Diabetes Antibody Standardization Program

First evaluation of assays for autoantibodies to IA-2β

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OBJECTIVE—Autoantibodies to IA-2β (IA-2βA) are important risk markers of type 1 diabetes. We report the first Diabetes Antibody Standardization Program (DASP) evaluation of IA-2βA assays.

RESEARCH DESIGN AND METHODS—Thirteen laboratories from nine countries received coded sera from 50 patients with newly diagnosed type 1 diabetes and 100 healthy blood donors. IA-2βA results were analyzed using receiver operating characteristic (ROC) curves. Concordance of antibody levels was compared using counts per minute (cpm), local and standard curve–derived common units.

RESULTS—Median laboratory-assigned sensitivity was 47% (interquartile range [IQR] 45–51), specificity 98% (IQR 95–99), adjusted sensitivity at 95% specificity 50% (IQR 49–53), and area under the ROC curve 0.70 (IQR 0.69–0.73). Use of common IA-2βA units improved concordance between assays compared with local units and cpm ($P < 0.0001$).

CONCLUSIONS—IA-2βA assays in multiple laboratories worldwide achieved good concordance and high specificity for type 1 diabetes. IA-2βA are suitable for inclusion in autoantibody testing for risk assessment in prediabetes.

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The Diabetes Antibody Standardization Program (DASP) was established to assess proficiency and harmonize the measurement of islet autoantibodies in laboratories throughout the world, as well as evaluate novel antibody assay developments (1–4). It has been shown that antibodies to islet antigen 2 (IA-2A) are associated with a high risk of progression to type 1 diabetes (5–8), and detection of additional antibodies binding to the homolog protein IA-2β identifies a subgroup of individuals at particular risk of rapid disease development (9–11). Although highly predictive, autoantibodies to IA-2β (IA-2βA) are, however, less widely used than other islet autoantibodies. To assess the sensitivity, specificity, and concordance of IA-2βA assays in a broader range of laboratories, these markers were included for the first time in the 2007 DASP proficiency evaluation.

RESEARCH DESIGN AND METHODS

Study design

The evaluation included 13 participating laboratories in nine countries (listed in Supplementary Appendix A). Each received uniquely coded sets of frozen 100-μL aliquots of sera from 50 patients with newly diagnosed type 1 diabetes and 100 healthy control subjects. Of the 50 patients, 1 was subsequently found to have long-standing, insulin-treated diabetes and was therefore excluded from the analysis. The laboratories also received nine serial dilutions of serum from an IA-2βA–positive patient with newly diagnosed type 1 diabetes (IDS005) and an IA-2βA–negative serum. In 12 laboratories, these standards were included in each assay. All subjects gave informed consent, and the investigations were carried out in accordance with the Declaration of Helsinki as revised in 2000.

An IA-2β clone provided by V. Lampasona (Center for Translational Genomics and Bioinformatics, San Raffaele Scientific Institute, Milan, Italy; aa 644–1015, cloned from human pancreatic islet cDNA) was used in 11 laboratories. One laboratory used an IA-2β construct cloned by J. Hutton (Barbara Davies Center, University of Colorado, Denver, CO; aa 640–1015), and another used a construct cloned by W.A. Hagopian (University of Washington, Seattle, WA; aa 633–1004). All laboratories performed radio-binding assays with in vitro transcription/translation of ^{35}S-methionine–labeled antigen (Promega Corporation, Madison, WI).

Laboratories were asked to test the sera using their usual method and to provide details of the local assay. All laboratories reported results for each serum as raw counts per minute (cpm). Local units calculated according to the laboratory's own protocol were reported by 10 laboratories, and 11 laboratories designated sera as IA-2βA positive or negative using the local cutoff.

Data analysis

Laboratory-assigned sensitivity and specificity were determined for IA-2βA positivity based on the local cutoff. Receiver operating characteristic (ROC) curves were generated, and area under the curve (AUC) was calculated for each assay to evaluate discrimination between patients and healthy control subjects. Adjusted

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*A complete list of participating laboratories can be found in Supplementary Appendix A online.

