Routine islet autoantibody testing in clinically diagnosed adult-onset type 1 diabetes can help identify misclassification and the possibility of successful insulin cessation.

Short running title: Islet autoantibodies in adult onset type 1 diabetes

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Abbreviations

AA	Islet Autoantibody
ADA	American Diabetes Association
AGLT	Adjuvant Glucose Lowering Therapy
BMI	Body Mass Index
EASD	European Association for the Study of Diabetes
GAD	Glutamic Acid Decarboxylase
IA-2	Insulin Antigen-2
IQR	Interquartile range
MODY	Maturity-Onset Diabetes of the Young
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
T1D-GRS	Type 1 Diabetes Genetic Risk Score
UCPCR	Urinary C-Peptide Creatinine Ratio
ZNT8	Zinc Transporter 8

Abstract

Objective

Recent joint American and European diabetes association guidelines recommend routine islet autoantibody testing in all adults newly diagnosed with type 1 diabetes. We aimed to assess the impact of routine islet autoantibody testing in this population.

Research Design and Methods

We prospectively assessed the characteristics and progression (annual change in Urine C-peptide Creatinine Ratio (UCPCR)) associated with islet autoantibody status (GAD, IA-2 and ZNT8) in 722 adults (≥ 18 years old at diagnosis) with clinically diagnosed type 1 diabetes and duration <12 months. We also evaluated changes in treatment and glycaemia over 2 years after informing participants and their clinicians of autoantibody results.

Results

24.8% (179/722) of participants diagnosed with type 1 diabetes were autoantibody negative. This group had genetic and C-peptide characteristics suggestive of a high prevalence of non-autoimmune diabetes: lower mean type 1 diabetes genetic risk score (islet autoantibody negative versus positive: 10.85 vs 13.09 (p<0.001) (type 2 diabetes 10.12)); lower annual change in C-peptide (UCPCR) -24% vs -43% (p<0.001).

After median 24-months follow up, treatment change occurred in 36.6% (60/164) of autoantibody negative participants: 22.6% (37/164) discontinued insulin, with a HbA1c similar to those continuing insulin (57.5 vs 60.8mmol/mol [7.4 vs 7.7%], p=0.4) and 14.0% (23/164) added adjuvant agents to insulin.

Conclusions

In adult-onset clinically diagnosed type 1 diabetes, negative islet autoantibodies should prompt careful consideration of other diabetes subtypes. When routinely measured negative antibodies are associated with successful insulin cessation. These findings support recent recommendations for routine islet autoantibody assessment in adult-onset type 1 diabetes.

Introduction

Identifying type 1 diabetes in adults can be challenging. Clinical features that can help distinguish type 1 and 2 diabetes frequently overlap, and the high prevalence of type 2 diabetes means even classical features of type 1 diabetes, such as low BMI or ketoacidosis, may not confirm the diagnosis (1-5). As a result, misclassification in adults is common: studies using biomarker-based approaches suggest that approximately 1 in 3 adults developing type 1 diabetes are initially diagnosed as type 2 diabetes, and 1 in 6 diagnosed with type 1 diabetes do not have this condition (6-9).

Recommendations for education, treatment and monitoring in different diabetes subtypes vary markedly. Misclassifying type 2, or maturity-onset diabetes of the young (MODY) cases as type 1 diabetes can therefore result in suboptimal clinical management, inappropriate education, potentially unnecessary insulin treatment, and reduced access to agents shown to have cardiovascular benefits in type 2 diabetes. Routine C-peptide testing in those with clinically diagnosed type 1 diabetes has recently been shown to lead to reclassification and insulin withdrawal in many patients, but C-peptide testing is likely to have limited utility at diagnosis, as levels may be retained at diagnosis in type 1 diabetes (6, 10).

Islet autoantibody testing may assist differentiation of type 1 diabetes from other diabetes subtypes (11-13), and has maximum utility at diagnosis, as levels can decline in longstanding disease. Recent guidance by the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) have now recommended islet autoantibody testing at diagnosis in all adults with clinically suspected type 1 diabetes, however the clinical impact of routine testing in this population has not been directly assessed (14).

We aimed to assess whether absence of positive islet autoantibodies in recent onset clinically diagnosed adult-onset type 1 diabetes is suggestive of misclassification and whether feedback of routinely measured islet autoantibodies is associated with subsequent change in patient treatment.

Methods

We used longitudinal data from the prospective StartRight study, https://clinicaltrials.gov/ct2/show/NCT03737799, to explore the effect of islet autoantibody status on clinical, biochemical and genetic characteristics in patients with clinically diagnosed type 1 diabetes treated with insulin from diagnosis. A reference population from the same study, of islet autoantibody negative participants diagnosed with type 2 diabetes and not treated with insulin from diagnosis, were also included in this analysis.

