged 20–39 years, this strategy conferred a 51% risk, whereas using ICAs as the second test gave 86% sensitivity and a 74% risk. Primary screening for IA-2 and/or GAD antibodies followed by testing for IAA and/or ICA antibodies achieved the highest sensitivity in both age-groups and conferred a 63% risk. In contrast, ICAs of ≥20 JDF U (the inclusion criteria for the European Nicotinamide Diabetes Intervention Trial) gave 48% sensitivity and 35% risk.

CONCLUSIONS — ICA testing can be replaced as a primary screening measure by IA-2/GAD or IAA/GAD antibody testing. The sensitivity of ICAs (used alone or in combination with IAAs) gives them a useful role in second-line testing. Combination testing could reduce the size of screening populations needed for recruitment in future intervention trials by ~50% compared with testing based on ICAs alone.

Diabetes Care 22:1796–1801, 1999

Islet cell antibodies (ICAs) have until recently formed the basis of risk assessment in relatives of children with type 1 diabetes and hence recruitment into intervention trials such as European Nicotinamide Diabetes Intervention Trial (ENDIT) and Diabetes Prevention Trial 1 (1,2). Because more than 100,000 first-degree relatives will be tested in the course of those two studies, screening represents the major expense of any large intervention trial. Several studies have recently suggested that ICAs could be replaced by testing for antibodies to GAD65 and the protein tyrosine phosphatase IA-2, which contribute the major part of ICA immunofluorescent staining (3–6). Simple radiobinding assays are available for these markers (7–9), whereas the ICA assay is labor intensive and operator dependent. A recently developed microassay for insulin autoantibodies (IAAs) has further extended the scope of high-throughput testing (10). Testing strategies that use combinations of antigen-specific markers should improve the efficiency of screening procedures by increasing the proportion of future cases identified and by identifying groups at higher risk. This would reduce the size of the screening population required for future intervention trials, which could save money and manpower and limit the number of those exposed to the potential risks of new therapy.

The aim of this study was to determine the most effective strategy for antibody-based recruitment of family members into intervention trials and to establish whether ICAs have a role in this context. Because the distribution of autoantibody markers at clinical diagnosis of diabetes varies with age (11–13), we also considered whether different testing strategies are needed for children and adults.

RESEARCH DESIGN AND METHODS

Subjects

The Bart's-Oxford prospective population-based family study has recruited parents and siblings of patients with type 1 diabetes diagnosed before age 21 years from within the Oxford Regional Health Authority area in England since 1985 (14). By 31 October 1996, 1,168 families had been recruited, and 3,655 eligible nondiabetic first-degree relatives had been identified. Serum samples were available from 3,197 of these relatives (1,994 parents and 1,203 siblings). The remaining family members were considered too young, did not consent to venipuncture, or were no longer living with the family. The median age of these relatives was 33.4 years (range 0.25–87.4). Of the relatives, 1,513 were male and 1,684 were

From Diabetes and Metabolism, Division of Medicine, University of Bristol, U.K.
Address correspondence and reprint requests to Dr. PJ. Bingley, Diabetes and Metabolism, Medical School Unit, Southmead Hospital, Bristol BS10 5NB, U.K. E-mail: polly.bingley@bristol.ac.uk.
Received for publication 22 April 1999 and accepted in revised form 8 July 1999.
Abbreviations: CV, coefficient of variation; ENDIT, European Nicotinamide Diabetes Intervention Trial; IAA, insulin autoantibody; ICA, islet cell antibody; JDF U, Juvenile Diabetes Foundation units.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

