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Biomarkers associated with severe hypoglycaemia and death in ACCORD

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Abstract

Aims and hypothesis—In patients with Type 2 diabetes, intensive glycaemic control is associated with hypoglycaemia and possibly increased mortality. However, no blood biomarkers exist to predict these outcomes. Using participants from the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study, we hypothesized that insulin deficiency and islet autoantibodies in patients with clinically diagnosed Type 2 diabetes would be associated with severe hypoglycaemia and death.

Methods—A nested case–control study design was used. A case ($n = 86$) was a participant who died with at least one episode of severe hypoglycaemia, defined as hypoglycaemia requiring assistance, at any point during ACCORD follow-up. A control ($n = 344$) was a participant who did not die and did not have severe hypoglycaemia during follow-up. Each case was matched to four controls (glycaemic intervention arm, race, age and BMI). Baseline insulin deficiency (fasting C-peptide 0.15 nmol/l) and islet autoantibodies [glutamic acid decarboxylase (GAD), tyrosine phosphatase-related islet antigen 2 (IA2), insulin (IAA) and zinc transporter (ZnT8)] were measured. Conditional logistic regression with and without adjustment for age, BMI and diabetes duration was used.

Results—Death during ACCORD in those who experienced at least one episode of severe hypoglycaemia was associated with insulin deficiency [OR 4.8 (2.1, 11.1): $P < 0.0001$], GAD antibodies [OR 2.3 (1.1, 5.1): $P = 0.04$], the presence of IAA or baseline insulin use [OR 6.1 (3.5, 10.7): $P < 0.0001$], which remained significant after adjusting for age, BMI, and diabetes duration. There was no significant association with IA2 or ZnT8 antibodies.

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Competing interests

None declared.

Author contributions

LSC, MEM and ES designed the research. SMM provided essential data for analysis. LSC, HC, MEM, and ERS analysed the data. LSC, HC, MEM, SMM and ERS wrote the manuscript. LSC, HC, MEM, SMM and ERS contributed to the discussion and reviewed/edited the manuscript. LSC and ERS are the guarantors for the manuscript and have the primary responsibility for the final content and had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and approved the final manuscript.

Conclusions—In patients with Type 2 diabetes, C-peptide or GAD antibodies may serve as blood biomarkers predicting higher odds of subsequent severe hypoglycaemia and death. (Clinical Trial Registry No: NCT00000620, www.clinicaltrials.gov for original ACCORD study)

Introduction

Intensive glucose control to lower HbA_{1c} to near-normal levels has many benefits in patients with Type 2 diabetes, including reduction in microvascular disease [1] and possibly macrovascular events [1]. Intensive glucose control in Type 2 diabetes is also associated with increased mortality in some [2], but not all [3,4] interventional studies of glycaemic control. However, intensive glucose control is associated with a higher frequency of severe hypoglycaemia (SH), defined as hypoglycaemia requiring assistance [5,6] and SH is associated with increased risk for major macrovascular events (death from a cardiovascular cause, nonfatal myocardial infarction for nonfatal stroke), major microvascular events (new or worsening nephropathy or retinopathy), death from cardiovascular cause and death from any cause [6].

Despite the general agreement that SH is associated with adverse outcomes, including death [7], there are no established blood biomarkers that predict whether patients with Type 2 diabetes might be at high risk for SH and death. Long-standing Type 2 diabetes is associated with insulin deficiency and reduced physiological response to hypoglycaemia [8]. There is a growing appreciation that certain patients with long-standing Type 2 diabetes can develop insulin deficiency approaching patients with Type 1 diabetes [9], which may [10] or may not [11] be related to autoimmunity. Consequently, we propose that blood biomarkers of insulin deficiency and islet autoimmunity may identify a subpopulation of patients with Type 2 diabetes at high risk for SH and death. Using data collected during the Action to Control Cardiovascular Risk in Diabetes (ACCORD), we hypothesized that profound insulin deficiency and islet autoantibodies would be significantly associated with SH and death in patients with previously diagnosed Type 2 diabetes.

