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T1D Autoantibodies: Room for Improvement?

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Abstract

Purpose of review—Type 1 diabetes (T1D) is now predictable by measuring major islet autoantibodies (IAbs) against insulin (IAA) and other pancreatic β-cells proteins including GAD65 (GADA), islet antigen 2 (IA-2A) and zinc transporter 8 (ZnT8A). The assay technology for IAbs has made great progress; however, several important aspects still need to be addressed and improved.

Recent findings—Currently a radio-binding assay (RBA) has been well established as the 'gold' standard assay for all 4 IAbs. New generation of non-radioactive IAb assay with electrochemiluminescence technology has been shown to further improve sensitivity and disease specificity. Recently, multiplexed assays have opened the possibility of more efficient screening in large populations. Identification of potential new autoantibodies to neo-antigens or neo-epitopes post-translational modification is a new important field to be explored.

Summary—Individuals having a single positive autoantibody are at low risk for progression to T1D, while individuals expressing two or more positive autoantibodies, especially on multiple tests over time, have nearly 100% risk of developing clinical T1D when followed for over 2 decades. More efficient and cost effective IAb assays will hopefully lead to point-of-care screening in the general population.

Keywords

autoantibodies; type 1 diabetes; biomarkers; prediction; assays

Introduction

Type 1 diabetes (T1D) is one of the most common and serious long-term diseases often beginning in childhood. The incidence of T1D is increasing worldwide at 3–5% each year and has doubled in the last two decades [1,2]. Although T1D is mainly a T-lymphocytes mediated -destruction of insulin producing beta cells within pancreatic islets, appearance of islet autoantibodies (IAbs) in peripheral blood is currently the most reliable marker to assess the autoimmune process leading to T1D; it marks the onset of beta cell autoimmunity and determines the disease risk. IAbs usually appear years before overt clinical disease and children with 2 or more of four major islet autoantibodies to insulin (IAA), GAD65

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Conflicts of interests

None.

(GADA), islet antigen 2 (IA-2A), and zinc transport 8 (ZnT8A), have a 70% risk of developing clinical T1D in 10 years and a nearly 100% progression to clinical disease when followed for over 2 decades [3]. Many national and international screening studies for T1D have being launched in relatives and general population, and these longitudinal studies on pre-clinical T1D have helped to 1) better understand the natural history of T1D development; 2) define the onset of islet autoimmunity to identify potential triggers; 3) identify individuals for current and upcoming trials to prevent T1D; and finally 4) prevent life-threatening diabetic ketoacidosis.

Through the laboratory proficiency programs and harmonization efforts [4–7], a radio-binding assay (RBA) has been well established as the current 'gold' standard assay for all 4 IAbs. A newly validated nonradioactive IAb assay using electrochemiluminescence (ECL) detection for both IAA and GADA demonstrated higher sensitivity and disease specificity than RBA in several clinical studies, with superior detection of early seroconversion to islet autoimmunity and better discrimination of high-affinity, high-risk autoantibodies from those low-affinity, low risk signals less or non-relevant to the disease [8,9,10,11**,12**,13*, 14**]. With the platforms of ECL assay and liquid-phase ELISA recently published, multiplexed assays combining multiple antibody assays in one well are currently being tested [15**,16**,17,18*]. However, improvements in IAbs assays are still needed to help with large scale screening in general populations, more efficiently at a lower cost. In this review, we will further discuss two fundamental aspects, sensitivity and disease specificity of autoantibodies for prediction of the risk and the rate of T1D progression. Potential autoantibodies to modified epitopes on protein molecules, post translationally modified and chimeric peptides may be a new interesting field to explore. Finally, we will briefly discuss the new generation of autoantibody assay, a multiplexed assay, which will hopefully allow large scale population screening and point-of-care clinical needs.

Text of review

Early identification of T1D autoimmunity

IAbs are currently the most reliable biomarkers of the autoimmune process leading to T1D and almost all individuals with 2 or more IAbs will develop clinical T1D over time. The IAbs usually develop sequentially, not simultaneously, and IAA is usually the first IAb to appear in young children followed from birth, which has been further confirmed by recent data from clinical studies [19]. Seroconversion with appearance of the first IAb in the peripheral blood circulation may mark the initiation of islet autoimmunity, an important check point as prospective studies such as The Environmental Determinants of Diabetes in the Young (TEDDY) analyze possible environmental factors contributing to the start of the autoimmune process. Children followed from birth, either from relatives of patients with T1D or general population, usually present with IAA as their first IAb, but older children and young adults often present with GADA first, especially subjects with DR3-DQ2 haplotype [20*], which has led to the current idea of heterogeneous etiology of T1D development, with different primary β-cell autoantigens, varying according to age and genetic background. The current standard RBA for IAbs have been greatly improved through the laboratory proficiency programs and harmonization efforts. However, the IAA assay has

