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# Comparison of Islet Autoantibodies in 'Pre-diabetes' and Recommendations for Screening

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## Introduction

Overwhelming evidence that insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease has accumulated since autoantibodies to cytoplasmic antigens (ICA) in the pancreatic islet cells were first described a decade ago. In addition, other autoantibodies reactive to cell surface antigens of islet cells, a product of the β-cell insulin (IAA) and an immunoprecipitable protein of approximately 64 kDa (64KA) have been identified using various methods and sources of islet tissue. The frequencies of these islet-reactive antibodies are highest near the time of diagnosis of IDDM and fall progressively thereafter. At the onset of IDDM, ICA have been detected in 60–80%, IAA in 35–60% and 64KA in 78–90% of newly diagnosed patients. These islet autoantibodies have been identified in individuals many years before the onset of clinical diabetes and may, therefore, serve as markers for the autoimmune destruction of β cells [1–4]. This report describes our experience in an ongoing screening program of relatives of IDDM and a group of school children using ICA and other autoantibodies in identifying 'prediabetic' patients.

## Methods

### *Patients*

Briefly, ICA were initially determined on sera obtained as part of a screening program from a high-risk population of relatives of IDDM probands ($n=6,874$) or from a general population of healthy school children and their families from Pasco County, Florida ($n=8,446$) [1]. Sera from newly diagnosed IDDM patients have been obtained routinely at the University of Florida.



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### *Islet cell autoantibodies (ICA)*

ICA were determined by indirect immunofluorescence using unfixed, 'snap frozen' human pancreas. Sera were considered positive when the intensity of the fluorescence and pattern of staining was the same as or greater than an in-house standard serum that had been calibrated to approximately 20 JDF units, using the international standard [5].

### *Insulin binding assay (IAA)*

The reference range for IAA was obtained by using the entire population of one school ($n=295$) [6]. The assay for IAA was a modification of the assay kindly provided by Dr Jerry Palmer (Seattle, WA), using $^{125}$I A-14 purified monoiodinated human insulin (Eli Lilly, Indianapolis, Indiana) [7]. The concentration of the ligand had been optimized to improve the discrimination between the control population and the patients with ICA. The intra-assay and interassay binding coefficients of variability were approximately 10% and 25%.

### *Determination of 64K autoantibodies (64KA)*

The assay for 64KA was a modification of the original method described by Baekkeskov *et al.* [8]. All fluororadiographs were analyzed independently by two observers who did not know sample identity. Both 64KA-positive and negative-control sera were used in each assay.

## Results

### *Predictive value of ICA*

In our study, unaffected relatives of IDDM probands are contacted annually for a history of developing diabetes and a serum sample is obtained biennially. Among these relatives, 16.8% of the ICA+ relatives have developed IDDM since the beginning of this study. In a large school population study in Pasco County, Florida, of 4,838 children, a similar number (13%) have developed IDDM in the last 3 years. However, a more appropriate method of analyzing the risk of IDDM from ICA in these populations requires the use of a life-table analysis which takes into account the duration of follow-up.

The life-table analysis for ICA+ siblings in comparison to all ICA+ relatives is demonstrated in Figure 1. The curve for all ICA− relatives or siblings is not shown since the actuarial estimate of probability of developing IDDM in ICA− relatives was less than 0.01%. As shown, the probability of developing IDDM in ICA+ siblings was nearly 55% by 5 years, but only 35% in the entire population of relatives.

### *Correlation of ICA with first phase insulin release*

In previous studies the first-phase insulin release after an i.v. bolus of glucose has been shown to be associated with progression to disease. In Figure 2 the first-phase insulin response is plotted as a function of the percentile of a reference population's

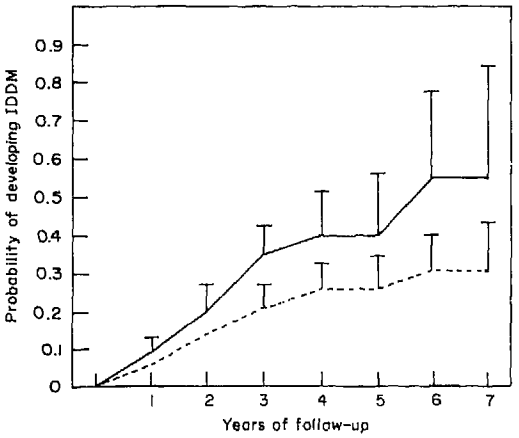


Figure 1. Life table analysis. Predictive value of ICA. Comparison of all relatives with siblings. ——, ICA+ siblings only ($n=50$); – – –, ICA+ all relatives ($n=25$).

