Islet Cell Antibodies and Glutamic Acid Decarboxylase Antibodies, But Not the Clinical Phenotype, Help to Identify Type 1½ Diabetes in Patients Presenting With Type 2 Diabetes

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This study was undertaken to determine which type 1 diabetes-associated autoantibodies and what clinical characteristics are most useful to identify patients with type 1½ diabetes. We studied 125 patients, recently diagnosed clinically with type 2 diabetes for the presence of islet cell antibodies (ICA), insulin autoantibodies (IAA), antibodies to glutamic acid decarboxylase(GADAb), and IA-2a (IA-2Ab). Patients with a diagnosis of type 2 diabetes who met all of the following criteria at diagnosis were studied: age ≥ 30 years, no history of ketonuria or ketoacidosis, and not requiring insulin treatment. Thirty-six patients (29%) were positive for at least 1 antibody. Thirty-two (26%) were ICA positive and 20 (16%) GADAb positive. Insulin autoantibodies and IA-2Ab occurred less frequently in 2 (1.6%) and 8 (6.4%) patients, respectively. There was no significant difference in the ages at diagnosis between the Ab(+) and Ab(−) patients, age in years (range) 47.2 (32 to 64) versus 51.2 (31 to 77), respectively, $P = .06$. Body mass index (BMI) was different in the 2 groups, with Ab(+) patients being less obese, BMI (range) 28.3 kg/m^2 (17.6 to 54.9) versus 32.0 kg/m^2 (19.2 to 68.8), respectively, $P = .01$. Clinical presentation of diabetes was more commonly symptomatic with polyuria and polydipsia in Ab(+) patients, while in Ab(−) patients, diagnosis was more often incidental, $P = .002$. However, more than 95% of patients overlapped in both age and BMI irrespective of antibody status. Similarly, 42% of Ab(+) patients had their diabetes diagnosed incidentally, while 29% of Ab(−) patients presented with polyuria and polydipsia. We therefore conclude that screening with antibodies, mainly ICA and GAD, but not age, BMI, or clinical presentation should be used to identify type 1½ diabetes.
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TYPE 1 AND TYPE 2 diabetes are different pathophysiologically. The disease process in classical type 1 diabetes is an autoimmune destruction of the pancreatic beta cells.[1] In contrast, the disease process in classical type 2 diabetes is not autoimmune in nature, the central defects being a decreased sensitivity to insulin action[2] and a poorly understood, but noninflammatory, beta cell lesion, which diminishes insulin secretory capacity.[3] In clinical practice, the diagnosis of type 1 versus type 2 diabetes is made phenotypically using variables such as age at onset, apparent abruptness of onset of hyperglycemia, presence of ketosis, degree of obesity, prevalence of other autoimmune diseases, and apparent need for insulin replacement. This clinical distinction of type 1 versus type 2 diabetes is recognized to be imperfect.

There is also a third group of individuals who phenotypically are similar to classic type 2 diabetes patients, but who are positive for 1 or more of the autoantibodies commonly seen in the type 1 disease process, namely islet cell antibodies (ICA), insulin autoantibodies (IAA), autoantibodies to glutamic acid decarboxylase (GADAb), and antibodies to the tyrosine phosphatase IA-2 (IA-2Ab). Different terminologies have been used to describe these patients, including latent autoimmune diabetes in adult (LADA),[4,5] slowly progressive type 1 diabetes,[6,7] latent type 1 diabetes,[8] type 1½ diabetes,[9-11] and Ab(+) type 2 diabetes. At the present time, it is unclear which term is the most appropriate. The main objective of this study was to determine what (clinical) criteria, if any, help to identify patients with type 1½ diabetes at the time of diagnosis of their hyperglycemic state.