proven the most difficult to standardize with relatively wide discrepancies between laboratories in Islet Autoantibody Standardization Program (IASP) workshop, and has not yet achieved a satisfactory level of sensitivity and specificity. A newly developed IAA assay with ECL technology has been validated in multiple clinical studies with higher sensitivity and earlier detection of seroconversion by years than RBA. In the Diabetes Autoimmunity Study in the Young (DAISY), all pre-diabetic children who were followed to clinical T1D from birth were found almost to have IAA as their first IAb by ECL assay at seroconversion (9); as the data is limited to only one study, it needs to be further validated in other large prospective studies. In addition, IAA has been found to correlate inversely with age in both prevalence and level, with lower frequency and lower levels in older children and adults, which obviously adds more difficulties to effectively identify IAA among individuals beyond childhood. In summary, IAA is an important early marker for initiation of islet autoimmunity and the sensitivity of IAA assay might be a key issue in resolving the current puzzle of T1D etiology in terms of primary autoantigen(s). This will be an essential question to be answered in the near future to better understand the underlying mechanisms of initiation of the autoimmune process leading to T1D in both children and adults with different genetic backgrounds. It is also critical to precisely pinpoint the exact time of initial seroconversion as studies such as TEDDY investigate potential environmental triggering factors involved in T1D pathogenesis. In addition, all current IAA assays are not able to distinguish natural IAA from induced insulin antibodies (IA) by exogenous insulin usage, and both IAA and IA are high affinity antibodies, although the epitope binding sites might be different. It would be helpful to have an assay that distinguishes IAA from induced IA to further aid T1D research as current T1D trials, often require the confirmatory presence of at least one IAb.

Predicting rate of T1D progression

Natural history studies of T1D have shown that T1D may begin with seroconversion of single IAb, conversion to multiple IAbs, and finally progression to overt clinical diabetes. At initial screening either in first degree relatives of patients with T1D or in general population, IAA or GADA are often detected in isolation while IA-2A and ZnT8A are rarely present as a single IAb. Children with single IAb only have a 15% risk of developing T1D [3] after 15 years of follow-up and predictive values with either single IAA or single GADA are low. In several longitudinal studies including DAISY, TrialNet, and TEDDY, our group has shown that prediction of T1D risk in subjects with single IAb can be improved by using ECL assay, which is more disease specific [8,10,11**]. Figure 1 illustrates better positive prediction values of single IAb by ECL assay compared to RBA for both IAA and GADA (both $p< 0.0001$) in the TrialNet Pathway to Prevention study. Cumulative risk of T1D development in subjects with single IAb by ECL assay is significantly higher than RBA for both GADA ($p= 0.0009$) and IAA ($p< 0.0001$) (Figure 2). Furthermore recent evidence suggests that subjects with either single IAA or single GADA confirmed by ECL assay have metabolic changes of impaired glucose tolerance [14**] comparable to subjects with multiple IAbs, while ECL negative subjects showed no signs of impaired glycemia during a median follow-up of 4.7 years. TrialNet Pathway to Prevention subjects who were ECL-positive had an overall risk of progression to diabetes within 6 years of 58% compared to 5% for those ECL-negative subjects (Figure 3A) ($p<0.0001$). Survival curves analyzing participants positive for

1 IAb by RBA showed a much higher risk for development of diabetes by year 6 for subjects who were ECL positive (23% if both ECL-IAA and ECL-GADA positive, 18% if only ECL-IAA positive and 21% if only ECL-GADA positive) compared to those subjects who were ECL negative (4%) (Figure 3B) ($p<0.0001$). When looking at subjects positive for ≥2 IAbs by RBA, the risk for development of diabetes was again much higher for those subjects who were ECL positive: 61% by 6 years if both ECL-IAA and ECL-GADA positive, 69% by 4 years if only ECL-IAA positive and 51% by 6 years if only ECL-GADA positive compared to 22% for those subjects who were ECL negative (Figure 3C) ($p=0.02$).

