Latent Autoimmune Diabetes of Adults (LADA) is likely to represent a mixed population of autoimmune (type 1) and non-autoimmune (type 2) diabetes

Angus G Jones PhD[1,2], Timothy J McDonald PhD[1,3], Beverley M Shields PhD[1], William Hagopian PhD[4], Andrew T Hattersley MD[1,2]

1. Institute of Biomedical and Clinical Science, College of Medicine and Health, University of Exeter, Exeter, EX2 5DW
2. MacLeod Diabetes and Endocrine Centre, Royal Devon and Exeter NHS Foundation Trust, Exeter EX25DW, UK
3. Blood Sciences, Royal Devon and Exeter NHS Foundation Trust, Exeter EX25DW, UK
4. Pacific Northwest Research Institute, Seattle, WA USA

Corresponding Authors (joint):

Angus Jones, NIHR Clinician Scientist and Associate Professor, Institute of Biomedical and Clinical Science, College of Medicine and Health, University of Exeter, Exeter, EX2 5DW, UK,

Email: angus.jones@exeter.ac.uk

Andrew T Hattersley, Professor of Molecular Medicine, Institute of Biomedical and Clinical Science, College of Medicine and Health, University of Exeter, Exeter, EX2 5DW, UK,

Email: A.T.Hattersley@exeter.ac.uk

Telephone**:** 01392 408260

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Abstract

Latent autoimmune diabetes of adults (LADA) is typically defined as a new diabetes diagnosis after 35 years of age, presenting with clinical features of type 2 diabetes, in whom a type 1 diabetes associated islet autoantibody is detected. Identifying autoimmune diabetes is important since the prognosis and optimal therapy differ. However, the existing LADA definition identifies a group with clinical and genetic features intermediate between typical type 1 and type 2 diabetes. It is unclear whether this is due to a) true autoimmune diabetes with a milder phenotype at older onset ages which initially appears similar to type 2 diabetes but later requires insulin, b) a disease syndrome where the pathophysiologies of type 1 and type 2 diabetes are both present in each patient, or c) a heterogeneous group resulting from difficulties in classification. Herein, we suggest difficulties in classification are a major component resulting from defining LADA using a diagnostic test, islet autoantibody measurement, with imperfect specificity applied in low prevalence populations. This yields a heterogeneous group of true positives (autoimmune type 1 diabetes) and false positives (non-autoimmune type 2 diabetes). For clinicians this means that islet autoantibody testing should not be undertaken in patients who do not have clinical features suggestive of autoimmune diabetes: In an adult without clinical features of type 1 diabetes, it is likely that a single positive antibody will represent a false positive result. This is in contrast to patients with features suggestive of type 1 diabetes, where false positive results will be rare. For researchers this means that current definitions of LADA are not appropriate for the study of autoimmune diabetes in later life. Approaches are needed which increase test specificity, or prior likelihood of autoimmune diabetes, to avoid inclusion of participants who have non autoimmune (type 2) diabetes. Improved classification will allow both improved assignment of prognosis and therapy, as well as an improved cohort upon which to analyse and better understand the detailed pathophysiological components acting at onset and during disease progression in late onset autoimmune diabetes.

Introduction

Latent autoimmune diabetes of adults (LADA) is typically defined as patients diagnosed with diabetes over 35 years of age presenting with clinical features of type 2 diabetes in whom a type 1 diabetes associated autoantibody is detected. This identifies a group with clinical and genetic features that are intermediate between typical type 1 and type 2 diabetes, and has been termed 'slowly evolving immune-mediated diabetes' by the WHO, under the category of hybrid forms of diabetes (1; 2). The identification of this intermediate phenotype has led to the idea that autoimmune diabetes in middle age and old age typically progresses more slowly than autoimmune disease in children and young adults (1).

