

Metabolic Syndrome and autoimmune diabetes: ACTION LADA 3

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Objective— To estimate whether prevalence of Metabolic Syndrome in adult European diabetes patients is associated with type of diabetes.

Research design and methods— A consecutive series of patients attending hospital-based diabetes clinics were assessed for the frequency of Metabolic Syndrome and compared with population-based controls as part of the Action LADA study. In total, 2011 subjects (age range 30 – 70 years) were studied, including 1247 recent-onset type 2 diabetes patients without glutamic acid decarboxylase autoantibodies (GADA), 117 non-insulin requiring patients with GADA off insulin therapy at least 6 months post-diagnosis, designated latent autoimmune diabetes of adults (LADA), 288 type 1 diabetes patients and 359 normal subjects.

Results— Frequency of Metabolic Syndrome was significantly different in type 1 diabetes (31.9%) and LADA (41.9%) ($p=0.015$), but in both conditions was less frequent than in type 2 diabetes (88.8%) ($p<0.0001$ for each). Eliminating glucose as a variable, prevalence of Metabolic Syndrome was similar in autoimmune diabetes (type 1 diabetes and/or LADA) (17.3%) and controls (23.7%) but remained more common in type 2 diabetes (47.8%) ($p=0.001$ for all groups). In both type 1 diabetes and LADA, individual components of Metabolic Syndrome were similar but less common than in type 2 diabetes patients ($p<0.0001$ for each).

Conclusions— Prevalence of Metabolic Syndrome is significantly higher in type 2 diabetes than in adults with LADA or type 1 diabetes. Excluding glucose as a variable, Metabolic Syndrome is not more prevalent in autoimmune diabetes than in controls. Metabolic Syndrome is not a characteristic of autoimmune diabetes.

Abbreviations: • NCEP, National Cholesterol Education Program • WHO, World Health Organization • IU, International Units • GADA, glutamic acid decarboxylase autoantibodies • LADA, latent adult-onset autoimmune diabetes • IDF, International Diabetes Federation. • Histocompatibility (HLA) • Confidence Intervals (CI) • Triglycerides (TG)

Type 1 diabetes is an autoimmune disease in which insulin deficiency results from immune-mediated destruction of insulin secreting islet cells. The majority of patients with type 1 diabetes have autoantibodies in their peripheral blood and these autoantibodies can predict the disease. Autoimmune diabetes, as characterized by these autoantibodies, such as glutamic acid decarboxylase autoantibodies (GADA), is the most prevalent form of diabetes in children, and also occurs in a proportion of patients who initially present with adult-onset non-insulin requiring diabetes, also called latent adult onset autoimmune diabetes (LADA)(1).

Since glucose disposal and blood glucose are determined by both insulin secretion and insulin action it follows that insulin sensitivity could be important in the pathogenesis of autoimmune diabetes. Insulin sensitivity has not been studied in details in autoimmune diabetes, though studies suggest that its loss may occur in established disease as well as in the prediabetic phase (2-5). Loss of insulin sensitivity is difficult to assess epidemiologically, but is reflected in the cluster of metabolically related cardiovascular risk-factors which together comprise the Metabolic Syndrome and include altered glucose levels, central obesity, dyslipidaemia and hypertension. Several groups, including the International Diabetes Federation (IDF) and the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (6), have proposed their own definitions for the Metabolic Syndrome.

LADA is clearly distinct from type 2 diabetes, in that LADA is associated with Histocompatibility (HLA) genes, diabetes-associated autoantibodies,

reduced insulin secretion, no need of insulin therapy initially after diagnosis and less prevalent Metabolic Syndrome (7-9). The key uncertainty is whether LADA is distinct from type 1 diabetes (1,10,11). That is, whether LADA is one end of a rainbow of pathophysiological variation encompassing autoimmune diabetes with a similar frequency of Metabolic Syndrome to childhood onset type 1 diabetes; or, whether LADA is a distinct form of autoimmune diabetes which resembles type 2 diabetes showing evidence of insulin resistance with a high frequency of Metabolic Syndrome (1). Therefore, the aim of this study was to test whether type 2 diabetes and autoimmune diabetes (incorporating type 1 diabetes and LADA) have a higher frequency of Metabolic Syndrome than normal subjects and our hypothesis was that they would.

PATIENTS AND METHODS

The study design is cross-sectional and includes adult diabetic patients and controls (aged 30 – 70 years) from 5 European cities (London, Belfast, Lyon, Barcelona and Rome) examined between 2004 and 2007. All patients came from 5 European hospital-based centers involved in ACTION LADA, a European Union-funded multi-centre European study with the aim of identifying immune and clinical risk factors for adult-onset autoimmune diabetes (www.actionlada.org). Patients were designated with diabetes according to standard criteria and LADA was defined as patients aged 30- 70 years of age with GADA who did not require insulin treatment for at least 6 months post-diagnosis (7,8). Type 1 diabetes patients and normal subjects fulfilling the inclusion criteria were ascertained consecutively

from 3 of these 5 European centers (London, Barcelona and Rome). The control subjects came from health centers in local communities.