Progression to diabetes diagnosis after seroconversion varies tremendously from several months to over a decade. Factors involved in rate of progression are poorly understood, although some determinants have been observed to be associated with rate of progression including younger age at seroconversion, number of autoantibodies, higher levels of IAA [21] and more recently higher levels of IA-2A [22*], but not GADA. In children with confirmed positive IAbs, the time of progression from single to multiple IAbs is influenced by family history of T1D and the presence of the high-risk HLA-DR3-DQ2/DR4-DQ8 genotype, while family history and HLA genotypes did not influence the time of progression to T1D in children with multiple IAbs [23**]. The rate of conversion from single to multiple IAbs was significantly higher in children with younger age less than 5 years in a recent study of the BABYDIAB/BABYDIET and the rate of conversion from single to multiple IAbs was highest in the first 2 years after seroconversion and declined rapidly after 4 years of follow-up ($p = 0.003$) [23**]. Development of diabetes was also faster in children who became multiple IAbs within 2 years of initial seroconversion than in children who became multiple IAbs above 2 years after seroconversion. Although positivity for multiple IAbs is accurate in predicting risk for T1D, we currently do not have the ability to predict the rate of progression to diabetes in individuals with islet autoimmunity. Adding other biomarkers to IAbs such as age, T and B or other cellular immune markers, metabolic changes, and genetic background, will likely help to predict rate of progression from islet autoimmunity to clinical T1D.

Neo-antigens and neo-epitopes

There is increasing evidence both in animal models and in humans that post-translational modification (PTM) is a characteristic of antigens in many autoimmune diseases. A recent study [24**] published in Science showed that the proteasome-generated spliced peptide pool accounts for one-third of the entire HLA class I immunopeptidome in terms of diversity and one-fourth in terms of abundance, which may have profound implications for the concept of self/nonself peptide presentation, derived from human or pathogen proteomes, with direct implications for autoimmunity. The β-cell is highly susceptible to endoplasmic reticulum (ER) and oxidative stress and is potentially a site of alteration in protein alternative splicing and PTMs [25,26]. There is supporting evidence in T1D that β-cell antigens undergo modifications through these mechanisms, which might be involved in the killing of β-cells [27–31]. Very recently, diabetes-inducing CD4 T cell clones isolated from NOD mice were found to recognize epitopes formed by covalent cross-linking of proinsulin peptides to chromogranin A and islet amyloid polypeptide present in β-cell secretary granules [32**, 33**,34*]. These hybrid proinsulin peptides are antigenic for CD4 T cells

from both mice and human, and CD4 T cells from the residual pancreatic islets of organ donors who had T1D were also found to recognize these proinsulin hybrids. The studies of modification of β-cell antigens have been limited to T cells so far with no current studies reported on B cells and autoantibodies. If PTM plays a critical role in initiation and development of the disease, identification of autoantibodies to these neo-antigens or neo-epitopes will be an important approach to gain new evidence of T1D pathogenesis and to help understand the underlying mechanism of loss of immune tolerance in T1D. These autoantibodies will also be new valuable biomarkers for T1D.

General population screening

Large screening of IAbs in general population, especially in young children, is currently in progress in the research setting [35*,36*]. Four biochemically defined IAbs are available and important in prediction and evaluation of risk of progression to T1D in both relatives of patients with T1D and general population. The screening methods using current standard RBA with single IAb measurement are laborious and inefficient for large scale screening. With the platforms of ECL assay and liquid-phase ELISA recently published [15–18], multiplexed assays combining multiple antibody assays in one well are being tested to help with large scale screening in general populations. There are at least 4 advantages with multiplex assays (using 4-plex assay as example) compared with current single radioassays: 1) higher throughput capacity: one lab technician is able to complete the assays for 3 to 4 times the number of samples that can be handled with current single RBA; 2) lower volume of serum: multiplex assay needs 3 to 4 times less serum than current RBA; 3) lower cost: multiplex assay will cost 30% less than total cost for current 4 single RBAs; 4) non-radioactive: as the multiplex ECL assay does not require radioactivity, we envision increased access to IAbs measurement with this methodology across academic and commercial laboratories.