Methods

Participants

The ACCORD study is uniquely suited for this analysis, because it was a randomized, multicentre, double 2*2, factorial design study examining the effect of glycaemic control (intensive vs. standard), blood pressure control and lipid control on cardiovascular morbidity and mortality in patients with clinically diagnosed Type 2 diabetes [12]. When SH was defined as hypoglycaemia requiring any assistance and either glucose < 2.77 mmol/l or symptoms that promptly resolved with treatment, the annual incidence in the ACCORD study was 5.05 events/100 person-years in the intensive group and 1.51 events/100 person-years in the standard group [5]. The ACCORD study enrolled 10 251 participants with long-standing Type 2 diabetes with either diagnosed cardiovascular disease or at least two cardiovascular risk factors (in addition to diabetes), for intervention and subsequent follow-up. All participants were randomized to either standard glycaemic control [HbA_{1c} target 53–63 mmol/mol (7.0–7.9%)], or intensive glycaemic control [HbA_{1c} target 42 mmol/mol (< 6.0%)]. Partway (median follow-up of 3.7 years) through the study, participants receiving

intensive glycaemic control [median HbA_{1c} 46 mmol/mol (6.4%)] were transitioned to standard therapy due to higher total and cardiovascular mortality in the intensive group compared with the standard treatment group. After the transition to standard therapy, the median HbA_{1c} in the intensive group [median HbA_{1c} 55 mmol/mol (7.2%)] remained lower than in the standard therapy group [median HbA_{1c} of 60 mmol/mol (7.6%)] by study conclusion (5 years of follow-up) [13].

Study design

The ACCORD data were reanalysed using a nested case–control design to explore whether baseline insulin deficiency, as measured by C-peptide and islet autoantibodies, was associated with at least one episode of SH, defined as hypoglycaemia requiring any assistance, and mortality during follow-up. A case ($n = 86$) was defined as a participant who died during ACCORD with at least one episode of SH at any point during the follow-up period. Total mortality was defined as death from any cause (with independent adjudication) during the study duration or through a National Death Index search. Because the focus of our analysis is on mortality that occurred subsequent to SH, we did not look at total mortality independent of SH, or at participants who did not die in the months after having SH.

A control ($n = 344$) was defined as an ACCORD participant alive at the time of the case death without SH at any point during the follow-up period. Each case was matched to four controls (exact matching on glycaemic intervention arm, and race and optimal distance matching [14] on age and BMI). This approach to matching finds the set of case–control matches that are closest among all possible pairings and minimizes an overall distance measure. Computerized matching of cases with controls was achieved using the DIST and VMATCH macros [14]. The study was noted to be exempt by the University of Minnesota Institutional Review Board and was approved by the ACCORD Presentation and Publication Committee. The study participants gave informed consent for participation in the ACCORD study as well as any approved substudies by the ACCORD Presentation and Publication Committee.

ACCORD criteria for Type 2 diabetes

For the ACCORD study, the participants were clinically screened for eligibility by history and physical examination. The patients enrolled in the ACCORD study were required to have a clinical diagnosis of Type 2 diabetes, defined by the 1997 American Diabetes Association criteria, for at least 3 months prior to enrolment. They were also required to be on a stable diabetes treatment programme for at least 3 months prior to enrolment. The ACCORD study specifically excluded patients with a history consistent with Type 1 diabetes, secondary causes of diabetes or gestational diabetes from enrolment. C-Peptide and islet antibodies were measured from the baseline study samples after the completion of the ACCORD study and were not used to determine initial ACCORD study eligibility.

C-Peptide as a surrogate for insulin deficiency

C-Peptide was measured from baseline serum samples. Insulin deficiency was approximated by fasting C-peptide levels. We used fasting C-peptide levels to categorize participants as

either insulin sufficient (C-peptide > 0.15 nmol/l) or insulin deficient (C-peptide ≤ 0.15 nmol/l), which is consistent with the literature [15]. Participants on insulin at baseline were also classified into insufficient/insulin-deficient categories by C-peptide criteria. Table 1 summarizes the classification criteria.

The C-peptide assay is an immunoenzymometric assay performed on a Tosoh AIA 1800 analyser, with an intra-assay coefficient of variation of < 2.0%, inter-assay coefficient of variation of < 5.0% and lower limit of detectability of 0.02 nmol/l. This sensitivity limit was established in the laboratory as part of the method validation and was verified for each reagent lot.