In this perspective we show how the definition of LADA predominantly using an imperfect diagnostic test, GAD65 autoantibody (GADA) measurement, in low prevalence populations will result in a heterogeneous group of true positives (type 1 diabetes) and false positives (type 2 diabetes). This mixed group would explain how the clinical features (e.g. BMI, HbA1c, time to insulin treatment) of a group of individuals with LADA average out to be intermediate between the two subtypes, an alternative explanation of the intermediate phenotype observation. We explore how observations in descriptions of LADA more strongly support the presence of individuals with type 1 and 2 diabetes rather than an intermediate condition. While we focus on GADA because of its predominant role in the definition of LADA, the issues described will affect any diagnostic test with imperfect specificity, including other islet autoantibody assays.

A historical perspective – LADA as an intermediate phenotype between typical type 1 and type 2 diabetes.

The recognition that pancreatic islet autoantibodies play a key role in defining the discrete and separate aetiologies of Type 1 and Type 2 diabetes occurred in the 1970s (3; 4). It was recognized

that the islet cell autoantibodies (ICA) could be used to confirm a diagnosis of Type 1 diabetes in children and young adults. Subsequent studies demonstrated that some older patients with initially non-insulin dependent diabetes were also ICA positive and that this subgroup had more rapid progression to insulin (5-7). Following the discovery of insulin antibodies in 1983 (8), GADA was the second specific islet autoantibody to be recognised in 1990 (9). Subsequently, GAD autoantibodies were described in a minority of patients who were diagnosed after 30 years of age and thought to have Type 2 diabetes, without initial insulin requirement (10; 11). This group of patients was called latent autoimmunity of adults (LADA) (11). These individuals were found to require insulin treatment earlier and more frequently than people diagnosed with diabetes at a similar age without GAD autoantibodies (GADA) (12). However, this was far from the 100% rate of requiring insulin treatment seen in young subjects with positive antibodies who had typical Type 1 diabetes (13). This led to the concept of LADA as adults with autoimmune diabetes and a less-aggressive disease, with an intermediate phenotype between Type 1 diabetes in children and Type 2 diabetes in middle and old aged adults (1). The LADA definition was supported by genetic studies showing that there was an over representation of HLA and non-HLA childhood Type 1 susceptibility alleles as well as of Type 2 diabetes susceptibility alleles in this group (1; 14-16).

The definition of LADA in some cohorts includes autoimmunity defined by a number of different islet autoantibodies, however the vast majority of cases are identified by positive GADA alone due to other antibodies being less frequently tested, and positive results infrequent in GADA negative older adults (17; 18). A number of studies have attempted to define the pathophysiology, epidemiology and complication risk of these patients (1). This has been limited by a lack of standardisation of antibody tests used to define patients, particularly the titre deemed to represent positivity, though considerable effort has been undertaken to address this issue with improvement in assay performance in recent years. While recent reviews have recognised the heterogeneity of this

condition the broad concept of LADA as an intermediate form of autoimmune diabetes has persisted

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Measurable GAD autoantibodies occur in non-diabetic control populations: a detectable GAD autoantibody does not always confirm autoimmune diabetes aetiology.

Like many immunological tests, the presence of detectable GADA does not confirm the presence of disease – detectable GAD autoantibodies are present in people without diabetes (with the level dependant on the assay, population, and threshold used), with presence of GAD alone associated with low risk for development of type 1 diabetes (19-24). This can been termed 'biological false positive' or 'diabetes irrelevant' islet autoantibodies, with previous studies demonstrating these antibodies may have different epitope specificity (20). The term 'biological false positive' is used to describe detectable islet autoantibodies not associated with autoimmune disease; this does not usually imply a test error and it is widely recognised that antibodies for many autoimmune conditions may be present in healthy people who do not have associated pathology or, in the majority of cases, go on to develop the associated disease (22; 25-27).

The proportion of those who do not have autoimmune aetiology diabetes who test positive for an antibody is determined by the test specificity, which will depend upon both the assay characteristics, and the threshold chosen to define positivity – for example an assay threshold yielding 95% specificity in similar control subjects would be expected to be positive in 5% of the population, including those with non-autoimmune (type 2 or monogenic) diabetes.