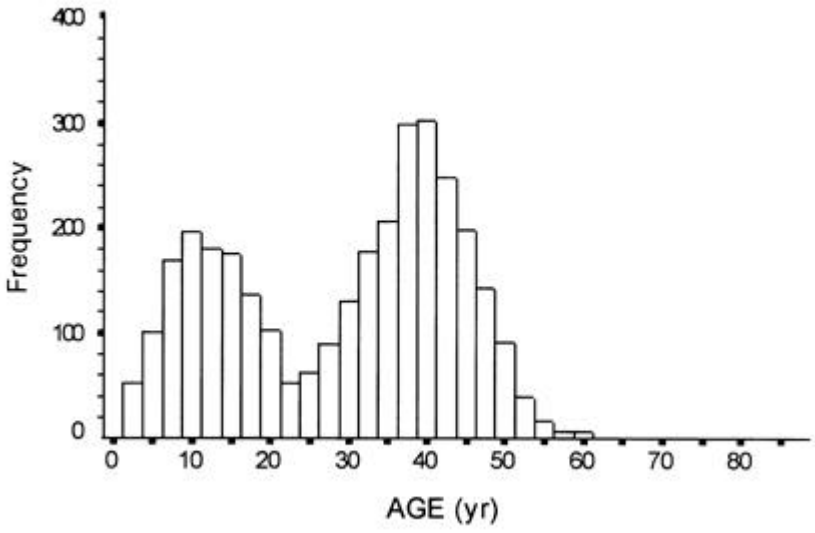

Figure 1—*Age of family members at the time of collection of the initial sample.*

female. The age distribution of the relatives is shown in Fig. 1.

Families were followed by annual visits or telephone calls, and the diabetes status of every family member was ascertained on each occasion. During follow-up, 26 family members developed diabetes requiring insulin treatment within 12 months of diagnosis, and 14 developed diabetes controlled by diet and/or oral hypoglycemic agents for at least 12 months. The latter subjects were classified as having type 2 diabetes and were excluded from the risk analyses.

Methods

ICA, IAA, GAD antibodies, and IA-2 antibodies were measured in the entry samples of all participants.

Assays

ICAs. ICAs were measured in undiluted sera by indirect immunofluorescence as previously described (15). End-point titers of test samples were converted to Juvenile Diabetes Foundation units (JDF U) by comparing them with a standard curve of $\log_2$ JDF U versus $\log_2$ of the end-point titer of the standard sera. The threshold of detection was 4 JDF U. The interassay coefficient of variation (CV) was 10% at 13 JDF U and 4.3% at 80 JDF U. The assay achieved 78.4% sensitivity with 98% specificity in the First IDS Combined Antibody Workshop (16).

IAAs. Antibodies to ^{125}I-labeled insulin were measured as previously described, using a format similar to that used to measure GAD and IA-2 antibodies (10). Immune complexes were isolated with protein A sepharose (Pharmacia Biotech AB, Uppsala, Sweden). Bound counts for each sample were calculated after subtraction of background counts, and the results were expressed in arbitrary units derived from a standard curve. The curve was constructed from nine doubling dilutions of serum from a patient with longstanding type 1 diabetes in normal human serum that ranged from 0.39 to 100 U. Sera with insulin binding >0.4 U were tested in a