Islet autoantibodies measurements

Islet autoantibodies were measured from baseline serum samples. Measured islet autoantibodies include antibodies against glutamic acid decarboxylase (GAD), tyrosine phosphatase-related islet antigen 2 (IA2), insulin (IAA) and zinc transporter (ZnT8). Baseline samples were measured for GAD and IA2 at the Northwest Research Laboratories (NWRL) University of Washington, central laboratory of the ACCORD study. The NWRL is one of the laboratories where the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) standardized procedure for GAD and IA2 autoantibodies have been implemented. The procedure was developed in cooperation with the NIDDK Harmonisation Programme and the Diabetes Autoantibodies Standardisation Programme (DASP). In the most recent DASP workshop, the sensitivity and specificity of the GAD assay were 82% and 93.3%, respectively. The sensitivity and specificity of the IA2 assay were 62% and 100%, respectively. For the GAD assay, participants with ≥ 33 DK units/ml were considered GAD positive and < 33 DK units/ml were considered GAD negative. For the IA2 assay, participants with ≥ 5 DK units/ml were considered IA2 positive and < 5 DK units/ml were considered IA2 negative.

ZnT8 and IAA analyses were performed at the Barbara Davis Center, University of Colorado. The ZnT8 results are expressed as index. Based on a receiver-operating characteristics curve for 100 controls and 50 patients with recent onset of diabetes, a cut-off at 0.02 index was determined. In the most recent DASP workshop 2010, the ZnT8 assay showed specificity and sensitivity of 100% and 64%, respectively. Similarly, the IAA analysis results are expressed as index, and a 0.01 index has been determined as the cut-off for controls. In the most recent DASP workshop, the IAA assay showed specificity and sensitivity of 56% and 99%, respectively. For ZnT8, the participants with > 0.02 index were considered ZnT8 positive and ≤ 0.02 index were considered ZnT8 negative. For IAA, participants with either > 0.01 index or baseline insulin use were considered IAA positive and ≤ 0.01 index were considered IAA negative. Because insulin-treated patients have been observed to acquire anti-insulin antibodies as a result of treatment, [16], we made the conservative assumption that participants on insulin at baseline would likely have detectable IAA. These participants were thus categorized as having detectable IAA. Participants not on baseline insulin were categorized by the IAA level measured from the baseline sample. The cut-off criteria are summarized in Table 1.

Statistical analysis

We used a nested case–control design in our study with the matched sets defining strata. Baseline variables used for matching were summarized using descriptive statistics for cases and controls without hypothesis testing. Other baseline characteristics were compared between cases and controls using Mantel–Haenszel test statistics to adjust for the correlations within strata. For each baseline risk factor (presence of insulin deficiency and antibodies), we fit separate conditional logistic regression models to examine its association with case/control status (Model 1). Because age and BMI were matched as continuous variables, we also fit multivariable conditional logistic regression models specifically adjusting for age and BMI, as well as self-reported baseline duration of diabetes (Model 2).

Because all participants who were insulin deficient had at least one measurable antibody, we evaluated the potential additive effect of antibodies and insulin deficiency relative to the referent insulin-sufficient group. We created a three-category variable indicating: (1) no insulin deficiency and absent antibodies, (2) presence of at least one antibody only, and (3) presence of both insulin deficiency and at least one antibody. The magnitude of associations between baseline risk factors and SH followed by death were quantified by odds ratios and associated *P*-values. Although the approach used for matching minimized an overall distance measure between cases and controls, some individual matches were not very close. To address this possible concern, we conducted a sensitivity analysis using only those controls in which the cases and controls were matched within 5 years on age and 2.5 kg/m² on BMI. All analyses were performed using SAS 9.3 (Cary, NC, USA) and a value of *P* < 0.05 was considered statistically significant.