SUBJECTS AND METHODS

Subjects

These studies were approved by the Human Subjects Review Committee of the University of Washington. One hundred and twenty five patients clinically diagnosed with type 2 diabetes using the 1997 American Diabetes Association (ADA) criteria[12] by endocrinologists in the greater Seattle area were enrolled into the study if they met all of the following: (1) age ≥ 30 years at diagnosis of diabetes, (2) no history of ketonuria or ketoacidosis, and (3) not requiring insulin treatment at diagnosis. All patients had been diagnosed with diabetes within 12 months of blood sampling. Demographic characteristics and information regarding clinical presentation were obtained at the time of diagnosis. Clinical presentation was defined as symptomatic if the presenting symptoms were polyuria and/or polydipsia. Serum was collected and studied for ICA, IAA, GAD65Ab and antibodies to IA-2a (IA-2Ab).

Antibody Assays

ICA assay. This assay was performed as previously described.[13] All sera with detectable ICA were end-point titered. The lower detection limit of our ICA assay is 1 Juvenile Diabetes Foundation (JDF) unit and the 99th percentile, positivity threshold, was established at 4 JDF units based upon approximately 4,000 normal school children.[14] Our laboratory has participated in a total of 8 International Diabetes Society Workshops (IDW) and the International Diabetes Society

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(IDS)-sponsored proficiency programs for ICA with an average sensitivity of 80% and a specificity of 100% every time. In the IDS-sponsored Combined Antibody Workshop,[15] our ICA assay had a specificity of 98% and a sensitivity of 76%. Our ICA assay has been validated most recently in a serum exchange with the Diabetes Prevention Trial—Type 1 Diabetes (DPT-1) ICA core lab. In this exchange the sensitivity of our assay was 85% with a specificity of 100%.

IAA Assay

IAA levels were determined as previously described.[16] Monoiodinated A14 human insulin tracer with an average specific activity of 300 μCi/μg was used with displacement by cold insulin. IAA was not measured if a patient was on insulin at the time of antibody testing. A subject was considered IAA positive if the insulin specific binding was greater than 3 standard deviations above the mean of approximately 100 normal controls.[16] We have participated in the IDW, the IDS-sponsored workshops, and proficiency programs for IAA with a sensitivity of about 100% and specificity of 100%.

GAD65Ab Assay

GAD65 autoantibodies were detected in a radiobinding immunoassay on coded serum samples.[17] Briefly,[35] S-GAD65 was produced in an in vitro-coupled transcription and translation system with SP6 RNA polymerase and nuclease treated rabbit reticulocyte lysate (Promega, Madison, WI). In each analysis, triplicate samples of trichloroacetic acid (TCA) precipitable^{35} S-GAD65, 20,000 cmp, were diluted in 60 μL immunoprecipitation buffer (20 mmol/L Tris, 150 mmol/L NaCl, 0.15% (vol/vol) Tween 20, 0.1% (wt/vol) aprotinin, 10 mmol/L benzamadine, 0.1% (wt/vol) bovine serum albumin (BSA) pH 7.4) before the addition of 2.5 μL human serum (final dilution, 1:25). Free^{35} S-GAD65 was separated from the antibody-bound tracer by protein A-Sepharose and several washes of 20 mmol/L Tris, 150 mmol/L NaCl, 0.1% (vol/vol) Tween 20, 0.1% (wt/vol) BSA (pH 7.4) using 96-well plates containing 0.65 μm hydrophilic polyvinylidene difluoride filters (Millipore, Bedford, MA). The immunoprecipitated radioactivity was counted after transferring the filters to glass scintillation vials using a Multiscreen Multiple Punch System (Millipore). The levels of GAD65 were expressed as a relative index (GAD index) using 1 positive serum (JDF World Standard for ICA) and 3 negative standard sera from healthy subjects. The GAD index was calculated, and a positive was considered at $\geq$0.09 which is the 99th percentile based on 200 normal controls. In the IDS-sponsored GAD65 Ab serum exchange and the Combined Antibody Workshop, our laboratory scored 100% for both sensitivity and specificity.