GAD assays prior to the last decade have not always been as technically reliable as more recent assays: median GAD specificity for the 39 laboratories participating in the 2010 Diabetes Autoantibody Standardisation Programme was 94%, with specificity ranging from 68 to 100% (20). This means that at that time, in a population without any autoimmune diabetes (such as those with

true type 2 diabetes), using an assay with average specificity for that exercise, a median of 6% would be GAD islet antibody positive, but this will vary from 32% to <1% depending on the assay and threshold used. This alone may explain much of the heterogeneity in LADA prevalence and characteristics in reported literature: Studies using an islet autoantibody assay and cut off with limited diagnostic specificity are will include many participants with positive islet autoantibodies who have diabetes that is not of autoimmune origin. In these cases, therefore, prevalence of LADA will be high, and characteristics less classical for type 1 diabetes when compared with studies using high specificity assays. There has been a marked improvement in assays performance in recent years, with median specificity improving to 98.9% for participating laboratories in the 2018 IASP standardisation exercise, however variation in performance persists. (28)

The implications of positive islet autoantibody will be very different in populations with high and low prevalence of autoimmune diabetes.

The prevalence of autoimmune diabetes in the population tested with an islet autoantibody will markedly alter the implications of a positive result, even where a high specificity assay is used. The positive predictive value (the proportion (%) who have the disease when the test is positive) of a biochemical test can be dramatically different dependent on the background prevalence of the disease it is aiming to detect. In cases where the disease prevalence is low, the positive predictive value (PPV) of a test will be lower. This supports the idea that those who test positive for GADA in a population with low prevalence of type 1 diabetes will be a mixture of true positives (type 1 diabetes) and false positives (type 2 diabetes). This is in marked contrast to populations with a high prior likelihood of type 1, such as children and adolescents presenting with diabetes, where false positives will be low.

We illustrate this point by examining the proportions of true and false positive GAD autoantibody results in two populations: one with 95% autoimmune aetiology diabetes and one with 5% autoimmune aetiology diabetes. Based on the median performance of the 2010 Diabetes Autoantibody Standardisation Program, GAD autoantibodies had 94% specificity and 86% sensitivity for detecting autoimmune diabetes. Figure 1 shows how in a population with high prevalence of autoimmune diabetes, out of 100 individuals, we would anticipate 95 autoimmune and 86% of these will be GAD positive, but we would not expect any false positives (6% of 5 = 0.25). In contrast, for a low (5%) prevalence population, out of 100 patients, we would expect only 5 to be autoimmune, and 4 of these GAD positive, but we would also anticipate a similar number of GAD positive non-autoimmune (6% of 95 = ~6). While GADA assays have continued to improve in recent years, even with a high performance assay false positive results will remain common where autoimmune diabetes is infrequent. Using the 2018 islet antibody program median performance (specificity of 98.9%, specificity 69%), with 5% prevalence of autoimmune diabetes, based on the same calculations, 23% of those with a positive GADA will have a false positive result.

The positive predictive value a test of a given specificity and sensitivity, at a given disease prevalence can be calculated this equation (29):

Positive predictive value = $\dfrac{\text{sensitivity x prevalence}}{(\text{sensitivity x prevalence}) + ((1 - \text{specificity}) \times (1 - \text{prevalence}))}$

We can use this to calculate the positive predictive value in populations with different prevalence of type 1 diabetes, for assays with different performance (Figure 2). If we use autoantibodies in a patient who is diagnosed with diabetes under the age of 20, when approximately 95% of patients will have type 1 diabetes, using this prevalence the positive predictive value can be calculated to be >99% (Figure 2) even when using a low specificity assay and threshold. This means that in this

setting, false positive tests are rare (<1%). This explains why, in children where most diabetes is type 1, a positive islet autoantibodies will confirm that the diagnosis is highly likely to be type 1 diabetes.