Participants

1798 adult (≥ 18 years) participants diagnosed with diabetes mellitus (of any type, excluding gestational diabetes, known secondary diabetes and pregnancy at the time of recruitment) within the previous 12 months were recruited from 55 National Health Service (NHS) sites in the United Kingdom. Recruitment took place between August 2016 and February 2020. Participants were followed annually for two years from recruitment. Eligible participants

were identified through: routine clinical care appointments, screening of primary and secondary care records and self-referral after advertisements in various clinical settings, with the majority (57%) of participants identified though secondary care specialist teams (who manage almost all of those with type 1 diabetes in the UK). To ensure a sufficient number of participants with late onset type 1 diabetes the study population was enriched for older individuals with type 1 diabetes by aiming for equal recruitment of those treated with and without insulin in those over age ≥50 at diabetes diagnosis. A study flow diagram is presented in **Supplementary Materials Figure 1**.

Definition of diabetes type

Clinically diagnosed type 1 diabetes was defined as a self-reported clinical diagnosis with concurrent treatment with insulin at recruitment (N=722/1798). Confirmed type 2 diabetes was defined as a self-reported clinical diagnosis of type 2 diabetes with absence of insulin treatment at recruitment, and negative islet autoantibodies (N=731/1798). Participants were considered to have 'suspected type 1 diabetes' when they were treated with insulin at recruitment and the self-reported diagnosis indicated clinical uncertainty (for example 'uncertain type' 'possible type 1 diabetes' 'likely type 1 diabetes' 'possible type 2 diabetes') (N=91/1798).

Participants were excluded from analysis (14.1% of recruited participants [N=254/1798]) if (1) they had a reported diagnosis of type 2 diabetes and were either receiving insulin at recruitment (N=85) or not receiving insulin but were islet autoantibody positive (N=84), or (2) where there was clinical uncertainty (or other diabetes type) in a participant's self-reported diagnosis and they were not receiving insulin at recruitment (N=85) (see **Supplementary Materials Figure 1)**.

Data collection

Diabetes type, initial diabetes treatment, symptoms at diagnosis (thirst, polyuria and patient reported weight loss) and concurrent auto-immune conditions were self-reported at recruitment/baseline (median duration of diabetes at recruitment 4.0 months). At baseline participants medical notes and laboratory records were reviewed by a research nurse to confirm diagnosis details, and biochemistry (HbA1c, glucose) at diagnosis. Diagnosis notes were examined for evidence of diabetic ketoacidosis (DKA), including discharge diagnosis, measured ketones and pH. At study recruitment a non-fasted (within 1-5 hours of a meal) blood sample was collected and analysed for: serum C-peptide, islet autoantibodies (glutamic acid decarboxylase [GAD], zinc transporter 8 [ZNT8], islet antigen 2 [IA2]) and DNA extraction performed for generation of a type 1 diabetes genetic risk score. Baseline BMI was calculated from weight and height measured at the baseline visit.

At each annual visit including recruitment, participants collected a urine sample for urine c-peptide creatine ratio (UCPCR) measurement (15, 16). Samples were collected post home meals. Participants were asked to fully empty their bladder immediately pre-meal and collect urine in containers with boric acid preservative 2 hours after meal completion. The sample was then posted directly to the Exeter Clinical Laboratory for analysis.

Participants were then contacted by telephone or email 1 year (within a minimum/maximum range of 10-16 months) and 2 years (within a minimum/maximum range of 22-28 months) post recruitment, to record concurrent treatment, treatment changes and health service

utilisation, including hospital admission for diabetes related illness. Post recruitment HbA1c results were obtained by the research team from healthcare records.

Laboratory Analyses

Laboratory results at diabetes presentation, and post recruitment HbA1c values, were obtained from participants healthcare records as previously stated. All other biochemical analysis was undertaken by the academic department of blood sciences at the Royal Devon and Exeter Hospital, Exeter, UK. GADA, IA2A and ZNT8A islet autoantibodies were measured using the RSR ELISA assay (Cardiff, U.K) on the Dynex DS2 automated ELISA system (Dynex Technologics, Worthing, U.K.). Islet autoantibodies were considered positive if exceeded the 97.5th centile of a cohort of 1559 non-diabetic population controls: GADA ≥11 units/mL, IA2A ≥7.5 units/mL and ZNT8A ≥65 units/mL in those aged up to 30 years and ≥10 units/mL in those aged ≥30 years (17, 18). These assays and thresholds have 97.5% (GADA and ZNT8A) and 99% (IA2) specificity based on the above analysis of the 1559 people without diabetes in our local population. In the 2020 international islet autoantibody standardisation program certification of the Exeter clinical laboratory these assays and thresholds have 99% specificity for all three islet autoantibodies (GADA, IA2A and ZNT8A). Sensitivity was 74% for both GADA and ZNT8A and 72% for IA2A.

C-peptide (blood and urine) was measured using the automated Roche diagnostics (Manheim, Germany) E170 immuno-analyser (limit of detection 3.3 pmol/l, inter- and intra-assay coefficients of variation < 4.5 % and < 3.3 %, respectively). Urine creatinine (for UCPCR) was analysed using the Jaffe method on the Roche P800 modular analyser.

Reporting of islet autoantibody results to participants and clinicians.

Islet autoantibody results were reported to both the participant and the treating primary and secondary (if applicable) care clinician following the study baseline visit. The result reporting forms included basic advice on result interpretation, with the reporting forms shown in **Supplementary Figures 5 & 6**. Baseline C-peptide, and follow up results such as UCPCR, were not reported. The management of each participant was left entirely to the discretion of the clinician responsible for their care.