Table 1—*Results for IA-2β autoantibody assays*

Laboratory no.	Laboratory-assigned sensitivity (%)	Laboratory-assigned specificity (%)	AUC	95% CI	*P* value	AS 95 (%)	Results reported	IA-2β clone
109	ND	ND	0.77	0.69–0.87	<0.0001	63	local units	Milan
115	39	98	0.72	0.62–0.82	<0.0001	53	cpm	Milan
116	47	99	0.70	0.60–0.80	<0.0001	53	local units	Milan
118	ND	ND	0.68	0.57–0.79	<0.0001	53	cpm	Milan
120	53	96	0.73	0.63–0.82	<0.0001	55	local units	Milan
121	47	99	0.69	0.58–0.79	<0.0001	49	local units	Milan
126	51	98	0.67	0.55–0.78	0.001	49	local units	Seattle
132	43	99	0.74	0.65–0.83	<0.0001	47	local units	Milan
133	47	99	0.69	0.59–0.80	<0.0001	53	local units	Denver
150	53	94	0.75	0.66–0.84	<0.0001	53	cpm	Milan
153	50	98	0.74	0.65–0.84	<0.0001	52	local units	Milan
213	47	92	0.71	0.61–0.81	<0.0001	47	local units	Milan
504	37	92	0.65	0.55–0.75	0.004	20	local units	Milan

ND, not designated.

sensitivity 95, i.e., sensitivity at 95% specificity, was derived from the ROC curve coordinates.

Reported cpm was adjusted for variation in nonspecific binding by subtraction of the cpm of the IA-2βA–negative standard. Common IA-2βA units were derived from a logarithmic standard curve constructed from nine serial dilutions of the IA-2βA–positive standard serum ranging from 130.5 to 0.5 units. For quantitative analysis, values >130.5 or <0.5 IA-2βA units were replaced with 131 and 0.5 IA-2βA units, respectively. A combined ROC curve was compiled from the median values for each patient and control sample from all assay measurements expressed as IA-2βA units.

Concordance between IA-2βA assays was assessed by linear regression of the sample rank in individual assays against the median rank of samples based on adjusted cpm, local units, and common IA-2βA units. Variances of regressions were compared by *F* test.

The association between IA-2βA units and IA-2A levels was analyzed using nonparametric Spearman correlation. For all statistical analyses, performed with SPSS 15.0, two-tailed *P* values < 0.05 were considered significant.

RESULTS—A summary of the results of each IA-2βA assay is given in Table 1. The median laboratory-assigned sensitivity based on local cutoff was 47% (interquartile range [IQR] 45–51%) and the median laboratory-assigned specificity was 98% (IQR 95–99%). The median AUC was 0.70 (IQR 0.69–0.73, $P < 0.0001$) and median adjusted sensitivity 95 was 50% (IQR 49–53%). The AUC of the combined ROC curve derived from median IA-2βA units for each sample was 0.74 (95% CI 0.64–0.84, $P < 0.0001$). A threshold of 1.82 common IA-2βA units gave 53% sensitivity with 98% specificity (Supplementary Fig. 1).

Samples from 22 patients and 1 control individual were reported positive in ≥75% of assays. Two additional patient samples were reported positive in ≥50% of assays, and three other patient samples and one control sample were positive in ≥25% of assays (Supplementary Fig. 2*A* and *B*). For 126 samples there was agreement on positive/negative status in ≥75% of assays.

There was significant agreement in ranking of patient samples by cpm ($r^2 = 0.23$, $P < 0.0001$) and local IA-2βA units ($r^2 = 0.37$, $P < 0.0001$), but there were large variations between assays (data not shown). Use of common IA-2βA units improved concordance compared with both cpm and local IA-2βA units ($r^2 = 0.75$, $P < 0.0001$; *F* test, $P < 0.0001$; Supplementary Fig. 3).

Common IA-2βA units were closely correlated with the local IA-2A units ($r_S = 0.691$, 95% CI 0.643–0.734, $P < 0.0001$). In the 11 laboratories that provided positive/negative designations for both IA-2βA and IA-2A, a median of 22 patient samples (IQR 20–24) were positive for IA-2A and IA-2βA, 11 patient samples (IQR 8–14) were IA-2A positive but IA-2βA negative, whereas a single patient sample was IA-2βA positive but IA-2A negative in two laboratories.

CONCLUSIONS—The first DASP proficiency testing for autoantibodies against IA-2β confirmed a strong association between IA-2βA and type 1 diabetes. Participating laboratories, including some with limited or no previous experience in using the assay, were able to achieve a comparable sensitivity at high specificity using IA-2βA radio-binding assays, as well as good concordance in reporting results. The introduction of common IA-2βA units significantly improved the concordance between laboratories, in spite of the use of different assay protocols or antigens. In conclusion, IA-2βA assays performed in multiple laboratories across the world reveal a high specificity for type 1 diabetes, suggesting that, with appropriate assurance of assay reproducibility, these markers are suitable for inclusion in autoantibody testing to assess risk of type 1 diabetes.

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