However, with increasing age, the amount of type 2 diabetes increases dramatically, such that in middle age (40-60 years), less than 5% of incident patients have type 1 diabetes (30). In this setting false positive results will be common, even with a high specificity assay (Figure 2). Therefore the group of patients defined by positivity for GADA will consist of two subpopulations: adult-onset autoimmune diabetes (true positives) and adult-onset type 2 diabetes (false positives). In this case, the phenotype of the group will lie between the phenotype of type 1 and type 2 diabetes, with the proportion of those with autoimmune (Type 1) and non-autoimmune (Type 2) diabetes varying with assay performance and prior prevalence. The characteristics of those with positive GADA will therefore be somewhere between type 1 and type 2 diabetes, but this does not reflect a subgroup with a true intermediate phenotype, but rather the average of the two subpopulations.

The prevalence of LADA when islet autoantibody specificity is robustly characterised suggests that the majority of those meeting current definitions of LADA do not have autoimmune diabetes

An estimate of the proportion of a LADA population who are unlikely to have autoimmune aetiology diabetes can be obtained by examining the extent to which the prevalence of antibody positive individuals in a population with apparent type 2 diabetes exceeds the expected prevalence in a population without diabetes. Figure 3 shows the proportion of 3 populations testing positive for GADA using the same assay and laboratory: non-diabetic controls, new onset clinically diagnosed type 2 diabetes and longstanding type 2 diabetes with absence of early insulin requirement. This assay and threshold had 100% specificity in both the 2018 islet autoantibody standardisation programme (IASP) (n<100), but has 97.5% specificity in this much larger (n=1500) control sample, meaning 2.5% of those without autoimmune diabetes will test positive. In newly diagnosed type 2 diabetes, and longstanding type 2 diabetes respectively, 4.1% and 3.3% of participants were GADA

positive, only modestly higher than the expected 2.5% positive rate in those without autoimmune aetiology diabetes. This is consistent with the majority of GADA positive diabetic individuals in these cohorts having diabetes that is not of autoimmune aetiology, despite use of a high performing modern assay. Previous large studies of LADA that have reported standardisation program assay performance have been broadly consistent with this finding (18; 31-33).

Studies of LADA are more consistent with a heterogeneous population of type 1 and Type 2 diabetes rather than a single intermediate phenotype

We can test if observations of LADA patients fit more with a heterogeneous population of Type 1 and Type 2 patients rather than a single intermediate phenotype. If this is the case then factors altering the specificity and sensitivity of the antibody test, and altering prevalence of type 1 diabetes in the population tested, will alter the positive predictive value of the antibody test and hence alter the proportion of false positive (type 2 patients) within the cohort defined by positivity for the antibody test. The lower the prior prevalence, or lower the test threshold (and so specificity) the more false positive (type 2 diabetes) and the less true positive (type 1/autoimmune aetiology) patients will be contributing to the phenotype. Hence the combined cohort will be more type 2 like, for example diagnosed older, have an increased BMI and be less likely to progress to insulin. These predicted changes can be compared to the changes seen in cohorts of LADA patients.

a) How do alterations in the definition of antibody positivity alter the phenotype in LADA?

The specificity and sensitivity of autoantibody testing can be altered by changing the number of antibodies tested or the titre of GADA considered as positive. These changes in sensitivity and specificity in turn alter the positive predictive value. We would predict that with less specific antibody tests, not only does the number of LADA patients increase, but also the phenotype moves to being more type 2 like.

Studies that have examined the relationship between titre and/or number of autoantibodies and clinical phenotype in late onset initial non-insulin requiring diabetes are summarised in Table 1. As shown in Table 1 a number of studies have shown that those who have low titres have clinical, biochemical and genetic characteristics more similar to type 2 diabetes, in contrast to those with higher titres (13; 18; 32-38). In addition low titres often become negative during follow up, in contrast to those positive with high titres (39). Where late onset autoimmune diabetes is defined by two positive autoantibodies (which will markedly increase specificity) a patient group is identified with very high rates of rapid insulin requirement, and the clinical and genetic characteristics of young onset type 1 diabetes (13; 14).