Type 1 diabetes genetic risk score

A type 1 diabetes genetic risk score (T1D-GRS) based on 67 single nucleotide polymorphisms (SNPs) associated with type 1 diabetes, and accounting for interactions between 18 HLA DR-DQ haplotype combinations, was generated as previously described (19). SNPs were directly genotyped by LGC genomics, as previously described (20). In this study the T1D-GRS was only included for participants with a White European ethnicity, as it has not been fully validated for other ethnicities. This result was not reported to the participant or clinician.

Statistical analysis

Due to the skewed nature of the C-peptide and UCPCR data, all values were natural log transformed for analysis in line with previous studies (21-24), which also allowed for a linear fit when assessing change over time after diagnosis. The geometric mean and 95% confidence intervals for these variables is presented in the tables. For modelling annual

UCPCR the intercept and slopes were determined using mixed effect models as described previously (21), with random effects at the patient level to allow each patient to contribute multiple C-peptide values at different time points. The benefit of this random-intercept, random-slope model is that it allows for variability between individuals in terms of both C-peptide level at diagnosis (the intercept) and in the percentage change in C-peptide over time (the slope). Groups defined by reported diabetes type, treatment and antibody status were separately assessed using an interaction term within the mixed effects model.

Due to the slope being on a log scale, coefficients were interpreted in terms of the percentage change per year (Calculated from the exponential of the ß-coefficient-1). The half-life of C-peptide was calculated from $\log_e(0.5)/ß$. The variability of individual slopes in the longitudinal models was determined using the SD range (calculated by back transforming the ß-coefficient ± 1 SD of the slope).

Comparisons of clinical characteristics between islet autoantibody positive and negative participants were assessed using two sample t tests for continuous data and Pearson Chi-squared test for categorical data. The relationship between type 1 diabetes genetic risk score and longitudinal C-peptide loss (annual change in natural log UCPCR) was assessed using mixed effects models with random effects at the patient level, with percentage change per year calculated from the beta coefficient as described above. Statistical significance was defined as $p < 0.05$. All analysis was carried out in Stata version 14.2 (StataCorp LLC, Texas, USA) and GraphPad Prism version 9.1.2 (GraphPad Software, California, USA).

Results

Of included participants 46.8% (722/1544) were classified as having clinician diagnosed type 1 diabetes. Of these participants 24.8% (179/722) were negative for all three islet autoantibodies. A detailed breakdown of the islet autoantibody composition is shown in **Supplementary Materials Figure 2**. Of the included participants 47.3% (731/1544) were classified as having confirmed type 2 diabetes and 5.9% (91/1544) as 'suspected type 1 diabetes'. Treatment status at approximately 1 year and/or 2 years post recruitment was available in 91.1% (N=1407/1544) of individuals – see **Supplementary Materials Figure 1,** with median follow up duration 24 months (IQR 22, 26).

The clinical & genetic characteristics of islet autoantibody negative clinically diagnosed type 1 diabetes suggests the inclusion of individuals with misclassified (type 2) diabetes.

Compared to islet autoantibody positive participants diagnosed with type 1 diabetes, autoantibody negative participants were older (age at diagnosis, autoantibody negative versus positive respectively, 42.7 [95% CI 40.6, 44.8] vs 38.2 [95% CI 37.0, 39.4] years) with a higher BMI (27.4 [95% CI 26.6, 28.2] vs 25.0 [95% CI 24.7, 25.4] kg/m^2), a lower prevalence of concurrent autoimmune conditions (3.9% [95% CI 1.1, 6.8] vs 16.3% [95% CI 13.2, 19.4]) and more likely to be male (72.9% [95% CI 66.3, 79.5] vs 50.5% [95% CI 46.2, 54.7]) (all p <0.001), as shown in **Table 1**. Rates of DKA and osmotic symptoms were broadly similar, however glucose at diagnosis was higher in antibody negative participants, and reported weight loss lower (both p<0.05). The characteristics of the control cohort diagnosed with type 2 diabetes are shown in **Supplementary Materials Table 1**.

The distribution of genetic susceptibility to type 1 diabetes, assessed by T1D-GRS, was bimodal in participants with islet autoantibody negative type 1 diabetes with a mean value of 10.85 (95% CI 10.43, 11.27) and intermediate between those with autoantibody positive type 1 diabetes (13.09 [95% CI 12.92, 13.25]) (p<0.001) and type 2 diabetes (10.12 [95% CI 9.93, 10.32]) (p<0.001), as shown in **Figure 1 part A**.

In participants with a clinical diagnosis of type 1 diabetes, negative islet autoantibodies are associated with a markedly lower rate of C-peptide decline

In the islet autoantibody negative type 1 diabetes cohort, the plasma C-peptide at recruitment was higher (607.6pmol/L [95% CI 500.5, 737.5]) than those with autoantibody positive type 1 diabetes (413.0pmol/L [95% CI 382.1, 446.4]) (p<0.001) but lower than the controls diagnosed with type 2 diabetes (1792.3pmol/L [95% CI 1710.9, 1877.6]) (both p<0.001). This cohort also had a slower progression of C-peptide loss: the annual change in UCPCR in islet autoantibody negative participants diagnosed with type 1 diabetes was -24% [95% CI -14, -32%], with a half-life of 2.6 years, compared to an annual decline of -43% [95% CI -39, -47%], in those who were autoantibody positive (half-life 1.2 years) (p<0.001), as shown in **Figure 1 part B**. Annual change in UCPCR in the control type 2 diabetes cohort was -6% [95% CI -1, -11%], half-life 11.2 years.