IA-2 Assay

Autoantibodies to IA-2a were measured under identical conditions as described for GAD65Ab.[17] The plasmid containing the cDNA coding for the cytoplasmic portion of IA-2a was kindly donated by Dr G. Eisenbarth, Barbara Davis Research Center, Denver, CO. The same JDF standard serum and control sera as for the GAD65Ab assay were used to calculate the IA-2 Ab index for each sample. IA-2 index was considered positive at $\geq$0.015, which is the 99th percentile based on 200 normal controls.

Statistical Analysis

The differences in body mass index (BMI) and age at diagnosis between Ab(+) and Ab(−) patients were compared using the unpaired *t* test. The differences in the age of the Ab(+) patients positive for 1 or more antibodies were compared by analysis of variance (ANOVA). The χ^2 test was used to compare differences in gender and clinical presentation in the Ab(+) and Ab(−) groups.

RESULTS

General Demographics

Among the 125 patients, 77 were male and 48 were female. One hundred and nine patients (87.2%) were Caucasian, 9 (7.2%) were African Americans, and 7 (5.6%) were either Native American or Asian Indians. The mean duration of diabetes at the time of screening was 3.8 months (SEM $\pm$ 0.30; range, 0 to 12 months). Sixteen of the 48 female patients (33.3%) were Ab(+), while 20/77 of the males (26%) were antibody positive $P = .38$.

Antibody Clustering

Thirty-six of the 125 patients (28.8%) were positive for at least 1 antibody (Ab+). Thirty-two (25.6%) were ICA positive, 20 (16%) were GADAb positive, 8(6.4%) were IA-2Ab positive, and 2 (1.6%) were IAA positive. Eighteen patients (14.4%), were positive for only 1 antibody, 10(8.0%) were positive for 2 antibodies, and 8 (6.4%) were positive for 3 antibodies. No patient was positive for all 4 antibodies. Among the 36 Ab(+) patients, 14 (38.9%) were positive for ICA alone, 2 (5.6%) were positive for GAD 65 Ab alone, and 2 (5.6%) were positive for IA-2 alone (Fig 1). Eighteen of the 36 Ab(+) patients (50.0%) had both ICA and GAD 65 Abs. Six of these 18 patients were also positive for IA-2 Ab, and 2 of the 18 were also positive for IAA. No patient was positive for IAA alone. Of the 17 non-Caucasian patients, only 3 (17.7%) were positive for antibodies, 2/9 (22.2%) African Americans were positive for ICA alone, and 1/7 (an Asian American) was positive for GADAb alone. Among the 32 patients positive for ICA, only 1 (3.1%) had a titer >20 JDF units, 2 (6.3%) had titers of 20 JDF

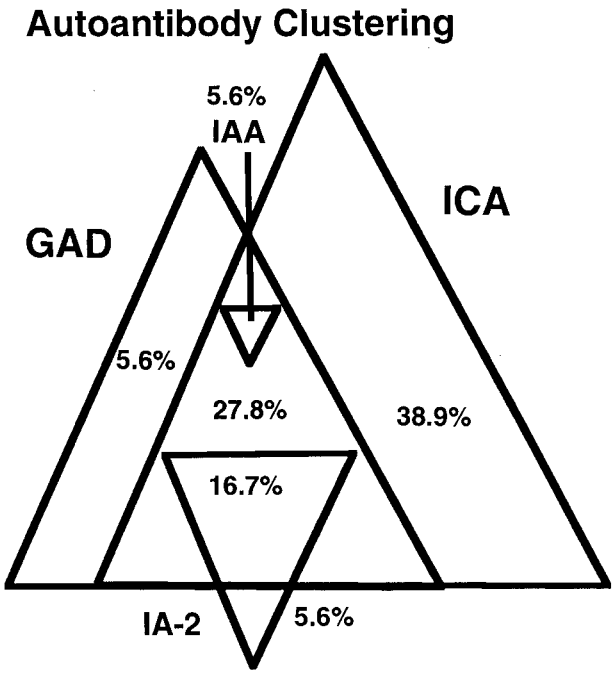

Fig 1. Clustering of autoantibodies in the 36 antibody-positive patients. Numbers (%) refer to the percentage of the 36 antibody-positive patients who were positive for the respective antibodies.

units, 6 (18.8%) had titers of 10 JDF units, 10 (31.3%) had titers of 4 JDF units, while the majority 13 (40.6%) had titers of 6 JDF units. As illustrated in Table 1, even at these low ICA titers, patients had other antibodies as well.