In populations with a much higher prior prevalence of type 1 diabetes, such as children and young adults with diabetes, the impact of altering test specificity using higher titres, or multiple positive islet autoantibodies, is modest (13; 40; 41). These findings are consistent with the influence of false positive results being greater in low prevalence populations (discussed below). In high prevalence populations even those with a weak positive antibody test will have a high probability of type 1 diabetes: test specificity will therefore be less critical to positive predictive value, and will have less effect on the characteristics of the test positive population (42).

b) *How do differences in the prevalence of type 1 diabetes in the population in which LADA is defined alter the phenotype?*

Factors that alter the prevalence of the proportion of type 1 patients will alter the positive predictive value of the antibody test and hence the proportion of type 1 and type 2 patients defined by antibody positivity. The easiest example is the age of the cohort that patients are taken from.

Type 2 diabetes is markedly more common with increased age therefore, as demonstrated in figure 1 and 2, a positive islet antibody will be far less likely to be a false positive result in younger people, where the prior probability of autoimmune diabetes is higher (30). This means the younger the

population tested, the more type 1 like it is predicted to be. This has been seen in many studies, for example in the UKPDS study of patients with a diagnosis of type 2 diabetes young single autoantibody positive participants had rapid progression to insulin, in marked contrast to older participants (13).

Other clinical criteria that make type 1 diabetes more or less prevalent will also alter the positive predictive value of a positive GAD islet autoantibody. For example, the frequently used exclusion of those patients treated with insulin within 6 months of diagnosis will reduce the number of true positives (type 1/autoimmune diabetes) and increase the number of false positives (type 2 diabetes), making the combined phenotype more type 2 like.   This effect has been clearly seen in large series when looking at clinical criteria like age of onset, BMI, and time to insulin treatment, where selecting a sub-population with lower prior probability of type 1 diabetes (for example an older, more obese, or non-insulin treated population) results in fewer GAD positives, and these participants will have characteristics mores similar to type 2 diabetes (18; 38; 43; 44). This is also apparent when using genetic susceptibility to type 1 diabetes: antibody positive 'type 2 diabetes' patients who lack genetic susceptibility to type 1 diabetes (and therefore have low prior probability) have low rates of early insulin requirement, in contrast to those at high type 1 diabetes genetic risk (45).

c) Bimodality of GAD autoantibody titres and differences in epitope specificity support the presence of 'true' (disease associated) and 'false' (disease irrelevant) positive islet autoantibody results

Antibody studies looking at both titre and epitope support LADA consisting of 2 sub-populations. A number of studies have reported a bimodal distribution of GAD autoantibody titre, suggesting two subpopulations with low and high GAD titre (18; 33; 34; 36; 38). Low titre individuals commonly become antibody negative on follow up, in contrast to those with high titre (39). Recently it has

been shown that positive GAD autoantibodies that are not associated with disease have different epitope specificity, and that in 'LADA' different epitope specificities identify different subpopulations: patients who are GAD positive to standard full length assays, but not to assays using GAD truncated to remove the N-terminus, have characteristics similar to antibody negative type 2 diabetes (20; 46; 47).

Definitions of late onset type 1 diabetes that are independent of islet autoantibody testing and clinical features do not suggest an intermediate phenotype.

Recent research has examined the characteristics of late onset autoimmune diabetes defined by examining the characteristics of excess diabetes occurring in those who are genetically susceptible (48). This technique does not suffer from either the problem of false positive islet antibody results, or the problem of presupposing characteristics if a definition is based on clinical features. In marked contrast to autoimmune diabetes defined by GAD testing, older participants with type 1 diabetes caused by excess genetic risk appeared to have near universal early insulin requirement, with 89% treated with insulin within the first year of diagnosis and 11% developing ketoacidosis. When type 1 diabetes in later life is defined by the development of severe, near absolute, insulin deficiency the clinical phenotype is very similar to that defined by this genetic technique (49).

The presence of a biomarker that can occur in the absence of disease should not define a disease state

It is our opinion that the presence of islet autoantibodies in populations with low Type 1 diabetes prevalence should not be considered to equate to a diagnosis of autoimmunity in that individual. Autoantibodies are a marker of autoimmunity and not the pathogenic agent (50). In other autoimmune diseases such as Systemic lupus erythematosus and Rheumatoid Arthritis, the presence of the highly associated autoantibody (which, like diabetes autoantibodies, may occur in healthy

control populations) is not sufficient to make a diagnosis on its own and other clinical, biomarker or imaging criteria are needed to be met to make the diagnosis (27).