Results

Characteristics of cases and controls

The characteristics of the cases and controls are described in Table 2. The quality of the optimal matching on the continuous age and BMI variables was excellent: 96.8% of age matches were within 5 years and the maximum difference in ages was 17 years. For BMI, 93.3% of matches were within 2.5 kg/m². Compared with controls, cases had diabetes of longer duration (15.9 years vs. 10.9 years, *P* < 0.0001) higher rates of insulin use (70% vs. 31%, *P* < 0.0001) and higher rates of cardiovascular events (55% vs. 32%, *P* < 0.0001) at baseline.

C-Peptide and islet autoantibody profile

Cases had a higher percentage of baseline insulin deficiency, as defined by fasting C-peptide than controls (14% vs. 3%, *P* < 0.0001). All participants with baseline insulin deficiency as defined by fasting C-peptide were taking insulin and were therefore considered positive for IAA. None of the participants with baseline insulin deficiency had antibodies against insulin. If IAA was excluded, GAD (55%) was the most common islet autoantibody in participants with baseline insulin deficiency.

Odds for SH and mortality based on baseline insulin deficiency and islet autoantibodies

Death during ACCORD with at least one episode of SH was significantly associated with baseline insulin deficiency, positive GAD antibodies, positive IAA and insulin use (Table 3). These associations remained significant after adjusting for age, BMI and diabetes duration. Of the islet autoantibodies, GAD and IAA were positively associated with SH and death; the other antibodies (IA2, ZnT8) were not associated (Table 3).

The number of autoantibodies detectable at baseline did not appear to have an effect on the risk for death following SH. The odds ratio for this outcome in participants with one detectable antibody was about the same [unadjusted OR 7.1 (3.9–12.9): $P < 0.0001$, adjusted OR 6.1 (3.1, 12.0): $P < 0.0001$] as in participants with two or more detectable antibodies (unadjusted OR 7.3, 95% CI 2.7–19.9, $P = 0.0001$, adjusted OR 6.8, 95% CI 2.2–20.8, $P = 0.0008$), relative to those participants free of detectable antibodies at baseline.

The risk for SH and subsequent death was greatest in those participants who had insulin deficiency and positive islet autoantibodies at baseline (Table 4). This relationship was driven by the use of baseline insulin because 100% of users were categorized as having positive IAA. In the setting of insulin sufficiency, the presence of one islet antibody increased the risk for SH and death [unadjusted OR 6.4 (3.5, 11.8): $P < 0.0001$, adjusted OR 5.6 (2.8, 11.0), $P < 0.0001$] this risk was further enhanced in the setting of insulin deficiency [unadjusted OR 13.3 (5.1, 35.0) $P < 0.0001$, adjusted OR 16.3 (5.0, 53.3): $P < 0.0001$]. We additionally explored whether an interaction might exist between baseline insulin treatment and the most common islet autoantibody (GAD). We found a significant interaction ($P = 0.038$ after adjusting for age, diabetes duration, BMI, baseline insulin use) between the presence of GAD antibodies and baseline insulin use. Specifically, if the ACCORD participant was not on insulin at baseline, the presence of GAD increased the risk for SH and mortality (OR 7.72, 95% CI 1.85–32.3). However, if a participant was on insulin at baseline, the presence of GAD did not significantly increase the risk of SH and mortality (OR 1.19, 95% CI 0.38–3.75).

The median time between SH and mortality was 1.75 years (25th to 75th percentile of 0.78–2.58 years). In terms of causes of death, the predominant causes were related to cardiovascular events (56%) and cancer (20%).

As a sensitivity analysis, we assessed whether excluding controls who were not well matched for age (within 5 years) or BMI (within 2.5 kg/m²) (31 controls removed) or excluding cases with no well-matched controls (4 cases removed) would alter the results. These results, even after adjusting for age, BMI and diabetes duration, were quantitatively similar to the full sample and our conclusions remained unchanged.

Discussion

In this study, we found that ACCORD cases who experienced SH and subsequent mortality had a higher prevalence of insulin deficiency and detectable islet antibodies at baseline than ACCORD controls who did not experience SH and remained alive over follow-up. Our findings suggest that fasting C-peptide and GAD antibody may serve as blood biomarkers in

patients with previously diagnosed Type 2 diabetes to predict prognosis with respect to hypoglycaemia risk and mortality.