In participants with a clinical diagnosis of type 1 diabetes and negative islet antibodies, lower Type 1 diabetes genetic susceptibility is associated with reduced C-peptide decline

In participants with positive islet autoantibodies Type 1 diabetes genetic susceptibility, as assessed by the T1D-GRS, was not associated with C-peptide (UCPCR) decline (p=0.3). In contrast in those with a clinical diagnosis of type 1 diabetes and negative islet autoantibodies higher T1D-GRS was associated with a higher rate of C-peptide loss (p=0.026), with a 1 unit increase in T1D-GRS associated with a 5.8% (95% CI 0.67, 11.1) greater annual decline in UCPCR.

Feedback of negative islet autoantibody results to participants and their clinicians is associated with successful insulin discontinuation

Follow up treatment status was available in 90.2% (164/179) and 89.4% (487/543) of those with clinically diagnosed type 1 diabetes that were negative and positive for islet autoantibodies, respectively. Median follow up duration was 24 months (IQR 22, 26). After feedback of a negative islet autoantibody result, treatment change was seen in 36.6% (60/164) of autoantibody negative participants: 22.6% (37/164) discontinued insulin and 14.0% (23/164) added adjuvant agents to continued insulin. This is shown in the treatment flowchart presented in **Figure 2**. Conversely, in participants where a clinical diagnosis of type 1 diabetes was reinforced by the presence of positive islet autoantibodies, only 7.8% changed therapy: 1.0% (5/487) discontinued insulin, while 6.8% (33/487) subsequently added adjuvant glucose lowering therapies (31 of 33 metformin) to continued insulin.

At the latest follow-up (median=24 months [IQR 22, 26]), the HbA1c was comparable between islet autoantibody negative participants discontinuing insulin relative to those continuing insulin treatment: 57.5 (95% CI 51.6, 63.5)mmol/mol (7.4 [6.9, 8.0]%) vs 60.8 (95% CI 56.2, 65.4)mmol/mol (7.7 [7.3, 8.1]%) (p=0.4), as shown in **Figure 3**. These were both comparable to those who had an adjuvant glucose lowering therapy added to concurrent insulin therapy, with a HbA1c at latest follow up of 57.3 (95% CI 44.8,

69.8)mmol/mol (7.4 [6.3, 8.5]%) (p>0.05 for both), however this group had a notably higher baseline HbA1c than those continuing insulin alone, or discontinuing insulin therapy (78.9 (95% CI 67.5, 90.3)mmol/mol (9.4 [8.3, 10.4]%) vs 68.7 (95% CI 64.0, 73.3) mmol/mol (8.4 [8.0, 8.9]%) and 67.9(95% CI 59.8, 76.0) (8.4 [7.6, 9.1]%), respectively). No ketoacidosis was reported after insulin withdrawal, however one participant changing treatment (from basal bolus insulin to basal insulin with oral agents) developed ketoacidosis concurrent with Covid-19 infection.

The characteristics of islet autoantibody negative participants diagnosed with type 1 diabetes, stopping and continuing insulin, are shown in **Supplementary Materials Table 2**. The mean T1D-GRS of those stopping insulin was 9.47 (95% CI 8.52, 10.42), comparable to the type 2 cohort (mean 10.12 (95% CI 9.93, 10.32)), and consistent with these participants having non-autoimmune diabetes and being initially misclassified. The characteristics of the 5 individual participants diagnosed with type 1 diabetes who stopped insulin following a positive islet antibody test are shown in **Supplementary Materials Table 3,** 3 of 5 were positive for multiple islet autoantibodies.

Where type 1 diabetes is suspected but uncertain, negative islet antibodies are associated with very high rates of insulin cessation.

Additional analysis evaluated the 91 study participants considered to have suspected type 1 diabetes, based on receiving insulin at recruitment and having a self-reported diagnosis indicating clinical uncertainty. Follow up treatment status was available in 90.1% (N=82/91) of these individuals.
Of these participants 68.1% (62/91) were islet autoantibody negative with their characteristics based upon autoantibody status shown in **Supplementary Materials Table 4**. Islet autoantibody negative participants with suspected type 1 diabetes had a higher baseline C-peptide than those who were autoantibody positive, 1159.4 (95% CI 967.0, 1390.2)pmol/L vs 747.8 (95% CI 550.9, 1015.2)pmol/L (p<0.05) with lower mean T1D-GRS 9.98 vs 12.60 (p<0.001).