Large scale screening can be hindered by geographical limitations, with families having to travel significant distances to study centers, as well as aversion to venous blood sampling particularly in young children. A simple, home-based procedure, without the need of a healthcare professional, could improve access to screening and potential prevention studies. Through the TrialNet study group, two pilot studies of feasibility for sampling on both dried blood spots (DBS) [37] and capillary blood samples (CBS) [38*] were performed. The IAbs were analyzed and compared with venous blood serum samples. Self-collected DBS or CBS were shown to be a feasible option, preferred by families over venous sampling, with the potential to facilitate screening for T1D risk. In the feasibility study on DBS samples, IAA was not performed since IAA assay on DBS samples was historically challenging with less sensitivity. In a very recent study [39*], conditions to elute autoantibodies from DBS on filter paper were optimized and stability of the DBS samples was assessed over time. Eluted DBS IAb measurements were performed for all 4 IAbs in new-onset T1D patients and in controls and compared to serum measurements using standard radioassays. Our results demonstrated that IAb measurements, including IAA, from DBS on filter paper strongly correlate to serum levels. Validation studies will be warranted to expand this approach into a general population screening test for T1D risk in the near future.

Conclusion

IAbs are currently the best biomarkers for T1D and have greatly contributed to the understanding of the natural history of the T1D autoimmune process and the prediction of risk in both first-degree relatives of T1D patients and general population. There are still many challenges and room for improvements, especially in 1) understanding the etiology of T1D in terms of primary autoantigens and to more precisely pinpoint the time of initiation of islet autoimmunity; 2) combining other biomarkers to predict or estimate rate of progression to disease; 3) exploring potential T1D autoantibodies to neo-antigens and neo-epitopes created by PTM mechanism; finally 4) developing an efficient and affordable tool for general population screening and point-of care.

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Key points

- IAA is an important early marker for initiation of islet autoimmunity and the sensitivity of IAA assay might be a key issue in resolving the current puzzle of T1D etiology in terms of primary autoantigen(s).
- Adding other biomarkers to IAbs such as age, cellular immune markers, metabolic changes, and genetic background, will likely help to predict rate of progression from islet autoimmunity to clinical T1D.
- Identification of autoantibodies to neo-antigens or neo-epitopes post translational modification (PTM) is a new important field which will likely help understand the underlying mechanism of loss of immune tolerance in T1D and provide new potential biomarkers for T1D.
- Multiplexed IAb assay combining multiple antibody assays in one well will be a useful tool for general population screening