Age at Diagnosis of Diabetes

All patients were over 30 years of age with a mean age (range in years ± SEM) of 50.0 (31 to 77 ± 0.95) years. The mean age of the Ab(+) patients was 47.2 (32 to 64 ±1.62) compared with 51.2 (31 to 77 ±1.14) in the Ab(−), $P = .06$ (Fig 2). Although there was no significant difference in the mean ages of Ab(−) patients and those positive for 1 antibody 51.2 (31 to 77 ± 1.14) years versus 52.8 (40 to 64 ± 1.93) years, $P = .55$, patients who had 2 or 3 antibodies were significantly younger than the antibody-negative patients, mean age, positive for 2 antibodies 42.4 years (32 to 58 ± 2.97) and positive for 3 antibodies 40.8 years (32 to 49 ± 2.17), $P < .05$ (Fig 3). However, as illustrated in Fig 2, over 98% of the patients in the Ab(+) and Ab(−) groups overlap in age distribution at 2 SDs from the mean.

BMI

The mean BMI (range ± SEM) in the Ab(−) patients was 32.0 kg/m^2 (19.2 to 68.8 ± 0.81) compared with 28.3 kg/m^2 (17.6 to 54.9 ± 1.30) in the Ab(+) group, $P = .01$ (Fig 4). Although, Ab(+) patients statistically have lower BMIs than Ab(−) patients, the mean BMI of the Ab(+) patients (the majority of whom were Caucasian) would be considered as obesity (BMI ≥ 27). It is also clear from Fig 4 that there is a wide distribution in the BMI's of Ab(+) and Ab(−) patients with over 97% overlap between the 2 groups at 2 SDs from the mean. For example, a patient with a BMI of 32 kg/m^2 could be antibody-positive, while 1 with a BMI of 25 kg/m^2 could be antibody-negative.

Clinical Presentation

The presentation of diabetes was symptomatic (defined as presence of polyuria and polydipsia as the main presenting symptom at diagnosis of diabetes) in 21/36 (58.3%) Ab(+) patients compared with 26/89 (29.2%) Ab(−) patients ($P = .002$). In the remaining patients in both groups, diabetes was diagnosed incidentally with either a routine screening blood or urine test. Again, despite this statistically significant difference, 15/36 (41.7%) Ab(+) patients had the diagnosis made incidentally, while nearly 30% of Ab(−) patients presented with polyuria and polydipsia at diagnosis.

Table 1. Relationship Between ICA Titers in JDF Units and Number of Patients Positive for Other Antibodies

ICA JDF Titer (no.)	GAD Positive	IA-2 Positive	IAA Positive
4 (10)	2	0	0
6 (13)	9	4	1
10 (6)	4	2	0
20 (2)	2	0	1
>20 (1)	1	0	0

NOTE. This table does not include the patients positive for GAD alone (n = 2) or IA-2 alone (n = 2).