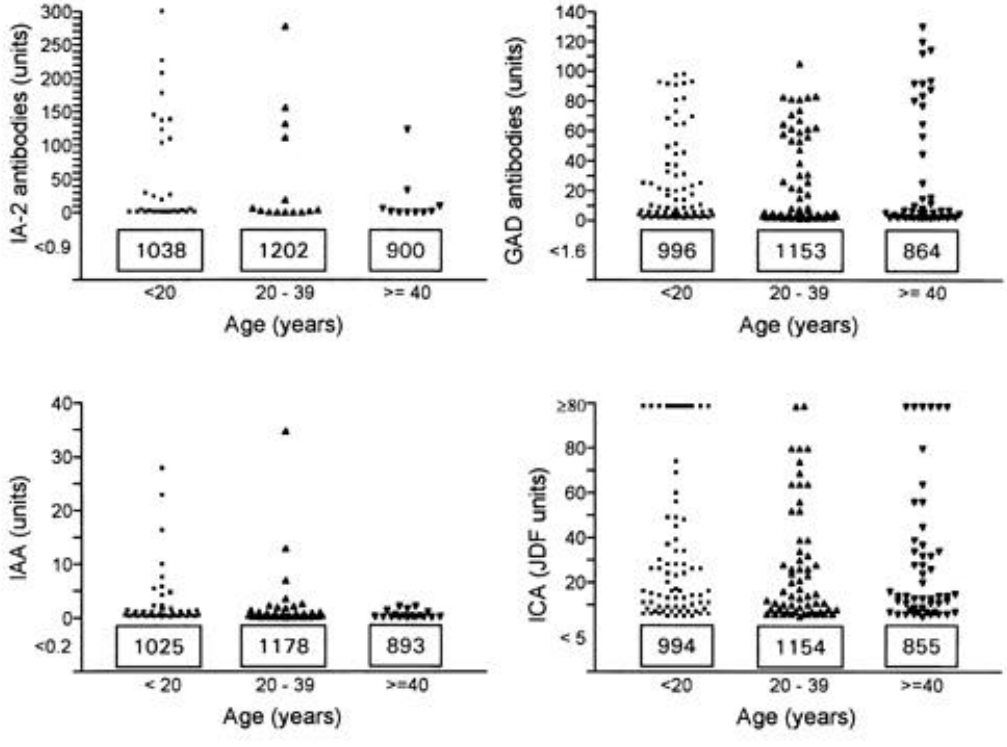

Figure 2—*The distribution of autoantibody levels in family members subdivided by age. The numbers in boxes indicate the number of family members with levels <97.5th percentile of 2,860 schoolchildren.*

Table 1—*Characteristics of family members who developed insulin-requiring diabetes and prevalence of antibody markers ≥97.5th percentile*

	Age-group (years)		
	<20	20–39	≥40
n	16	7	3
M:F	11:5	3:4	2:1
GAD antibodies	11	7	3
IA-2 antibodies	5	3	1
IAAs	9	2	0
ICAs	8	6	3
No markers	3	0	0
One marker	2	1	0
Two markers	5	2	2
Three markers	3	3	1
Four markers	3	1	0

Data are *n*.

competition assay in which further duplicate wells of each sample were incubated with 15,000 cpm of ^{125}I-insulin diluted in a buffer solution containing unlabeled human insulin (Humulin; Lilly, Basingstoke, U.K.). Specific bound counts were calculated for each sample by subtracting the counts of the tubes with excess unlabeled insulin from those with labeled insulin alone and were converted into arbitrary units as described above, including an additional standard at 0.2 U. The interassay CV of the screening assay was 17% at 0.5 U and 13% at 1.4 U, and the interassay CV of the competition assay was 31% at 0.6 U and 16% at 1.4 U. The assay achieved 58% sensitivity with 99% specificity in the samples included in the First IDS Combined Antibody Workshop (16).

GAD and IA-2 antibodies. Antibodies to in vitro translated [^{35}S]GAD65 and [^{35}S]protein tyrosine phosphatase IA-2$_{ic}$ were measured by immunoassay as previously described (12). Immune complexes were isolated on protein A sepharose. After washing, bound counts per minute were expressed as arbitrary units derived from a standard curve. The curve was constructed by using a positive serum with an arbitrary value of 100 U to test each assay undiluted and then diluted by 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, and 1/128. Values >100 U were beyond the steepest gradient of the standard curve and were reported as >100 U. The interassay CV of the GAD antibody assay was 17% for samples with 1.5 U and 9% for samples with 17 U of antibody. The interassay CV of the IA-2 antibody assay was 15% for samples with 1 U and 21% for samples with 9 U of antibody. The GAD antibody assay achieved 91% sensitivity with 99% specificity, and the IA-2 antibody assay achieved 74.4% sensitivity with 99% specificity in the First IDS Combined Antibody Workshop (16).

Statistical analysis

Life table analysis was used to estimate the cumulative risk of developing type 1 diabetes (17). The follow-up period for each individual was calculated from the date of the first sample, and the end of follow-up was defined as date of last contact, date of diagnosis, or date of entry into an intervention trial. Diabetes was defined according to World Health Organization criteria (18). Antibody prevalences were expressed in relation to percentiles derived from a population of 2,860 schoolchildren in the Oxford region (12). The 97.5th percentile corresponded to 5 JDF U for ICAs, 0.2 U for IAAs, 1.6 U for GAD antibodies, and 0.9 U for IA-2 antibodies. Distributions of autoantibody levels in family members subdivided into three age-groups (<20 years, 20–39 years, and ≥40 years) were compared by using the Kruskal-Wallis *H* test. *P* values were corrected for the number of antibodies compared ($n = 4$).