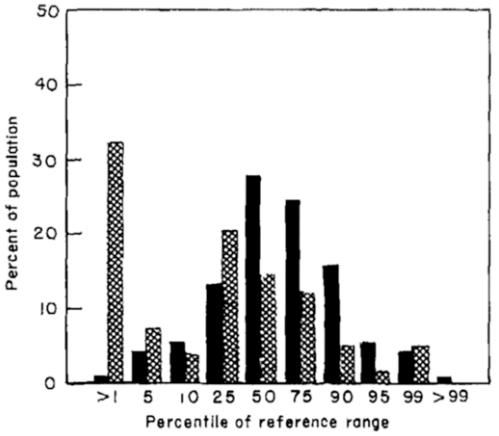


Figure 2. First phase insulin secretion. Sum of 1- and 3-min insulin concentration. ■, control ($n=153$); ▩, ICA+ ($n=84$).

response. In ICA+ individuals a significant skew of the insulin responses can be seen, with nearly 32% of ICA+ group below the first percentile. In addition, half of the patients with both ICA+ and IAA+ have insulin responses below the 1st percentile.

### *Comparison of islet cell autoantibodies*

Although ICA appears to be an excellent marker for the development of IDDM, the prediction is not absolute since several patients developed IDDM without prior identification of ICA. In Table 1, comparison of ICA, IAA, and 64KA as predictive markers for IDDM is shown. In newly diagnosed IDDM patients, 75% were ICA+ and 80% were positive for 64KA. In patients studied before IDDM subsequently

**Table 1.** *Comparison of islet cell autoantibodies*

| | New IDDM ($n=20$) | 'Pre-IDDM' ($n=22$) |
|---|---|---|
| ICA | 15 (75%) | 17 (77%) |
| 64KA | 16 (80%) | 20 (91%) |
| IAA | ND | 10 (48%) |

ND, no determined.

developed, 'pre-IDDM', 64KA were found to be most often positive, followed by ICA and IAA in decreasing frequency.

## Discussion

The ability to identify individuals prior to onset of IDDM is essential if the disease is ever to be prevented. ICA proved to be an excellent marker for identifying individuals prior to the onset of IDDM in both relatives of IDDM probands and the general population.

To determine whether everyone with ICA will develop IDDM requires continued follow-up in these population-based studies. In our study we have shown that the risk of developing IDDM appears higher in unaffected ICA + siblings in comparison to all ICA + relatives. The Barts Windsor study have shown an actuarial estimate of probability of developing IDDM at 8 years in their family studies similar to that in our siblings [4].

In our cohort of 29 patients eight developed IDDM without previous ICA, and 64KA was absent from only two of the 22 studied. The two who were negative for 64KA were older patients. Most importantly, all of the patients under 21 years who later developed IDDM and who were ICA negative were positive for 64KA. However, at the onset of IDDM the frequencies of both ICA and 64KA were similar. Thus 64KA may be the earliest markers of β destruction and may reflect the primary autoimmune response to the inciting antigen.

Unfortunately, present methodology for identifying 64KA is quite cumbersome and expensive and does not lend itself well to screening large populations. ICA will therefore remain the current standard for screening for IDDM, since IAA were not found in half the 'pre-IDDM' individuals, primarily adults. IAA have limited usefulness in screening for IDDM, but they have been shown to identify a subgroup of ICA+ patients with low insulin responses and thus to identify patients with more active disease. In NOD mice, the appearance of IAA appears to coincide with β-cell destruction, perhaps the same is true in human IDDM. However, screening for IAA in younger patients may be quite cost effective since the radioassay for IAA lends itself particularly well to mass screening.

The feasibility of intervention would be helped immensely if the timing to clinical disease could be accurately ascertained and an assay to monitor the activity of the disease developed.

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