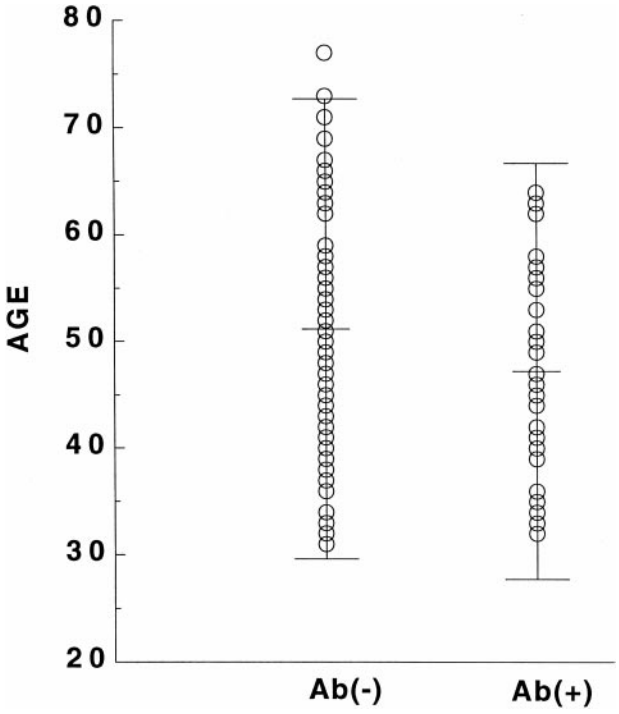

Fig 2. Age (mean ± 2 SD) at diagnosis of diabetes in antibody-positive and antibody-negative type 2 diabetes patients.

DISCUSSION

Classical, childhood onset type 1 diabetes has a relatively low prevalence in Caucasians, approximately 0.3%. In contrast, type 2 diabetes is much more common with a prevalence in Caucasians of 5% to 7%.[18] In Caucasian children of European descent, most diabetes is clinically type 1 with the disease process being autoimmune in nature. However, epidemiologic studies have suggested that the incidence rate of type 1 diabetes peaks twice, once close to puberty and again around 40 years of age,[19] and it has been suggested that the overall incidence rate of type 1 diabetes is approximately equivalent above and below the age of 20.[20] This relatively high incidence rate of type 1 diabetes in adults is often not appreciated, probably because of the over 10-fold greater frequency of type 2 diabetes in this age group.

In this study, we analyzed clinical phenotype and immune markers (presence or absence of antibodies) in patients diagnosed with type 2 diabetes to try to determine the best way to diagnose type 1½ diabetes. Our inclusion criteria excluded patients who had any evidence of ketosis or were thought to require insulin at the time of diagnosis of diabetes. Using 4 autoantibodies commonly associated with type 1 diabetes, we found that 29% of patients diagnosed with type 2 diabetes had the presence of 1 or more of these antibodies. ICA were the most common followed by GAD65Ab. Although ICA and GAD65Abs together were present in 50% of the Ab(+) subjects, all but 2 of the GAD-positive patients were also positive for ICA. Overall, IA-2 and IAA occurred infrequently and even when positive, these 2 antibodies were nearly always associated with the presence of multiple antibodies (Fig 1).

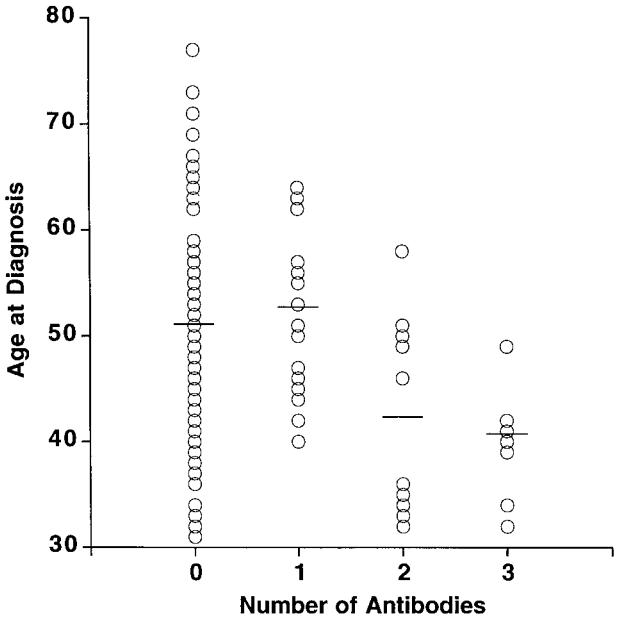

Fig 3. Age in years at diagnosis of diabetes in patients positive for zero, 1, 2, and 3 antibodies.