RESULTS

Distribution of autoantibodies

The distributions of ICAs, IAAs, GAD antibodies, and IA-2 antibodies in the three age-groups are shown in Fig. 2. IAAs, GAD antibodies, and IA-2 antibody levels were higher in the family members aged <20 years ($P < 0.0001$). ICA levels did not differ significantly ($P = 0.07$). Levels of ICAs, GAD antibodies, and IA-2 antibodies were significantly higher than in the schoolchildren in all age-groups ($P < 0.01$), but IAA levels were only higher in family members aged <20 years ($P < 0.0001$). The prevalence of antibody levels ≥97.5th percentile of schoolchildren in family members aged <20 years was 7% for ICAs, 4% for IAAs, 6.6% for GAD antibodies, and 4% for IA-2 antibodies. In family members aged 20–39 years, the prevalence of antibody levels ≥97.5th percentile was 4.9% for ICAs, 3% for IAAs, 5.2% for GAD antibodies, and 1.1% for IA-2 antibodies. In subjects aged ≥40 years, the prevalence was 5.3% for ICAs, 2% for IAAs, 5% for GAD antibodies, and 1.1% for IA-2 antibodies.

Progression to diabetes

After a median follow-up period of 3 years (range 0.6–11.3), 26 family members developed insulin-requiring diabetes. Their median age at study entry was 14.1 years (1.6–42).

Sensitivity of antibody markers

Of 16 family members aged <20 years who developed diabetes, 11 (69%) had GAD antibodies, 5 (31%) had IA-2 antibodies, 9 (56%) had IAAs, and 8 (50%) had ICAs ≥97.5th percentile of the schoolchildren. Of the seven family members aged 20–39 years, all had GAD antibodies, three (43%) had IA-2 antibodies, two (29%) had IAAs, and six (86%) had ICAs above this threshold. Of the three

Table 2—*Cumulative risk of diabetes within 10 years in family members aged <20 years and aged 20–39 years using ICA, IAA, GAD, and IA-2 antibody thresholds equivalent to the 97.5th and 99th percentile of schoolchildren*

	≥97.5th percentile		≥99th percentile	
	Cases identified (*n*)	Risk	Cases identified (*n*)	Risk
Aged <20 years				
IAAs	9	35 (14–55)	4	37 (12–62)
GAD antibodies	11	27 (12–43)	11	33 (16–50)
IA-2 antibodies	5	25 (5–45)	4	33 (5–62)
ICAs	8	17 (6–20)	7	28 (9–46)
Aged 20–39 years				
IAAs	2	8 (0–20)	0	—
GAD antibodies	7	16 (3–29)	7	26 (8–44)
IA-2 antibodies	3	28 (0–58)	3	42 (3–81)
ICAs	6	21 (4–38)	4	25 (2–47)

Data are % (95% CIs) or *n*.

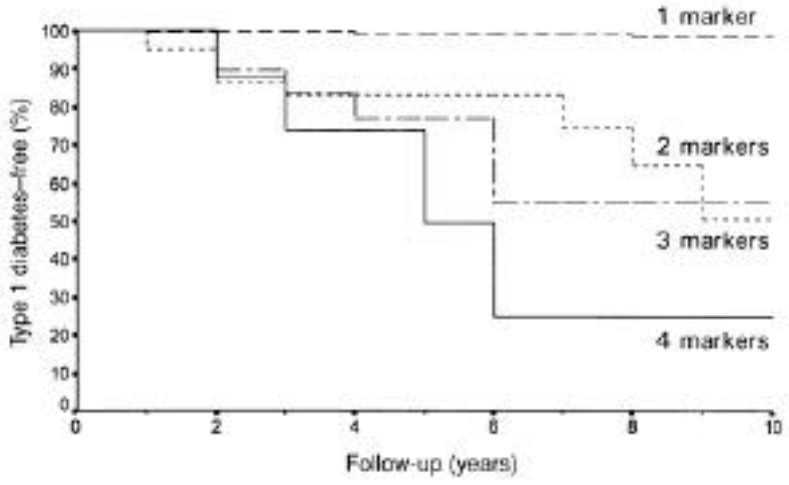

Figure 3—*The cumulative risk of diabetes in family members according to the number of autoantibody markers ≥97.5th percentile.*

family members aged ≥40 years, all had GAD antibodies and ICAs, one (33%) had IA-2 antibodies, and none had IAAs ≥97.5th percentile. The number of antibody markers ≥97.5th percentile in each individual according to age is summarized in Table 1. Overall, 15% had four markers, 27% had three markers, 35% had two markers, 8% had a single marker, and 12% had no markers ≥97.5th percentile at entry. The three individuals with no markers at entry were aged 1.6, 2.2, and 7.8 years at the time of sampling. A total of 22 out of 26 (85%) family members who developed diabetes had IA-2 and/or GAD antibodies ≥97.5th percentile, and 23 (88%) had IAAs and/or GAD antibodies ≥97.5th percentile.

