AUTOIMMUNE MARKERS

1. Use

> **Recommendation:** Islet cell autoantibodies are recommended for screening of non-diabetic family members who wish to donate part of their pancreas for transplantation to a relative with end stage, immune-mediated (type 1) diabetes. Islet cell autoantibodies are not recommended for routine diagnosis of diabetes nor for screening.
>
> Level of evidence: E

No therapeutic intervention has been identified that will prevent diabetes (204, 205). Therefore, although several autoantibodies have been detected in individuals with type 1 diabetes, measurement of these has very limited use outside of clinical studies. Because of the minimal indication for use of autoantibodies in routine management of patients with diabetes, this section will focus on the pragmatic aspects of clinical laboratory testing for autoantibodies at present and briefly address some areas of controversy.

A. Diagnosis/Screening

a. Diagnosis

In type 1 diabetes the insulin-producing b-cells of the pancreas are destroyed. In the vast majority of these patients, the destruction is mediated by T cells (1). This is termed type 1A or immune mediated diabetes (IMD) (Table 1). Islet cell autoantibodies comprise autoantibodies to islet cell-cytoplasm (ICA), to native insulin, referred to as insulin autoantibodies or IAA (208), to glutamic acid decarboxylase ($GAD_{65}A$) (209-211), and to two tyrosine phosphatases {insulinoma associated antigens IA-2A (212) and IA-2βA (213)}. Autoantibody markers of the immune destruction are usually present in 85-90% of individuals with IMD when fasting hyperglycemia is initially detected (1). Autoimmune destruction of the β-cells has multiple genetic predispositions and is modulated by undefined environmental influences. The autoimmunity may be present for months or years prior to the onset of symptoms. Patients with type 1A diabetes have a significantly increased risk of other autoimmune disorders including celiac disease, Graves' disease, thyroiditis, Addison's disease, and pernicious anemia (63). As many as 1:4 females with IMD have autoimmune thyroid disease while 1:280 patients develop adrenal autoantibodies and adrenal insufficiency. A minority of patients with type 1 diabetes (type 1B, idiopathic) have no known aetiology and no evidence of autoimmunity. Most of these patients are of African or Asian origin.

Approximately 10-15% of Caucasian adult patients who present with type 2 diabetes phenotype also have islet cell autoantibodies (214), particularly $GAD_{65}A$, which predict insulin dependency. This has been termed latent autoimmune diabetes of adulthood (LADA) (215). Although ICA- or $GAD_{65}A$-positive diabetic patients progress faster to absolute insulinopenia than do antibody-negative patients, many antibody-negative (type 2) diabetic adults also progress (albeit more slowly) to insulin dependency with time. There is no role for islet cell autoantibody testing in patients with type 2 diabetes because the institution of insulin therapy is based on glucose control.

b. Screening

Recommendation: Screening of relatives of patients with type 1 diabetes or of persons in the general population for islet cell autoantibodies is not recommended at present.

Level of evidence: E

The risk of developing IMD in relatives of patients with type 1 diabetes is ~ 5%, which is 15-fold higher than the risk in the general population (1:250-300 lifetime risk). Screening relatives of patients with IMD for islet cell autoantibodies can identify those at high risk of IMD. However, as many as 1-2% of healthy individuals have a single autoantibody and are at low risk of developing IMD (216). Because of the low prevalence of IMD (~0.3% in the general population), the positive predictive value of a single autoantibody will be low (205). The presence of multiple islet cell autoantibodies (IAA, $GAD_{65}A$, and IA-2A/IA-2βA) is associated with a risk of IMD > 90% (216, 217). However, until cost effective screening strategies can be developed for young children and effective intervention therapy to prevent the clinical onset of the disease become available, such testing cannot be recommended outside of a research setting.

Children and young adults with certain HLA-DR and/or DQB1 chains (**0602/*0603/*0301*) are mostly protected from IMD, but not from developing islet cell autoantibodies (218). Because islet cell autoantibodies in these individuals have substantially reduced predictive significance, consideration should be given to excluding them from prevention trials.

B. Monitoring/Prognosis

Recommendation: There is currently no role for measurement of islet cell autoantibodies in the monitoring of patients in clinical practice. Islet cell autoantibodies are measured in research protocols and some clinical trials as surrogate end-points.