Numerous studies have reported that anywhere from 5% to 30% of phenotypic type 2 diabetes patients are positive for at least 1 antibody commonly seen in type 1 diabetes and could therefore be classified as having type 1½ diabetes.[4,8,21-24] Antibodies in type 2 patients have been reported from many countries including Finland,[4] Australia,[5] New Zealand,[25] the US,[26] UK,[27,28] Germany,[21] and Sweden.[22,29] The 29% antibody positivity we found in type 2 diabetes patients is slightly greater than that previously reported. One reason for this may lie in the fact that this data is not population-based and therefore the percent positive for antibodies may not be totally representative and comparable to other results in the literature. Another reason may be because we have a very sensitive ICA assay and have set our cut-point for positivity at 4 JDF units. If we include only those with ICA $\geq$ 10 JDF units, we find that 9/125 (7.2%) of our patients had at least 1 antibody. Nonetheless, similar to Kobayashi et al,[30] we have found that most patients positive for ICA had low titers. At these low titers also, we believe that these patients are true positives since at even 6 JDF units, 9/20 (45%) of the GAD positives and 4/8 (50%) of the IA-2 positives would be identified (Table 1). We have also recently demonstrated that type 1½ diabetes patients, many with low ICA titers, commonly have T-cell responses to islet proteins similar to type 1 diabetes patients.[13]

Some studies have shown that Ab(+) type 2 patients tend to be younger at diagnosis of diabetes[6,31,32] and also tend to have lower body mass indices[8,31,33] than their Ab(−) counterparts. In our study, we found no significant difference in the age at diagnosis of diabetes between the Ab(+) and Ab(−) patients. Although patients with more than 1 antibody tended to be younger than patients with 1 or no antibody, we found that patients across all age groups could be either antibody-positive or negative. Similarly, not only was the mean BMIs in the Ab(+) patients in the obese range (28.2 kg/m^2), but patients among all the BMI ranges could be either Ab(+) or Ab(−).

Type 1 diabetes often presents symptomatically with polyuria, polydipsia, and occasionally polyphagia and is commonly accompanied by ketoacidosis. Although statistically more Ab(+) patients in our study presented with polyuria and polydipsia as compared with Ab(−) patients, in over 40% of the Ab(+) patients, the diagnosis of diabetes was made on routine screening tests, while nearly 30% of the Ab(−) patients had an symptomatic presentation. For individual patients therefore, neither age, BMI, nor acuteness of presentation can reliably predict antibody positivity in phenotypic type 2 diabetes.

Many studies have shown that Ab(+) type 2 diabetes patients have lower C-peptide values and/or their beta-cell function deteriorates more rapidly with time compared with their Ab(−) counterparts.[8,31,34] The failure rate for sulfonylureas is also high and more rapid in Ab(+) type 2 patients, usually in 3 to 5 years, as compared with the 6 to 8 years for Ab(−) patients.[21,27,35,36] Beta-cell function deteriorates not only in the nonobese ICA (+) type 2 patients, but also in obese ICA (+) type 2 patients.[34] Since Ab positivity is associated with loss in beta-cell function, it would be important to screen patients diagnosed with type 2 diabetes for the presence of antibodies to prevent inadvertent combining of 2 different populations of subjects.

If screening for antibodies in type 2 diabetes patients is to be recommended, which would be the best antibody(s) to measure? Of the 36 Ab(+) patients in our study, 89% (32/36) were ICA-positive and 56% (20/36) were GAD65Ab-positive. The