Cumulative risks

The cumulative risks of developing diabetes within 10 years associated with each of the four antibody markers in family members <20 years and 20–39 years by using thresholds set at the 97.5th and 99th percentiles are shown in Table 2. Only three family members aged ≥40 years developed diabetes, and in this age-group, the 10-year risk associated with antibodies ≥97.5th percentile was 9.8% (95% CI 0–24) for GAD antibodies and 9.9% (0–25) for ICAs. None of the 18 individuals aged ≥40 years with IAAs ≥97.5th percentile nor of the 10 individuals with IA-2 antibodies ≥97.5th percentile developed diabetes within 10 years.

Figure 3 shows the cumulative risk of developing diabetes according to the number of markers with levels ≥97.5th percentile. Individuals with two or more markers above this level had a 50% (31–70) overall risk of developing diabetes within 10 years.

Potential testing strategies

Table 3 shows the proportion of cases detected using either low-threshold ICA screening, IA-2/GAD antibody measurement, or IAA/GAD antibody measurement as the first-line test, followed by ICA, IAA, or IA-2 antibody measurement as the second-line test in family members subdivided according to age. Primary screening with low-threshold ICA testing (≥5 JDF U) identified 14 of 23 cases (61%), whereas primary screening with IA-2/GAD antibodies or IAA/GAD antibodies identified 19 (83%) and 20 cases (87%), respectively. Applying the higher ICA threshold used in the ENDIT (≥20 JDF U) in subjects who were positive on initial testing reduced the overall sensitivity to 48%, whereas the addition of ICA or IAA testing in family members who were positive on the IA-2/GAD antibody or IAA/GAD antibody primary screening reduced sensitivity to between 51 and 62%. All second-line testing strategies identified subgroups at higher risk, but there were no significant differences between the risks associated with the strategies.

Table 3—*Performance of alternative strategies for primary and secondary testing in family members aged <40 years*

	Aged <20 years		Aged 20–39 years	
	Cases identified	Cumulative risk of diabetes within 10 years	Cases identified	Cumulative risk of diabetes within 10 years
Overall	16	2.5 (1–4)	7	0.9 (0–1.4)
Primary testing				
ICA ≥5 JDF U	8 (50)	17 (6–28)	6 (86)	21 (4–38)
IA-2 and/or GAD antibodies	12 (75)	24 (11–27)	7 (100)	15 (4–26)
IAA and/or GAD antibodies	13 (81)	23 (11–35)	7 (100)	12 (3–21)
Secondary testing				
ICA ≥20 JDF U	7 (44)	38 (13–63)	4 (57)	27 (2–52)
IA-2 and/or GAD antibodies plus ICA	7 (44)	61 (29–93)	6 (86)	74 (34–100)*
IA-2 and/or GAD antibodies plus IAA	8 (50)	71 (39–100)	4 (57)	51 (8–94)
IAA and/or GAD antibodies plus ICA	8 (50)	62 (31–93)	6 (86)	36 (7–65)†
IAA and/or GAD antibodies plus IA-2 antibodies	5 (31)	46 (14–78)	3 (43)	64 (12–100)
IA-2 and/or GAD antibodies plus ICA ≥5 JDF U and/or IAA	9 (56)	57 (27–86)	6 (86)	74 (33–100)*

Data are % (95% CIs) or *n* (%). *8-Year cumulative risk; †5-year cumulative risk.

CONCLUSIONS — This study confirms the advantages of screening for diabetes risk by using combinations of autoantibody markers. Combining markers identified higher levels of risk and achieved higher sensitivity than any single marker. A total of 58 family members aged <40 years, including 74% of those who progressed to diabetes, had levels of at least two of the four antibody markers at ≥97.5th percentile, and this conferred a 58% cumulative risk of developing diabetes within 10 years. Testing all samples for all four antibodies however would be expensive and laborious, and equivalent performance can be achieved by using several strategies that involve two or more steps.