Practical implications for clinicians

For clinicians the take away message is that islet autoantibodies have far greater diagnostic utility when tested in patients with a clinical suspicion of Type 1 diabetes. In those with clinical features suggestive of type 1 diabetes a positive islet antibody result, using a modern validated assay, will usually confirm a diagnosis of autoimmune diabetes. However, in an adult without clinical features of type 1 diabetes, as we have shown, it is most likely that a single positive antibody will represent a false positive result. When considering a result at diagnosis prediction model approaches that combine islet antibodies with other features may offer a practical approach for a clinician to assess the predictive value of a positive autoantibody (51). Given the uncertainty at diagnosis in insulin-treated patients C-peptide measurement, preferably >3-5 years after onset, is critical to establish treatment requirements where diabetes subtype is uncertain (52; 53)

Implications for researchers

To address any research question related to autoimmune diabetes in adults it is essential that the population studied has autoimmune, rather than type 2 diabetes.

 Where autoantibodies are used to define autoimmune diabetes the performance of the assay used should be robustly demonstrated, as this is critical to interpretation of research findings (28). While modern islet autoantibody assay quality has increased, unusually high specificity is required where the prior probability of autoimmune diabetes is low. Therefore if antibodies alone are used to define autoimmune diabetes in a low risk population, such as adults with apparent type 2 diabetes, specificity should be increased. This could be achieved through requirement for multiple positive autoantibodies to define autoimmunity, and/or through the use of assays with restricted epitope

specificity (20; 46). An alternative approach would be to use clinical features or other biomarkers (such as genetic risk scores or C-peptide) to increase prior probability of autoimmune diabetes, either alone or through prediction models which combine multiple features. Ultimately the optimal approach will depend on the research question being addressed, for example the use of clinical features or genetic risk to increase prior probability will not be appropriate for studies assessing these outcomes, but may be appropriate for unrelated research questions.

Conclusion

Autoimmune diabetes in later life, and its diagnosis, is an important and challenging clinical problem. We have shown that observations in LADA, where autoimmune diabetes has been diagnosed on the basis of the presence of GAD islet autoantibodies in low prevalent Type 1 populations, can be predominantly explained by the test identifying a mixture of true positive (Type 1) patients and false positive (Type 2) patients. Specifically LADA patient's intermediate phenotype will at least partly reflect a combination of 2 heterogeneous populations with very different phenotypes, rather than a true intermediate subtype of diabetes. Improvement of the diagnostic approach by applying new findings from the field will greatly improve classification from the pioneering work that led to the first descriptions of LADA more than 25 years ago.

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Tables:

Table 1: Summary of studies reporting the impact of differing GADA titre and/or number of positive islet autoantibodies on the prevalence and associated characteristics of LADA. Higher titre and/or antibody number (and so higher test specificity) lead to characteristics more similar to T1D. dx = diagnosis, m= months, y = years, c-pep = C-peptide, TG = Triglyceride, avg = average.