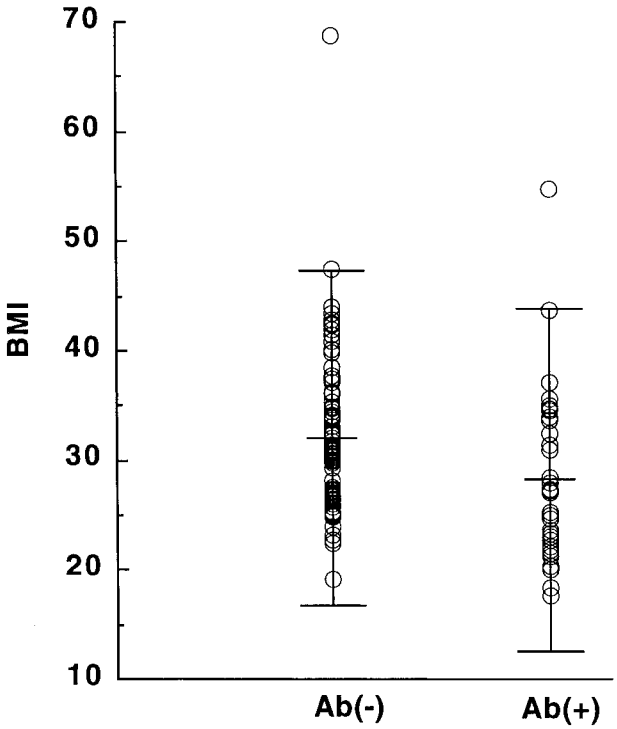

Fig 4. BMI (mean ± 2 SD) at diagnosis of diabetes in antibody-positive and antibody-negative type 2 diabetes patients.

occurrence of positive ICA is higher than that reported by Willis et al[37] who found 67% of insulin-deficient adult onset diabetics to be ICA or GADAb-positive. In our study, patients who were positive for only 1 antibody were most commonly positive for ICA (39%). These data suggest that if a single antibody was to be chosen as a screening test, it should be ICA. However, we know that in classic childhood onset type 1 diabetes, not only do multiple antibodies cluster, but also the risk of progression to type 1 diabetes in high-risk nondiabetic subjects is greater with multiple antibodies compared with any 1 antibody.[38-41] Moreover, the measurement of ICA is technically demanding, assays remain poorly standardized, and although commercially available, it is unknown how comparable the commercial assays are with well-validated research assays.[41] With the availability of radioimmunoassays for GADAbs,[17] which can be automated and standardized, we are now able to perform these assays easily,[23,42] and they are also commercially available, although not all yet validated. An advantage of testing for GADAb alone is that at least in classic type 1 diabetes, GADAbs can be detected for up to 10 years before,[43] and according to some, for up to 40 years after[23] the clinical diagnosis, while ICA tends to fall off with time post-diagnosis.[41] Nonetheless, there are also likely to be type 1½ patients who would be ICA (+), but GADAb(−), and this group would be missed if we tested for GADAb alone. For instance, if we measured only GAD65Ab in our study, we would miss the 14/32 (44%) patients positive for ICA alone. Several studies in type 2 diabetic patients have evaluated both ICA and GADAbs,[23,28,31,37,44] and in 2 of these, the presence of both ICA and GADAbs was found to be a better predictor of insulin deficiency than the presence of either of the 2 alone.[28,37] Therefore, although testing for ICA alone may be sufficient to screen for type 1 ½ diabetes, from a clinical perspective of potential insulin deficiency, we feel that both ICA and GAD65Abs should be used in trying to identify type 1½ diabetes.

In conclusion, unlike many studies that have suggested that antibody-positive type 2 diabetes patients tend to have a clinical phenotype in terms of age at diagnosis and BMI that is similar to type 1 patients,[6,8,31-33,45] results of our study show that clinical phenotype is not reliable in individual patients. Because up to a third of patients diagnosed clinically as type 2 diabetes may have immune markers of type 1 diabetes and because patients with these immune markers could have a more rapid progression to beta-cell failure, it may be prudent to screen type 2 diabetes patients with both ICA and GAD65Abs. Several interventions are currently being evaluated for their ability to block or inhibit the type 1 diabetes disease process in humans. If 1 or another of these interventions is found to be effective in classical type 1 diabetes, there will be intense interest to know whether the same intervention is also effective in patients with type 1½ diabetes. If so, providers would need to screen all type 2 diabetes patients to identify those with antibodies to offer treatment to them.

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