The first-line test should be simple, inexpensive, and adapted for high throughput. It should also be as sensitive as possible, and we have therefore used a threshold equivalent to the 97.5th percentile in schoolchildren rather than the more commonly used 99th percentile (12). GAD and IA-2 antibodies in combination can match the sensitivity of ICAs (3) and can be measured together in a single test (4,5,8). In our population, simultaneous testing for these two markers would have identified more cases than low-threshold ICA testing. However, this effect is not specific for the combination of GAD and IA-2 antibodies because we found that the combination of IAAs and GAD antibodies is equally sensitive for primary screening (although currently more demanding and costly than combined GAD and IA-2 antibody testing). Either of these primary screening strategies would have identified >85% of future cases of diabetes in this population compared with 61% identified by screening for low levels of ICAs.

The use of a second test in samples that were positive in the first screening step can identify a subgroup at higher risk but is inevitably associated with some decrease in sensitivity. This limitation is intrinsic to any screening procedure and would not be overcome by any of the strategies we applied. In our study, the use of ICAs as the second-line test after GAD/IA-2 antibody screening reduced sensitivity from 100 to 86% in family members aged ≥20 years, whereas IAA testing reduced sensitivity to 57%. To minimize the loss of sensitivity in a two-step procedure, the second test should be as sensitive as possible. For example, first-line testing with IAA/GAD antibodies followed by second-line testing for IA-2 antibodies (which have an overall sensitivity of <35%) would result in a high number of future cases being missed. In contrast, initial screening for IA-2/GAD antibodies followed by combined testing for ICAs and/or IAAs (which have a combined sensitivity of 70%) would identify 65% of future cases compared with the 48% identified with ICA criteria as used to define eligibility in the ENDIT (≥20 JDF U). The ENDIT group needed to screen more than 50,000 relatives to identify 552 individuals with ICAs ≥20 JDF U who fulfilled other criteria for eligibility and were willing to participate in the trial. Adjusting for differences in both sensitivity and risk, the alternative strategies described in this article could have potentially reduced the size of the screening population required to less than 20,000 relatives, which would allow at least two major trials to be conducted with the same screening effort.

The sensitivity of individual markers is affected by age. For example, IAAs ≥97.5th percentile were detected in the initial sample from 56% of the future cases in the youngest age-group but in none of those aged ≥40 years. However, GAD antibodies exceeded this threshold in all subjects aged ≥20 years and in ~70% of the younger group. These differences can be overcome at either the primary or secondary level of screening. One option is to use IA-2 and GAD antibody testing (which can be considered relatively age neutral) in the first line of testing followed by second-line testing with IAAs in younger family members and ICAs in older family members. Alternatively, IAAs/GAD antibodies could be used as the primary test for future subjects of all ages, followed by age-neutral ICA testing.

Comparison of testing strategies in this and all other studies is limited by a lack of statistical power. For example, we observed that ICAs ≥20 JDF U were associated with a 35% cumulative risk within 10 years versus a 65% risk with IA-2/GAD antibody screening followed by testing for ICAs and/or IAAs. The CIs for these estimates are wide, and sample size calculations indicate that a study population at least 12 times larger (i.e., 36,000 family members) would be required to provide adequate power to determine whether one strategy was associated with higher cumulative risk. These limitations must be considered in trial design, and the results of subgroup analyses should be viewed with even greater caution.

In conclusion, ICAs can be replaced as a primary screening measure by either GAD/IA-2 antibody or IAAs/GAD antibody testing. This approach offers considerably improved screening efficiency for future intervention trials. Whichever strategy is used, ICAs remain useful as a second-line testing procedure, mainly because of their unsurpassed sensitivity. Autoantibody profiles vary in different age-groups at diagnosis, and first-line testing with IA-2/GAD antibodies could logically be followed by IAA screening in subjects aged <20 years and by ICA screening in older age-groups. Alternatively, both IAAs and ICAs could be used in all age-groups. Improved strategies for disease prediction will play an important role in reducing the cost and increasing the efficiency of future intervention trials.