Following feedback of islet autoantibody results there was treatment change in 74.5% (41/55) of autoantibody negative participants with suspected type 1 diabetes: 52.7% (29/55) discontinued insulin, and 21.8% (12/55) added oral hypoglycaemic agents to continued insulin (**Supplementary Materials Figure 3**). In those continuing and discontinuing insulin, HbA1c at latest follow up was 53.1 (95% CI 42.5, 63.8)mmol/mol (7.0 [6.0, 8.0]%) and 51.3 (95% CI 45.5, 57.2)mmol/mol (6.8 [6.3, 7.4]%), respectively (p=0.7); both similar to those adding adjuvant glucose lowering therapies to continuing insulin treatment, 57.3 (95% CI 44.8, 69.8)mmol/mol (7.4 [6.3, 8.5]%) (both p>0.05), as shown in **Supplementary Materials Figure 4**. No islet antibody negative participants with suspected type 1 diabetes reported ketoacidosis during the follow up period.

Discussion

Our findings demonstrate that adults diagnosed with type 1 diabetes who are negative for islet antibodies have genetic and C-peptide characteristics that are intermediate between type 1 and type 2 diabetes. This is suggestive of substantial misclassification within this group, with this group likely to include those who have islet antibody negative autoimmune (type 1) diabetes, and those with non-autoimmune (predominantly type 2) diabetes who have been misclassified. Following feedback of a negative islet autoantibody result to

participants and their treating clinician, alteration of treatment regimen, including successful insulin cessation, was common. In contrast treatment change was rare in antibody positive individuals. In those treated with insulin from diagnosis with uncertain diabetes type, over half of those who were autoantibody negative discontinued insulin therapy. Insulin cessation was not associated with deterioration in glycaemic control.

To our knowledge this is the first study to examine the clinical impact of routine islet autoantibody measurement in all adults with a clinical diagnosis of type 1 diabetes. Our finding suggesting misclassification of clinically diagnosed type 1 diabetes in adults is consistent with a previous study defining diabetes by maintained insulin secretion (≥3 years diabetes duration). Foteinopoulou and colleagues showed that routine C-peptide testing of those with longstanding diabetes, followed by islet antibody and type 1 genetic risk score testing in selected individuals, was associated with reclassification of 11% of those developing diabetes as an adult, with reclassification rare (0.8%) in childhood onset diabetes (6). 22% of reclassified individuals successfully discontinued insulin therapy.

The analysis of clinical phenotypes associated with a diagnosis of type 1 diabetes in relation to islet autoantibody status have been previously explored. Bravis and colleagues identified a level of diagnostic heterogeneity associated with 268 islet autoantibody negative individuals (adults and children) in the multi-ethnic ADDRESS2 cohort within 6 months of a diagnosis of type 1 diabetes (25). Relative to the 1510 islet autoantibody positive participants the autoantibody negative participants tended to be older (median 31.4 versus 20.1 years old), have a higher BMI (median 25.5 versus 23.9kg/m^2) and associate with a male predominance (72% versus 56%). Although a mixed age cohort with a focus predominantly on clinical features, those highlighted in that study reflect several findings within our cohort. Interestingly, Thomas et al. looked at the genetic predisposition to type 1 diabetes in the ADDRESS2 cohort and demonstrated a significant reduction in genetic predisposition to type 1 diabetes of antibody negative adults (26). This was consistent with non-type 1 diabetes in 77% and 45% of antibody negative participants diagnosed aged >30 and 18-30 years, respectively. Islet autoantibody status did not affect genetic predisposition to type 1 diabetes in children, which may reflect the lower frequency of non-autoimmune diabetes in this population.

A key strength of the StartRight cohort is the routine measurement of GADA, IA2A and ZNT8A in all eligible adults, from primary and secondary care, recruited within 12 months of diabetes diagnosis regardless of perceived diabetes type. This was accompanied by detailed assessment of biomarkers, clinical features and treatment regimens from diagnosis and the proceeding 2 years, including an annual UCPCR, allowing for follow-up of beta-cell function. Routine feedback of islet autoantibodies, without specific clinical guidance, meant that we were able to observe whether the results of testing were associated with subsequent changes in standard clinical care. This is fundamental for the consideration of routine testing, as the impact of a test on patient care and outcomes will, alongside test cost and practicality, determine its utility.

A key limitation of this analysis is that there was no control arm in regards to participants and their treating clinicians who did not receive feedback of the islet autoantibody results. This means it is possible that participants may have changed treatment or stopped insulin without routine antibody testing, however the rarity of attempted treatment change in antibody positive participants does suggest that clinicians will take different actions where islet antibody status is known. A further limitation is that recruitment and antibody

assessment was up to 12 months after diagnosis of diabetes. While this is unlikely to meaningfully affect islet autoantibody prevalence in adults (27), it is likely that some participants will already have had islet auto antibodies measured in clinical care, potentially underestimating the impact of routine testing at diagnosis. This study relied on participants self-reporting of diabetes type (alongside insulin treatment from diagnosis) and annual treatment changes (including timings). It is possible reported diagnosis could have differed from clinicians' actual diagnosis as this was not confirmed from medical records, and that insulin withdrawal may not have been captured if temporary. Of note the study enriched recruitment for older people with type 1 diabetes, and therefore may not reflect the popualtion age distribution for type 1 diabetes. However despite this enrichment only 26% of our participants with clinically diagnosed type 1 diabetes were diagnosed after age 50, with UK population data suggesting 30% of all incident adult onset type 1 diabetes occurs in this age group (1). Our study population was mostly of European ancestry, with T1D-GRS analysis limited to this group, and further studies to assess the impact of ethnicity would be of great interest: diagnosing type 1 diabetes in adulthood can be particularly challenging in those whose ethnicity is associated with higher rates of type 2 diabetes in the comparatively young and thin (28). Consistent with this our study, and others, have demonstrated higher rates of negative islet antibodies in non-white ethnicity participants diagnosed with type 1 diabetes (25). Lastly there was no routine MODY testing, though the authors expect that the rates of MODY in this cohort of insulin treated diabetes, who are predominantly aged >30 at diagnosis, to be extremely low: in a similar cohort the prevalence in islet autoantibody negative adults diagnosed >30 was only 1% (26).