Study	n	Inclusion criteria	Antibody status and threshold (% of population)	% of study population	Positive predictive value for insulin treatment	Age at dx (years)	BMI(kg/m^2)	C-pep(nmol/l)	TG(mmol/L)
Turner 1997 (13)	1538*	Age 25-65, diagnosis of T2D	GAD and ICA neg	88%	14% at 6 y	-	-	-	-
			GAD 20-60u/L	4.0%	23% at 6 y	-	-	-	-
			GAD >=60u/L	7.1%	63% at 6 y	-	-	-	-
			GAD&ICA low titre	4.3%	77% at 6 y	-	-	-	-
			GAD&ICA high titre	3.0%	89% at 6 y	-	-	-	-
Tuomi 1999 (35)	1122	Clinical diagnosis of T2D	Gad <5 RU	90.8%	-	-	27**	0.62	2.0**
			GAD 5-38 RU	6.2%	-	-	27**	0.55	1.6**
			GAD >38 RU	3.0%	-	-	25**	0.27	1.5**
Davis 2000 (36)	1225	Diagnosis T2D, at >age 60; or no initial insulin	GAD neg	96.3%	11.2% at avg 3y	61.1	29.6	-	1.9
			GAD 20-60u/L	1.4%	17.6% at avg 2.3y	58.4	27.4	-	2.0
			GAD >60u/l	2.3%	46.4% at avg 5.3 y	59.7	27.0	-	1.3
Genovese 2006 (37)	881	Diagnosis T2D age 40-70, Hospital based.	Gad neg (<3 U)	95.4%	20.2% at avg 8.1y	52.0	29.2	-	-
			3-10 U	-	42% at avg 8.7y***	-	-	-	-
			>10 U	-	73.9% at 8.7y***	-	-	-	-
			GAD >3U & IA2	2.2%	78.9% at 8.7y***	-	-	-	-
Buzzetti 2007 (34)	4248	35-75y at diagnosis, not insulin treated, duration 6m to 6 y	GAD neg	95.5%	-	55.5	29.9	-	-
			GAD pos <32Units	2.3%	-	51.1	28.4	-	-
			GAD pos >32U	2.2%	-	49.1	26.2	-	-
Maoli 2010 (38)	5568	T2D age 35-70, no insulin 8m post diagnosis Duration<5y	GAD neg	95.1%	-	57.7	30.8	-	1.4
			GAD pos index<0.5	2.4%	-	55.4	28.8	-	1.3
			GAD pos index>0.5	2.5%	-	53.4	26.9	-	1.3
Hawa 2013 (18)	6156	Age 30-70, no insulin rx for 6m from diagnosis	GAD neg	90.2%	13.2% at avg 2.3y	54.9	30.9	-	2.0
			GAD 70-200 WHO U	2.2%	39.7% at avg 2.5y	47.9	28.5	-	1.8
			GAD >200 WHO U	6.5%	54.6% at avg 2.1y	47.0	26.7	-	1.2
Zhou 2013 (33)	4880	Onset >30y, no DKA, no insulin by 6m, recruit by 1y of dx	GAD negative	94.1%	-	51.4	24.8	0.64	1.8
			18 to 180 WHO U	4.3%	-	51.2	24.5	0.51	1.6
			>180 WHO U	1.6%	-	48.1	22.3	0.32	1.2
Maddaloni	17072	Onset age 30-70y, no DKA,	GAD & IA2A neg	97.3%	-	46.9	31.4	-	
			GAD >10u/ml	2.6%	21.8% at 5y	45.1	30.7	-	

| 2015 (32) | | no insulin rx by 6 mo of dx | GADA >10u/ml and IA2a 10u/ml | 0.1% | - | | 41.2 | 28.5 | - | |

- Not reported *randomised to non insulin therapy ** approximate value from article figure (reported graphically only) *** duration not reported separately, therefore duration for antibody positive overall provided

Figure Legends

Figure 1: Proportion of GAD positive individuals who have autoimmune aetiology diabetes in a 95% and 5% prevalence population. Expected results from testing 100 participants, using median GAD assay performance from the 2010 Diabetes Autoantibody Standardisation Program (assay specificity 94%, sensitivity 86%).

Figure 2: The effect of prior prevalence and assay performance on GAD positive predictive value. Calculated as described in section 3 for GAD assays with the following characteristics: 94% specificity 86% sensitivity (Diabetes Autoantibody Standardisation Program 2010 median performance), 97.5% specificity 74% sensitivity, 99% specificity 69% sensitivity (Diabetes Autoantibody Standardisation Program 2018 median performance).

Figure 3: Excess prevalence of GADA in participants with clinically diagnosed diabetes in comparison to a non-diabetic control population. GADA assessed using the RSR limited (Cardiff, UK) bridge ELISA by the Blood Sciences Department of Royal Devon and Exeter Hospital, Exeter, UK. A value >10 units was considered positive.