Acknowledgments — The Bart's-Oxford study is supported by the British Diabetes Association. P.J.B. is funded by the Juvenile Diabetes Foundation.

We thank the physicians and pediatricians in the Oxford region as well as the families taking part in the study for their continuing support. We also thank the project administrators, Hilary Gillmor and Suzanne Weeks, and the Bart's-Oxford study fieldworkers for their help: Kathryn Darvill, Louise Gorrod, Denise Morgans, Pam Sawtell, Rose Streeton, and Sallie Wall. We are grateful to Prof. Franco Bottazzo for invaluable help and support on this project and to Marion Shattock and Tracey Collins for technical assistance.

References

1. Gale EAM: Theory and practice of nicotinamide trials in pre-type 1 diabetes. *J Pediatr Endocrinol Metab* 9:375–379, 1996
2. Skyler JS, Marks JB: Immune intervention in type 1 diabetes mellitus. *Diabetes Rev* 1: 15–42, 1993
3. Bonifacio E, Genovese S, Braghi S, Bazzigaluppi E, Lampasona V, Bingley PJ, Rogge L, Pastore MR, Bognetti E, Bottazzo GF, Gale EAM: Islet autoantibody markers in IDDM: risk assessment strategies yielding high sensitivity. *Diabetologia* 38:816–822, 1995
4. Seissler J, Morgenthaler NG, Achenbach P, Lampeter EF, Glawe D, Payton MA, Christie MR, Scherbaum WA, for the DENIS Study Group: 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, 1996
5. Dittler J, Seidel D, Schenker M, Ziegler AG: GADIA2-combi determination as first-line screening for improved prediction of type 1 diabetes in relatives. *Diabetes* 47:592–597, 1998
6. Myers MA, Rabin DU, Rowley MJ: Pancreatic islet cell cytoplasmic antibody in dia-

betes is represented by antibodies to islet cell antigen 512 and glutamic acid decarboxylase. *Diabetes* 44:1290–1295, 1995
7. Petersen JS, Hejnaes KR, Moody A, Karlsen AE, Marshall MO, Hoier-Madsen M, Boel E, Michelsen BK, Dyrberg T: Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. *Diabetes* 43:459–467, 1994
8. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E: 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, 1995
9. Hawkes CJ, Wasmeier C, Christie MR, Hutton JC: Identification of the 37-kDa antigen in IDDM as a tyrosine phosphatase-like protein (Phogrin) related to IA-2. *Diabetes* 45:1187–1192, 1996
10. Williams AJK, Bingley PJ, Bonifacio E, Palmer JP, Gale EAM: A novel micro-assay for insulin autoantibodies. *J Autoimmun* 10:473–478, 1997
11. Vardi P, Ziegler AG, Mathews JH, Dib S, Keller RJ, Ricker AT, Wolfsdorf JI, Herskowitz RD, Rabizadeh A, Eisenbarth GS, Soeldner JS: Concentration of insulin autoantibodies at onset of type 1 diabetes: inverse log-linear correlation with age. *Diabetes Care* 11:736–739, 1988
12. Bingley PJ, Bonifacio E, Williams AJK, Genovese S, Bottazzo GF, Gale EAM: Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. *Diabetes* 46:1701–1710, 1997
13. Vandewalle CL, Coeckelberghs MI, De Leeuw IH, Du Caju MV, Schuit FC, Pipeleers DG, Gorus FK, for the Belgian Diabetes Registry: Epidemiology, clinical aspects, and biology of IDDM patients under age 40 years. *Diabetes Care* 20:1556–1561, 1997
14. Bingley PJ, Gale EAM: The incidence of insulin-dependent diabetes in England: a study in the Oxford region 1985–6. *Br Med J* 298:558–560, 1989
15. Bingley PJ, Bonifacio E, Shattock M, Gillmor HA, Sawtell PA, Dunger DB, Scott RDM, Bottazzo GF, Gale EAM: Can islet cell antibodies predict IDDM in the general population? *Diabetes Care* 16:45–50, 1993
16. Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, Eisenbarth GS, participating laboratories: Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. *Diabetes* 47:1857–1866, 1998
17. Bland JM: *An Introduction to Medical Statistics*. Oxford, U.K., Oxford University Press, 1995
18. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)