Our findings are clinically important as they suggest routine islet autoantibody testing in adults with diagnosed or suspected type 1 diabetes helps to identify a significant proportion who may be misclassified, and in some cases will be able to stop insulin treatment. In contrast one or more positive islet antibodies will usually confirm type 1 diabetes in this setting. While a small proportion of our study stopped insulin treatment misclassification may potentially result in a lifetime of unnecessary insulin treatment, and lack of access to effective treatment approaches in type 2 diabetes. Therefore, identifying possible misclassification through routine antibody testing at diagnosis has the potential to lead to marked benefit for individual patients, and, given the high cost of a lifetime of insulin treatment for type 1 diabetes, to be cost effective (6, 29). However a formal analysis of cost effectiveness, and longer term data on patient outcomes, would be needed to address this question. Our findings support recent ADA/EASD guidelines recommending the testing of islet autoantibodies in all adults with newly diagnosed suspected type 1 diabetes, and recommendations in this guidance that suggest that positive islet autoantibodies will usually confirm type 1 diabetes in this setting (14). It is important to emphasise that many islet autoantibody negative participants will still have autoimmune (or idiopathic) type 1 diabetes and develop severe insulin deficiency with absolute insulin requirement. Any attempts to withdraw insulin where there is uncertainty regarding diabetes subtype must be undertaken with marked caution and appropriate patient safeguarding to prevent ketoacidosis. It must also be recognised that some people with classical type 1 diabetes can temporarily discontinue insulin in the 'honeymoon period' but go on to absolute insulin requirement. Therefore careful ongoing monitoring of those discontinuing insulin following an initial diagnosis of type 1 diabetes is essential, and longer term follow up of this group is an important area for future research.

As this study did not directly intervene to attempt insulin withdrawal it is likely that there will be antibody negative individuals who remain misclassified and/or continue to receive

unnecessary insulin treatment. Therefore, it is important that, as recommended in recent ADA/EASD guidance, plasma C-peptide as assessed after 3 years duration in those with negative islet autoantibodies, to further clarify diabetes diagnosis and treatment requirements (10, 14).

For researchers our findings provide further evidence that clinically diagnosed type 1 diabetes in adults is likely to consist of a mix of those with and without autoimmune type 1 diabetes. This is likely to be because the very high prevalence of type 2 diabetes in adults will make robustly discriminating true type 1 diabetes from atypical presentations of type 2 diabetes challenging: low prior likelihood of type 1 diabetes may mean atypical presentations of type 2 diabetes (for example with ketoacidosis, marked hyperglycaemia or low BMI) are relatively common in comparison to classical presentations of type 1 diabetes (5, 26). Some reported characteristics of type 1 diabetes in older adults, such as low islet autoantibody prevalence, may potentially reflect the inadvertent study of those with and without autoimmune diabetes, and our findings, along with other research in this area, suggests a need to combine clinical diagnosis with a confirmatory antibody test in this setting (6, 25, 26, 30).

Conclusion

In adult-onset clinically diagnosed type 1 diabetes, negative islet autoantibodies should prompt careful consideration of other diabetes subtypes. When routinely measured negative antibodies are associated with successful insulin cessation. These findings support recent recommendations for routine islet autoantibody assessment in adult-onset type 1 diabetes

Contributors

RJE, NJT and AGJ designed the study. AVH, BAK, AC, TJM and AGJ researched the data. RJE, NJT, BMS and AGJ analysed the data. RJE, NJT and AGJ wrote the first draft of the report. All authors reviewed the draft and contributed to the revision of the report. AGJ is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Declaration of interest

The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

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Figure Legends:

Figure 1: The relationship between islet autoantibody status and A: T1D-genetic risk score (T1D-GRS) and B: short-term loss of beta cell function as defined by urine C-peptide creatinine ratios (UCPCR). A: A violin plot of T1D-GRS with the width of the greyed area indicating frequency, for participants diagnosed with: T1D that are AA negative (–VE), T1D that are AA positive (+VE) & type 2 diabetes (T2D) that are AA negative (-VE). The circle and associated error bars within each plot represent the mean T1D-GRS and 95% confidence intervals, respectively. Analysis is restricted to white European participants: N values represent the number participants in each group with available data. *P* values given are 2 sample t-tests. B: The plotted adjusted predictions for a mixed effects linear regression analysis of the natural log of the change in UCPCR from recruitment. The points represent the adjusted predictions of the UCPCR (nmol/mmol) and the error bars the 95% confidence intervals.