 * No known diabetes, HbA1c <48mmol/mol, n=1500 (45)

aged >18 at diagnosis, clinical diagnosis of type 2 diabetes, median duration 3 months (Jones et al unpublished, https://clinicaltrials.gov/ct2/show/NCT03737799)

^ Age ≥35 at diagnosis, clinical diagnosis of type 2 diabetes, absence of insulin requirement within 6 months of diagnosis (45)

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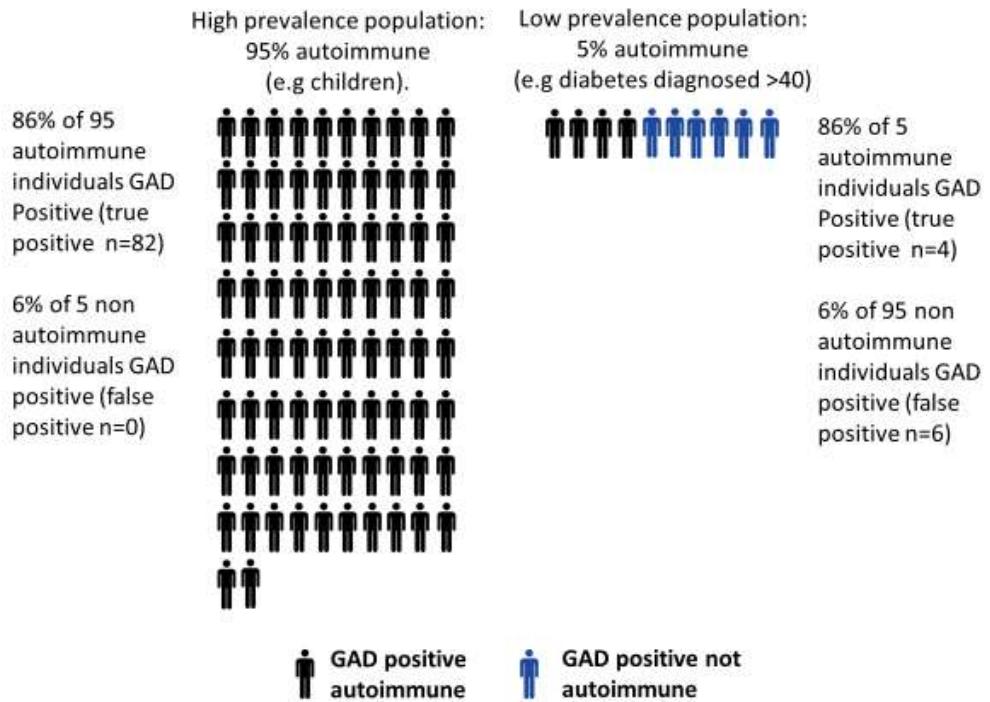
Figure 1

High prevalence population: 95% autoimmune (e.g children).

Low prevalence population: 5% autoimmune (e.g diabetes diagnosed >40)

86% of 95 autoimmune individuals GAD Positive (true positive n=82)

6% of 5 non autoimmune individuals GAD positive (false positive n=0)

86% of 5 autoimmune individuals GAD Positive (true positive n=4)

6% of 95 non autoimmune individuals GAD positive (false positive n=6)

GAD positive autoimmune

GAD positive not autoimmune

Figure 2

The effect of prior prevalence and assay performance on GAD positive predictive value

Positive predictive value (y-axis): 0.0% to 100.0%

Proportion autoimmune diabetes (x-axis): 98%, 95%, 90%, 80%, 50%, 20%, 10%, 5%, 2%

Specificity 94% Sensitivity 86% — Specificity 97.5% Sensitivity 74% — Specificity 99% Sensitivity 69%

Figure 3

Percent GADA positive

Control population without diabetes (n=1500)*
Newly diagnosed type 2 diabetes (n=883)#
Long duration type 2 diabetes (n=8606)^

■ Excess prevelence - autoimmune aetiology diabetes
■ Expected prevalence in absence of autoimmune diabetes (assay specificity 97.5%)