Level of evidence: E

No acceptable therapy has been demonstrated to prolong survival of islet cells once diabetes has been diagnosed or to prevent the clinical onset of diabetes in islet-cell-autoantibody-positive subjects (204). Thus, repeated testing for islet cell autoantibodies to monitor islet cell autoimmunity is not clinically useful at present. In islet cell or pancreas transplantation, the presence or absence of islet cell autoantibodies may clarify whether subsequent failure of the transplanted islets is due to recurrent autoimmune disease or to rejection (219). When a partial pancreas has been transplanted from an identical twin or HLA-identical sibling, appearance of islet cell autoantibodies may raise consideration of the use of immunosuppressive agents to try to halt recurrence of diabetes. Notwithstanding these theoretical advantages, the value of this therapeutic strategy has not been established.

Some experts have proposed that testing for islet cell autoantibodies may be useful in the following situations: a) to identify a subset of adults initially thought to have type 2 diabetes, but have islet cell autoantibody markers of type 1 diabetes and progress to insulin dependency (220); b) to screen non-diabetic family members who wish to donate a kidney or part of their pancreas for transplantation; c) to screen women with GDM to identify those at high risk of progression to type 1 diabetes and d) to distinguish type 1 from type 2 diabetes in children to institute insulin therapy at the time of diagnosis (221, 222). For example, some pediatric diabetologists are now treating children thought to have type 2 diabetes with oral medications, but treat autoantibody positive children immediately with insulin. However, it is possible to follow patients who are islet cell autoantibody positive to the point of metabolic decompensation and then institute insulin therapy. A small pilot trial from Japan suggests that insulin therapy in islet cell antibody positive patients may preserve C-peptide (a measurement of insulin secretion) compared to oral medications (223), but this observation requires confirmation.

During the review of this manuscript by a panel of experts, it became evident that there is wide variability in clinical practice regarding the use of islet cell autoantibodies. While some indicate that the results of autoantibody assays are clinically useful, others point to a lack of evidence. Although some clinicians, particularly those who treat pediatric patients, use autoantibody assays as outlined in the preceding paragraph, clinical studies are necessary to provide outcome data to validate this approach. There is no systematic review that addresses these questions.

2. Rationale

The presence of autoantibodies suggests that insulin therapy is the most appropriate therapeutic option especially in a young person. Conversely, in children or young people without islet cell autoantibodies, consideration may be given to a trial of oral agents and life style changes other than insulin therapy. There is not unanimity of opinion, but the presence of autoantibodies may alter therapy for subsets of patients, including Hispanic and African American children with a potential diagnosis of non-IMD, adults with autoantibodies but clinically classified as having type 2 diabetes, and children with transient hyperglycemia. The majority of non-diabetic individuals who have only one autoantibody will never develop diabetes. Although expression of multiple anti-islet cell autoantibodies is associated with greatly increased diabetes risk (216, 217), approximately 20% of individuals presenting with new onset diabetes express only a single autoantibody.

3. Analytical Considerations

Recommendation: It is important that autoantibodies be measured only in an accredited laboratory with an established quality control program and participation in a proficiency testing program.

Level of evidence: E

ICA are determined by indirect immunofluorescence on frozen sections of human pancreatic tails (224). ICA measure the degree of binding of imunoglobulin to the islets and are compared to a standard serum of the Immunology of Diabetes Workshop group (225). The results are reported in Juvenile Diabetes Foundation (JDF) Units. Positive results depend upon the study or context in which they are used, but many laboratories use 10 JDF units determined on two separate occasions, or a single result ≥ 20 JDF units, as significant titers which may convey an increased risk of IMD.

For IAA, a radioisotopic method that calculates the displaceable insulin radioligand binding after the addition of excess non-radiolabelled insulin (226) is recommended. Results are reported as positive when the specific antibody binding is greater than the mean + 2 (or 3) SD for healthy persons. Each laboratory needs to assay at least 100 healthy individuals to determine its own values. Many laboratories use a cut-off value between 80-110 milliunits/mL. An important caveat concerning IAA determination is that insulin antibodies develop following insulin therapy even in those persons who use human insulin.