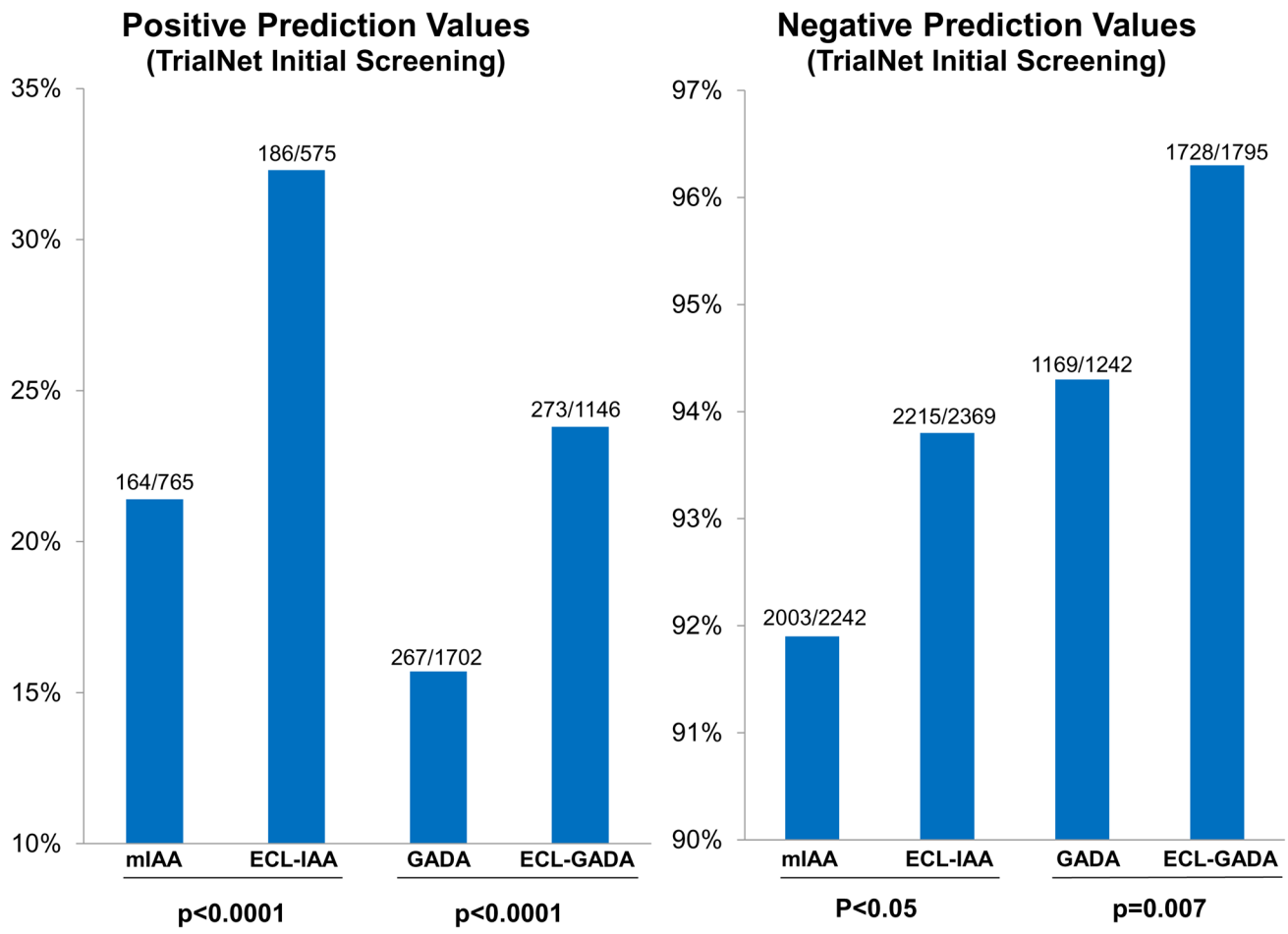

Figure 1.

Comparison of predictive values between ECL and radioassays on 2,944 subjects for their very first initial screening samples in TrialNet Pathway to Prevention Study.

Panel A: Comparison of positive predictive values between RBA-IAA (mIAA) and ECL-IAA ($p<0.0001$), and between RBA-GADA and ECL-GADA ($p<0.0001$).

Panel B: Comparison of negative predictive values between RBA-IAA (mIAA) and ECL-IAA ($p<0.05$), and between RBA-GADA and ECL-GADA ($p=0.007$).

[11] Diabetes Technol Ther. 2015;17:119–27.