For patients with Type 2 diabetes treated with insulin or insulin secretagogues, SH is a known complication [17]. However, identification of patients with Type 2 diabetes at greatest risk for mortality given a history of SH remains challenging. It has been suggested that patients with Type 2 diabetes and comorbidities might be at greatest risk, because patients with Type 2 diabetes who reported SH had higher rates of major microvascular events, major microvascular events, cardiovascular related mortality and total mortality than patients with Type 2 diabetes who did not report SH [6]. However, the lack of a dose–response relationship between SH frequency and number of co-morbidities raises concerns that these variables may not be tightly connected. Because SH is more common in study participants randomized to intensive vs. control Type 2 diabetes treatment [5,6], SH and the associated adverse outcomes have been speculated to be more frequent in patients trying to achieve a near-normal HbA_{1c}. Yet in ACCORD, SH was associated with death regardless of treatment arm and SH did not account for the full difference in mortality between the two treatment arms [18]. It has previously been reported that SH is not exclusive to near-normal HbA_{1c} [19], suggesting the need to look beyond HbA_{1c} to identify SH risk. This need is also supported by our previous findings that blood biomarkers indicating insulin deficiency and islet autoantibodies are associated with SH and inability to achieve good glycaemic control [HbA_{1c} target 42 mmol/mol (< 6.0%)] in ACCORD participants who receive intensive glycaemic treatment [20]. Given the timing between SH and mortality (median 1.75 years) reported in this study, SH does not seem to be driver for mortality, but rather, in the right clinical substrate, a harbinger for mortality. The current results extend our earlier findings on SH and glycaemic control [20] and suggest that these blood biomarkers may predict SH and mortality. Overall, this work suggest that fasting C-peptide and GAD antibody may serve as potential biomarkers in predicting outcomes in patients with clinically diagnosed Type 2 diabetes.

The association between insulin deficiency and SH in Type 2 diabetes has been described. Patients with long-standing Type 2 diabetes (> 5 years) have hypoglycaemia rates similar to patients with Type 1 diabetes [21]. This may be due to several reasons, including the inability to regulate endogenous insulin secretion in response to falling glucose levels [22], and a blunted counter-regulatory response to hypoglycaemia approaching patients with Type 1 diabetes [8]. Because patients with insulin-deficient diabetes have an attenuated response of glucagon to hypoglycaemia [22], the patient becomes increasingly reliant on the catecholamine response to recognize and respond to hypoglycaemia [22]. Catecholamines increase heart rate, lower blood pressure and serum potassium [23], and affect cardiac repolarization [24]. Strong associations between hypoglycaemia and cardiac ischaemia [25] and hypoglycaemia and cardiac arrhythmias [26] have been observed in patients with Type 2 diabetes.

Progressive β -cell dysfunction resulting in insulin deficiency is characteristic of long-standing Type 2 diabetes. In our study, the cases had longer duration of diabetes than the controls (15.85 vs. 10.87 years, $P < 0.0001$). However, this finding did not confound our reported associations. Although the mechanisms driving the decline remain uncertain, our

observations support the possibility for autoimmune islet destruction in some patients previously diagnosed with Type 2 diabetes. Approximately 10% of patients with presumed Type 2 diabetes have detectable islet autoantibodies [27] and are commonly labelled as latent autoimmune diabetes of adulthood [28]. Compared with patients with Type 2 diabetes and negative islet autoantibodies, these patients typically develop diabetes at a younger age, are generally leaner, and have similar mortality rates [29]. In the current study, the cases and controls were older (mean age 67) and heavier (mean BMI 32 kg/m²), resembling the typical Type 2 diabetes phenotype with mid–late life onset and associated obesity. There was a notable significant interaction between the presence of GAD and lack of baseline insulin use in increasing the likelihood of our adverse outcome. These observations suggest that the presence of insulin deficiency or evidence of islet autoimmunity in a patient with a typical Type 2 diabetes phenotype may identify individuals who are especially at risk for SH and subsequent death.