Figure 1

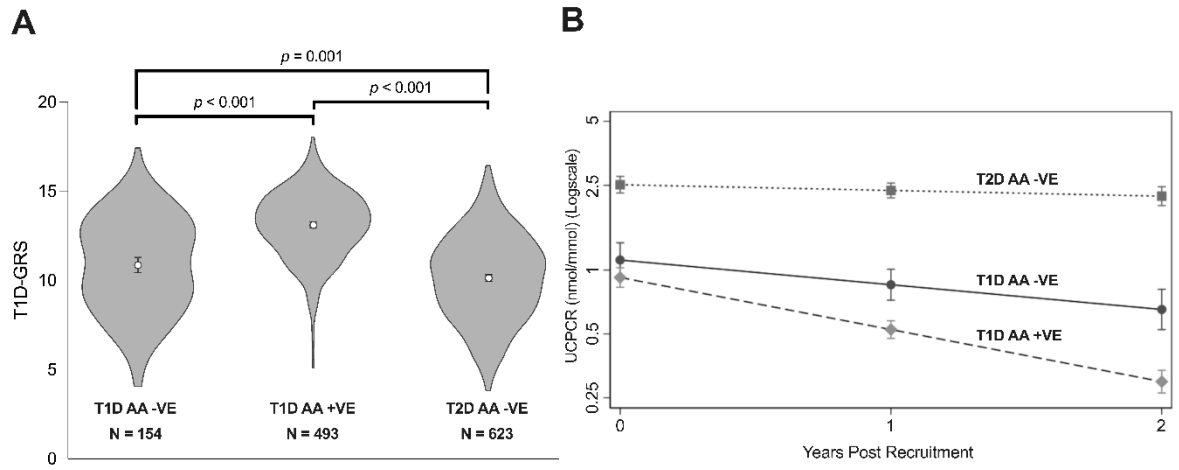

Figure 2: Treatment change following feedback of routinely measured islet autoantibodies to participants with type 1 diabetes and their clinicians. *Median follow up duration (post islet autoantibody assessment) 24 months (IQR 22, 26). Median diabetes duration at follow up, 29.6 months (IQR 25.8, 32.5). AGLT - adjuvant glucose lowering therapy; including either/or a: metformin, sulphonylurea SGLT-2 inhibitor or GLP-1 receptor agonist.