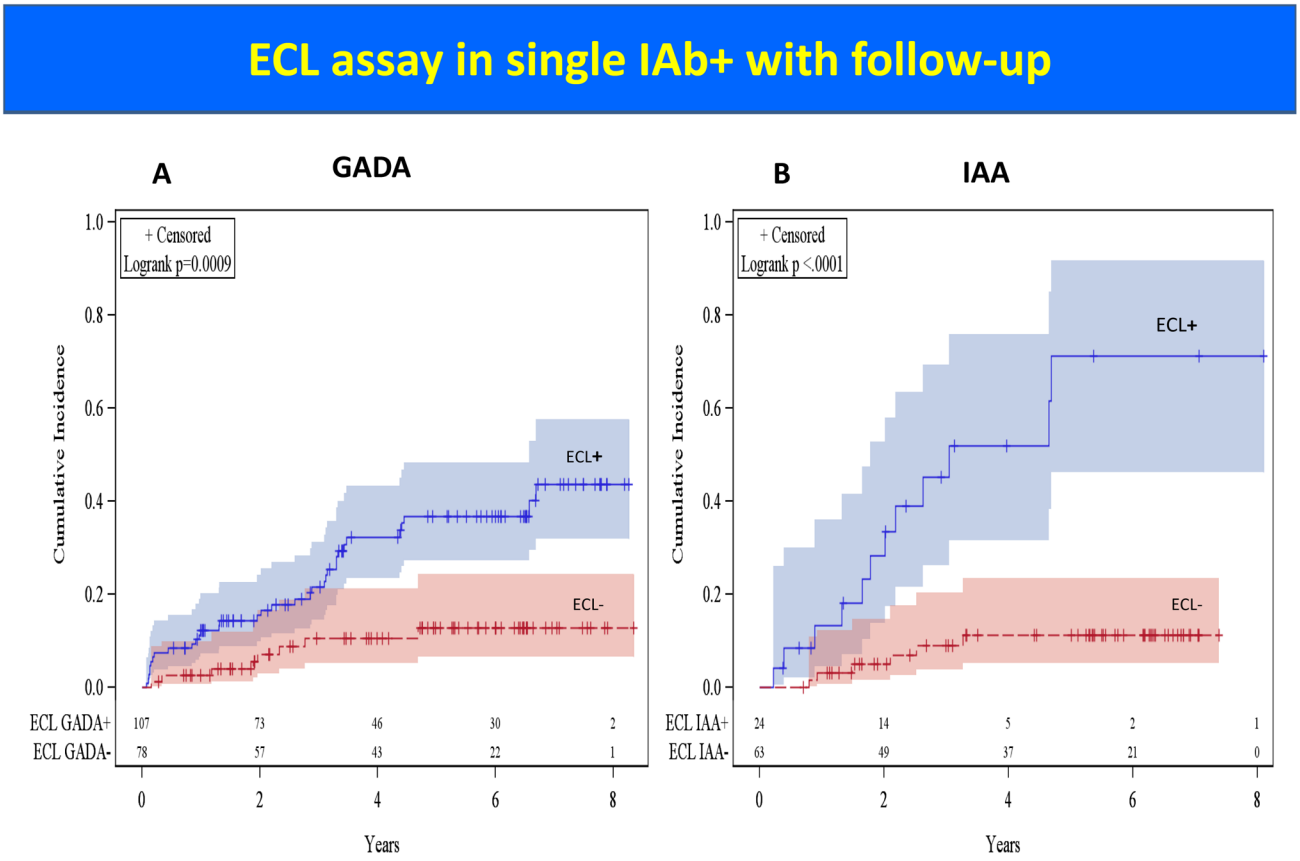

Figure 2.

Cumulative incidence of T1D development in subjects with single IAb in TrialNet Pathway to Prevention Study.

Panel A: Cumulative incidence of T1D development in subjects with single GADA positive by RBA, divided with ECL-GADA positive vs ECL-GADA negative (p= 0.0009).

Panel B: Cumulative incidence of T1D development in subjects with single IAA positive by RBA, divided with ECL-IAA positive vs ECL-IAA negative (p< 0.0001).

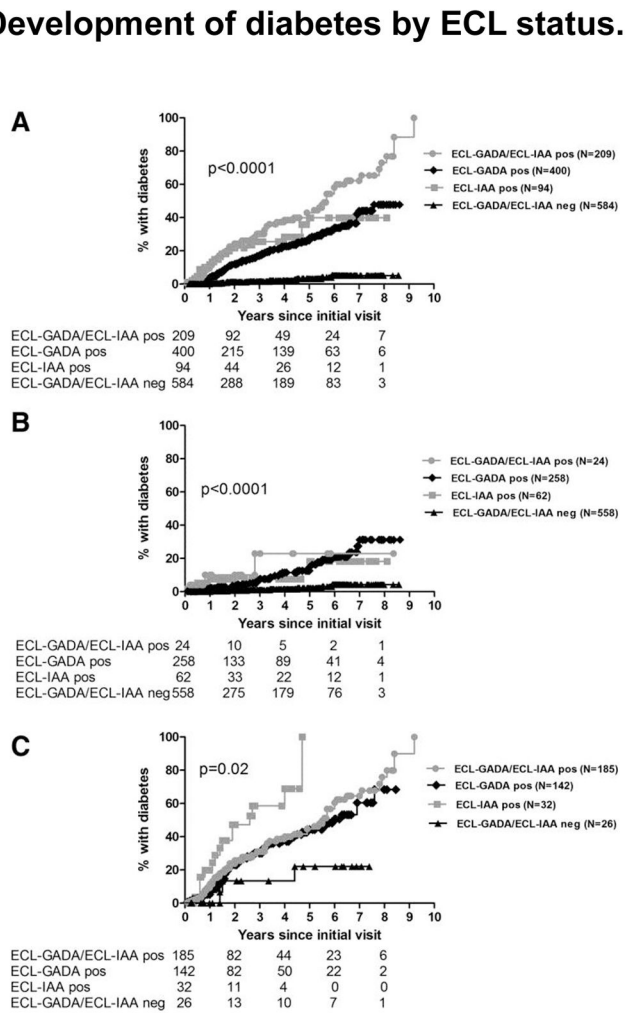

Figure 3.

Development of diabetes by ECL status. A: All subjects (n = 1,287). B: Subjects positive for one autoantibody by RIAs (n = 902). C: Subjects positive for two or more autoantibodies by RBAs (n = 385). Survival analysis was performed for the development of diabetes since initial visit according to ECL positivity using the log-rank test.

ECL-GADA/ECL-IAA pos, positive for both ECL-GADA and ECL-IAA; ECL-GADA pos, positive for ECL-GADA only; ECL-IAA pos, positive for ECL-IAA only; ECL-GADA/ECL-IAA neg, negative for both ECL-GADA and ECL-IAA.

[13] Diabetes Care. 2016;39:1738–44.