This study had several strengths. This study focused on patients with presumed Type 2 diabetes with high cardiovascular risk who participated in the ACCORD study. Outcome measures were obtained by standardized protocols with rigorous quality control with a standardized definition of hypoglycaemia events. The cases and controls were matched according to age, BMI, treatment intensity and duration of follow-up. Baseline islet autoantibodies were measured by a standardized laboratory. We acknowledge several limitations. We acknowledge that fasting C-peptide may be influenced by fasting glucose (study-reported mean of 9.5 ± 3.8 mmol/l) and insulin treatment. The ACCORD study could have inadvertently enrolled participants with latent autoimmune diabetes of adults or Type 1 diabetes, despite the presentation phenotypically similar to Type 2 diabetes. Because the blood biomarkers were measured in only a selected population from the ACCORD trial rather than all participants, only a small number of detectable islet antibodies (excluding IAA which we designated as positive in the setting of insulin use) were discovered. The generalizability of these significant findings remains limited and suggests the need for replication in a larger cohort. We could not separate mortality and SH given our study design, but the strong associations observed in our study remain clinically relevant and would be the basis for future studies. Because our study was a post-hoc analysis, we could not distinguish the causal vs. associative nature of the measured biomarkers. Lastly, due to the exploratory nature of the study, we chose the two groups that are most different (SH and death vs. no SH or death) for the case–control comparison. The reason for this selection is to establish whether important biomarkers might exist. We acknowledge that selecting this extreme difference may overestimate our choice of cases and controls, which is understandable given the study's exploratory nature. For future studies, we would recommend a more nuanced comparison between more clinically similar groups (e.g. SH and death vs. SH and no death).

The natural extension of these findings would be to use a prospective cohort design, rather than the current nested case–control design, to establish risk scores predicting SH-associated outcomes and the extent to which these identified blood biomarkers would provide added value.

Our observations have clear clinical relevance, because we describe a strong association between patients with previous diagnosed Type 2 diabetes who experienced SH and death and the prevalence of insulin deficiency, GAD antibodies and insulin use at baseline. These findings support an individualized approach to therapy, with a focus on minimizing SH in patients with positive biomarkers. Our findings suggest that future studies are needed to examine the relative benefits of hypoglycaemia reduction or β -cell preservation in presumed Type 2 diabetes, particularly in the setting of insulin deficiency or positive islet antibodies.

In patients with Type 2 diabetes, insulin deficiency, GAD antibodies and insulin use at one point in time are significantly associated with SH and subsequent death in the years following the measurement. When present, these biomarkers may predict poor prognosis and provide support for using therapies which minimized hypoglycaemia. Whether such treatments will reduce mortality in Type 2 diabetes remain an intriguing area for further study.

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What's new?

- In patients with clinically diagnosed Type 2 diabetes, there are no blood biomarkers predicting severe hypoglycaemia and death.
- Using the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study, we measured insulin deficiency (fasting C-peptide) and islet autoantibodies [glutamic acid decarboxylase (GAD), tyrosine phosphatase-related islet antigen 2 (IA2), insulin (IAA) and zinc transporter (ZnT8)].
- Death during ACCORD in those who experienced at least one episode of severe hypoglycaemia was associated with insulin deficiency, baseline insulin use and positive antibodies for GAD and insulin.
- In patients with Type 2 diabetes, C-peptide or GAD antibodies may serve as blood biomarkers predicting higher odds of subsequent severe hypoglycaemia and death.

Table 1

Measured blood biomarkers

Measured markers	Insulin deficient	Insulin sufficient
C peptide (nmol/l); normal range 0.17–1.0 nmol/l	0.15	> 0.15
Antibodies	Positive	Negative
GAD; DK unit/ml	33	< 33
IA2; DK units/ml	5	< 5
IAA; index	> 0.01 Also designated positive if baseline insulin use	0.01
ZnT8; index	> 0.02	0.02