For IA-2A and $GAD_{65}A$, a dual micro-method and RIA performed with ^{35}S-labeled recombinant human IA-2 and ^{3}H-labeled human recombinant GAD_{65} in a rabbit reticulocyte expression system is currently used by many laboratories (216). Methods for both $GAD_{65}A$ and IA-2A have recently become commercially available. $GAD_{65}A$, IA-2A and IA-2βA are reported as positive when the signal is > 99.7% (3SDs) of values in healthy controls (216). Comparison of multiple laboratories worldwide by a small number of quality control sera sent out from the laboratory of one of the authors (NM) revealed a concordance >90% for classification of individuals as antibody positive or negative. The Centers for Disease Control (CDC) is working with the Immunology of Diabetes Society to develop the Diabetes Autoantibody Standardization Program (DASP). A limited pilot proficiency testing program using samples obtained from patients with type 1 diabetes was begun recently. It is not yet clear whether this program will become generally available.

4. Interpretation

In newly diagnosed patients with type 1 diabetes, ICA is found in ~75-85%, $GAD_{65}A$ in ~ 60%, IA-2A in ~ 40% and IA-2βA in ~ 20%. IAA are positive in more than 90% of children who develop type 1 diabetes before age 5 years, but in fewer than 40% in individuals developing diabetes after age 12.

In some laboratories ICA is considered to be the most sensitive and specific single test for the detection of type 1 diabetes. However, the ICA assay is labor-intensive and difficult to standardize, and marked inter-laboratory variability in sensitivity and specificity has been demonstrated in workshops (207, 227). Few clinical laboratories are likely to implement this test. The immunoassays are more reproducible. Measurement of T cell reactivity in peripheral blood is theoretically appealing (because T cells mediate islet destruction), but the variability of such assays precludes their use in a clinical setting (228, 229).

Autoantibody-positivity is reported (by definition) in some healthy individuals despite an absence of family history of autoimmune diseases. Islet cell autoantibodies are no exception. If one autoantibody is found, the others should be assayed because the risk of IMD increases if two or more autoantibodies are positive (205, 217). For the standardized ICA, replicate titers in excess of 10 JDF units predict an increased risk of diabetes. Similarly, IAA above the mean + 3 SDs of healthy controls also predict an increased risk of diabetes, and when associated with ICA or another antibody, carry a risk of 20-50%.

The following suggestions have been proposed by Atkinson and Eisenbarth (204) as a rational approach to the use of autoantibodies in diabetes: a) antibody assays should have specificity >99%; b) proficiency testing should be documented; c) multiple autoantibodies should be assayed and d) sequential measurement should be performed. These strategies will reduce false positive and negative results.

5. Emerging Considerations

Since immunoassays for IAA, GAD and IA-2A/IA-2βA are now available, it is likely that a panel of these autoantibodies will eventually be used for screening purposes, possibly with ICA used for confirmatory testing. Because ICA may represent either GAD_{65} or IA-2 autoantibodies and ICA assays are difficult to standardize, some experts in the field do not use ICA testing at all. Cost considerations aside, the best screening would be through all of the above autoantibodies including ICA. However, this recommendation is controversial and some experts disagree.

It is likely that other islet cell antigens will be discovered, which could lead to additional diagnostic and predictive tests for IMD. For example, GLIMA-38 (230) is associated with IMD, but its prognostic significance has not been established. Autoantibody screening on finger-stick blood samples appears quite feasible in future. In those individuals who are islet cell autoantibody positive, *HLA-DR/DQ* genotyping will help define absolute risks of diabetes.

Several clinical trials to prevent IMD are being actively pursued (205). Such trials can now be done in relatives of patients with type 1 diabetes or in the general population on the basis of the islet cell autoantibody and/or HLA-DR/DQ genotype status. Risk can be assessed by islet cell autoantibodies alone, without the need for evaluation of endogenous insulin reserves as was done for the US DPT-1 trial. Autoantibody positivity rates are distinctly lower in the general population than in relatives of individuals with IMD, so that trials in the latter group are more economical. Potential interventional therapies (for IMD) undergoing clinical trials include oral or nasal insulin given to patients at the time of diagnosis of diabetes or to non-diabetic - but islet cell autoantibody positive - relatives of individuals with IMD. Trials of a vaccine based upon immunization by an insulin β-chain peptide or GAD_{65} are scheduled to begin soon. Additional trials of other antigen based immunotherapies, adjuvants, cytokines and T cell accessory molecule blocking agents are likely in the future (200). Decreased islet cell autoimmunity will be one important outcome measure of these therapies.