Figure 3

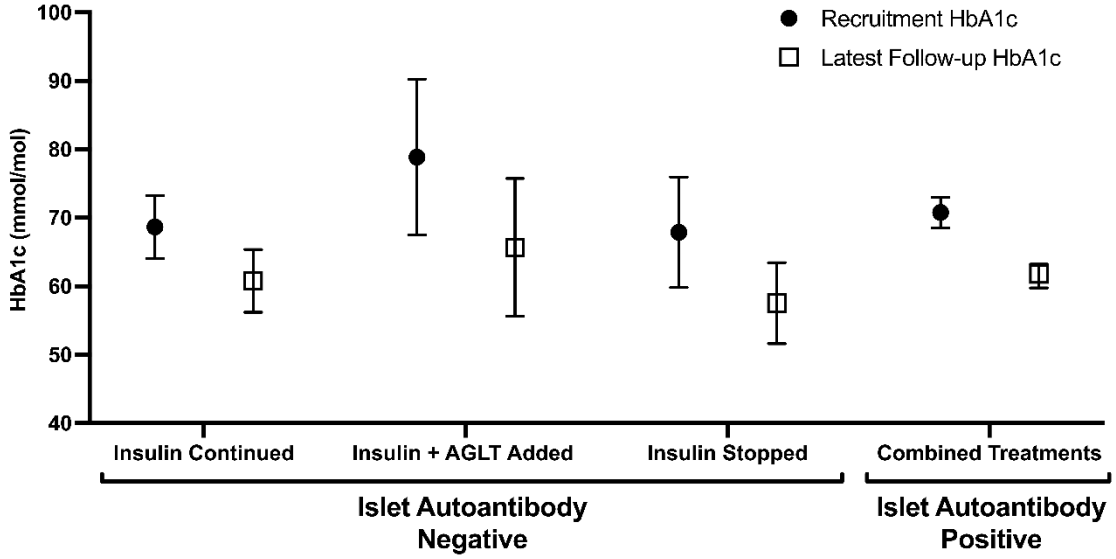

Figure 2

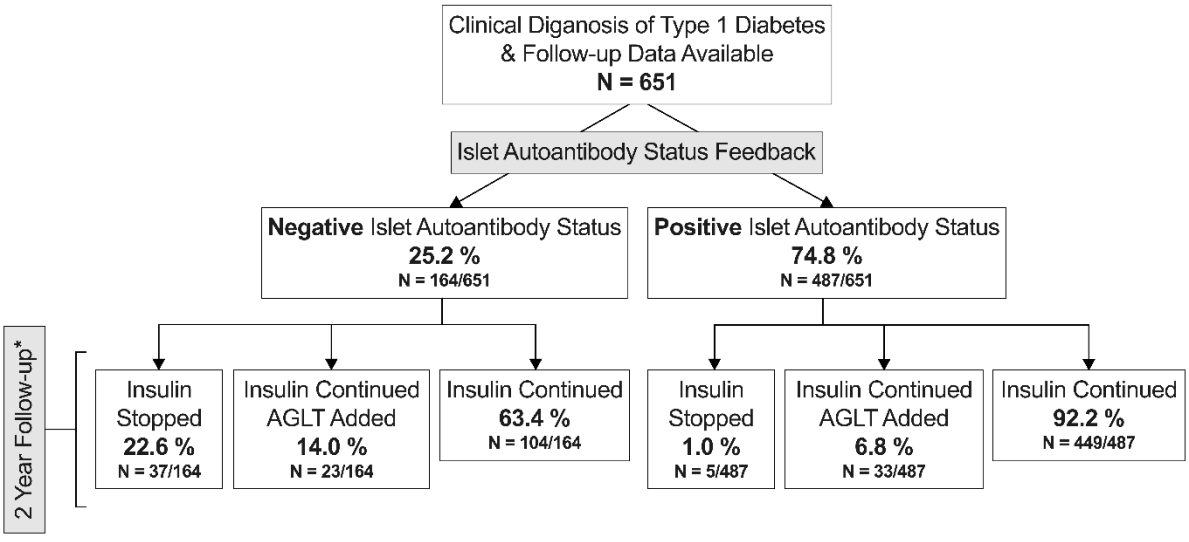

Figure 3: Mean HbA1c at study recruitment and latest follow up in participants with type 1 diabetes, by antibody status and treatment change. Error bars represent 95% confidence intervals. Recruitment HbA1c was assessed at a median 4 months diabetes duration. Last recorded HbA1c was assessed at a median 24 months later (median diabetes duration 29.6 months [IQR 25.8, 32.5]). The data represented is of islet autoantibody positive (N = 461/487) and negative (N=161/164) participants who had follow up data available for both treatment and a HbA1c > 3 months after any alteration. Combined Treatments – the collective available data irrespective of any treatment continuation or alteration, Insulin continued – participants whose Insulin was continued (N=101/104), Insulin stopped – participants whose Insulin was stopped (N=37/37), Insulin + AGLT added – participants whose Insulin continued alongside the addition of an adjuvant glucose lowering therapy (N=23/23), which included either/or a: metformin, sulphonylurea, SGLT-2 inhibitor or GLP-1 receptor agonist.

	Islet Autoantibody Positive (N = 543) mean (95% CI) or (where specified) % (95% CI)	Islet Autoantibody Negative (N = 179) mean (95% CI) or (where specified) % (95% CI)	*P* value
Clinical Features			
Male (%)	50.5 (46.2-54.7)%	72.9 (66.3-79.5)%	<0.001
Ethnicity (% White European)	91.1 (88.7-93.5)%	86.0 (80.9-91.2) %	0.05
Age at diagnosis (years)	38.2 (37.0-39.4)	42.7 (40.6-44.8)	<0.001
Duration of diabetes at recruitment (weeks)	20 (19-21)	17 (15-19)	0.03
BMI at recruitment	25.0 (24.7-25.4)	27.4 (26.6-28.2)	<0.001
DKA at diagnosis (% Yes)	20.9 (17.5-24.3)%	20.7 (14.7-26.7)%	0.9
Osmotic symptoms at diagnosis # (% Yes)	94.6 (92.7-96.5)%	91.1 (86.8-95.3)%	0.09
Weight loss pre-diagnosis (% Yes)	84.7 (81.6-87.7)%	76.0 (69.7-82.3)%	0.008
Other auto-immune condition (% Yes)	16.3 (13.2-19.4)%	3.9 (1.1-6.8)%	<0.001
Biochemical/Genetic Features			
HbA1c at diagnosis (mmol/mol)	105.4 (103.0-107.7)	109.8 (106.1-113.6)	0.06
Glucose at diagnosis (mmol/L)	21.3 (20.4-22.2)	23.6 (21.8-25.4)	0.02
Plasma C-Peptide at recruitment * (pmol/L)	413.0 (382.1-446.4)	607.6 (500.5-737.5)	<0.001
UCPCR at recruitment * † (nmol/mmol)	1.00 (0.90-1.11)	1.09 (0.85-1.39)	0.48
T1D-GRS ‡	13.09 (12.92-13.25)	10.85 (10.43-11.27)	<0.001

Table 1 - Clinical characteristics of participants with clinically diagnosed type 1 diabetes by islet autoantibody status (GADA, ZNT8, IA2). *P* values given for continuous variables are 2 sample t-tests and Pearson chi-squared for categorical variables. # One or more of polyuria, nocturia, polydipsia. * The C-peptide and UCPCR are geometric means with the statistics performed on the natural log transformed values. †UCPCR available in a subset of 431/543 islet autoantibody positive participants, and 136/179 islet autoantibody negative participants. ‡T1D-GRS from 154 and 493 islet autoantibody negative and positive participants of white European ethnicity. T1D-GRS, Type 1 Diabetes Genetic Risk Score; UCPCR, Urinary C-Peptide Creatinine Ratio; DKA, Diabetic Ketoacidosis.