Table 2

Baseline characteristics of cases and controls

Value (% for categorical variables, SD for continuous variables)	Cases (<i>n</i> = 86)	Controls (<i>n</i> = 344)	<i>P</i> -value for difference between cases and controls
Age	67 (8.3)	66.5 (7.6)	Matched
Gender (male)	55 (64%)	192 (56%)	0.15
Race (white)	57 (66%)	226 (66%)	Matched
BMI (kg/m ²)	32.3 (6.1)	32.3 (5.8)	Matched
Mean fasting glucose (mmol/l)	9.6 (3.8)	9.7 (2.9)	0.71
Mean IFCC HbA _{1c} (mmol/mol)	70 (13.1)	67 (12)	0.08
Mean HbA _{1c} (%)	8.6 (1.2)	8.3 (1.1)	0.08
Mean duration of diabetes (years)	15.85 (9.1)	10.87 (7.6)	< 0.0001
Insulin use at baseline	60 (70%)	105 (31%)	< 0.0001
Mean SBP (mmHg)	140 (21)	138 (18)	0.32
Mean DBP (mmHg)	71 (12)	74 (10)	0.04
Percentage in intensive treatment arm	59 (69%)	236 (69%)	Matched
Percentage with cardiovascular events at baseline	47 (55%)	111 (32%)	< 0.0001
Mean LDL (mmol/l)	2.8 (0.9)	2.8 (0.9)	0.47
Insulin deficiency (C-peptide 0.15 nmol/l)	12 (14%)	10 (3%)	< 0.0001
GAD positive	11 (13%)	21 (6%)	0.03
IA2 antibody positive	3 (3%)	3 (1%)	0.07
IAA positive or baseline insulin use	63 (73%)	107 (31%)	< 0.0001
ZnT8 positive	1 (1%)	5 (1%)	0.84
Presence of antibodies			< 0.0001
0	19 (22%)	226 (66%)	
1	59 (69%)	105 (31%)	
2	6 (7%)	10 (3%)	
3	1 (1%)	1 (0.3%)	
4	1 (1%)	2 (0.6%)	

A portion of the data was presented as a poster at the 2014 American Diabetes Association [30].

Table 3

Likelihood of death with at least one episode of severe hypoglycaemia given the combination of insulin deficiency status and autoantibody presence

		Odds ratio (95% CI) for death with at least one episode of severe hypoglycaemia		
				<i>P</i>
C-peptide		Insulin sufficient	Insulin deficient	
	Model 1 [*]	1 [Reference]	4.8 (2.1, 11.1)	<0.0001
	Model 2 [†]	1 [Reference]	5.4 (1.9, 15.3)	0.0014
GAD antibody		Negative	Positive	
	Model 1 [*]	1 [Reference]	2.3 (1.1, 5.1)	0.04
	Model 2 [†]	1 [Reference]	3.1 (1.3, 7.7)	0.01
IAA or baseline insulin use		Negative	Positive	
	Model 1 [*]	1 [Reference]	6.1 (3.5, 10.7)	< 0.0001
	Model 2 [†]	1 [Reference]	5.0 (2.6, 9.3)	< 0.0001
IA2		Negative	Positive	
	Model 1 [*]	1 [Reference]	4.0 (0.8, 19.8)	0.09
	Model 2 [†]	1 [Reference]	2.9 (0.5, 17.7)	0.25
ZnT8		Negative	Positive	
	Model 1 [*]	1 [Reference]	0.8 (0.1, 6.9)	0.84
	Model 2 [†]	1 [Reference]	0.7 (0.1, 6.2)	0.73

^{*} Unadjusted.

[†] Model 1 additionally adjusted for age, BMI and diabetes duration.

A portion of the data was presented as a poster at the 2014 American Diabetes Association. [30]

Table 4

Likelihood of death with at least one episode of severe hypoglycaemia given the combination of insulin deficiency status and presence of any autoantibody

Odds ratio (95% CI) for Death with at least one episode of severe hypoglycaemia			
			<i>P</i>
	Insulin sufficient and no autoantibodies	Insulin sufficient and at least one autoantibody present	
Model 1 *	1 [Reference]	6.4 (3.5, 11.8)	< 0.0001
Model 2 †	1 [Reference]	5.6 (2.8, 11.0)	< 0.0001
	Insulin sufficient and no autoantibodies	Insulin deficient and at least one autoantibody present	
Model 1 *	1 [Reference]	13.3 (5.1, 35.0)	< 0.0001
Model 2 †	1 [Reference]	16.3 (5.0, 53.3)	< 0.0001

* Unadjusted.

† Model 1 additionally adjusted for age, BMI and diabetes duration

A portion of the data was presented as a poster at the 2014 American Diabetes Association [30].