Inclusion criteria were diagnosis of diabetes (with at least 2 fasting blood glucose measurements ≥ 7 mmol/L); time from diagnosis < 5 years for all non-insulin requiring diabetes patients; all patients and controls were aged 30 – 70 years at examination. Patients came from Europe but with different ethnicity 91.7% Caucasian, 4.6% Middle Eastern, 1.4% Asian, 1.3% African and 1.0% of mixed race. Control subjects were obtained from local communities attending primary care centres for routine examination with similar age range and gender ratio to the patients and were all Caucasian. Exclusion criteria for both patients and controls were: incomplete data set, currently pregnant, renal disease with raised creatinine or proteinuria; acute illness at the time of testing; in addition, patients with non-insulin requiring diabetes > 5 years duration were excluded as well as control subjects with any clinical disease, therapy, or family history of autoimmune disease. Data on medication and risk factors were registered by the attending physician based upon the medical files. Serum and plasma samples were collected according to standard procedures and stored at -80°C .

Each subject was tested locally for waist circumference and blood pressure. Blood pressure was measured at least twice in the sitting positions. Lipids, and lipoproteins (serum total and HDL cholesterol and triglycerides) were determined by standardized assays at each center. All patients were tested for GADA in a central laboratory (London) in all initially non-insulin requiring patients as part of the European Union ACTION

LADA programme.

Diagnostic criteria for the Metabolic Syndrome—The Metabolic Syndrome was assessed according to the NCEP criteria (6) as follows: waist circumference in males >102 cm and females >88 cm, triglycerides (TG) >1.70 mmol/l, HDL cholesterol in males <1.00 mmol/l and in females <1.30 mmol/l, blood pressure 130/85 mmHg or antihypertensive medication, and fasting glucose ≥ 6.1 mmol/l. We chose to identify all diabetes patients in this study as fulfilling the criteria for hyperglycemia. Three of five criteria were required for the diagnosis of the Metabolic Syndrome. As raised glucose is one criterion for Metabolic Syndrome and all the diabetes patients, by definition, have a raised glucose, we reanalyzed the data, excluding glucose as a variable.

Antibody Measurement: The radioimmunoprecipitation assay for GADA uses human islet GAD₆₅ cDNA employing in vitro transcription and translation systems as previously described (12). All samples were centrally tested in London in duplicate including positive and negative control standard sera. Each assay for GADA included in-house standard serum from a pre-diabetic individual serially diluted to end-point equivalent at 70 World Health Organisation (WHO) units above which samples were scored positive. A separate positive serum sample (equivalent to the WHO standard of 200 WHO units) was used as an in-house control to standardise each assay for unit calculation. In the DASP antibody workshop our assay had a sensitivity of 74%, specificity 98% for GADA (13). In the latest DASP workshop (2007) our assay had a sensitivity of 80%, specificity of 98% for GADA (data unpublished). Positive samples were repeated to

confirm GADA positivity and reduce false positive rate. In order to compare the prevalence of Metabolic Syndrome with respect to GADA titre in LADA patients, we sought to divide them into two subgroups using a Q-Q plot of GADA positive patients.

Statistical analyses—The differences between groups were tested with χ^2 test or Fisher's exact test when appropriate. Quantitative variables were analyzed with General Linear Model Univariate, a post-hoc analysis was performed with Bonferroni test and data presented as mean (SD). A logistic regression analysis was performed to evaluate confounding by covariables, with adjustment for sex, age of onset, disease duration and ethnicity to calculate odds ratio for Metabolic Syndrome; three dummy variables were created to include three groups of patients with controls as a reference group. Data are presented as odds ratios with 95% confidence interval (CI). GADA levels, TG and HDL were log transformed to normalize distributions. All analyses were performed using SPSS statistical software for Windows. A P value <0.05 was considered statistically significant. Q-Q probability plots were used to analyze the distribution of GADA measurements for normality. Observed antibody values were plotted along the horizontal axis against expected normal values under normality on the vertical axis using Blom's proportion estimation formula. The study protocol is in accordance with the Declaration of Helsinki, and was approved by local ethics committee in each study center. Informed written consent was obtained from all subjects before blood sampling.

RESULTS

Of the 2011 subjects studied, the diabetes patients (n=1652), mean (SD)

age was 52.7 (10.3) years, mean duration of diabetes was 5.2 (6.7), 51.8% were males. Of controls (n=359), mean age was 53.5 (10.7) years and 51.0% were males. The demographics of the different groups are shown in Table 1. Of note, the mean age was significantly lower in type 1 diabetes 43.8 (9.8) years than in LADA 49.7 (10.4) years ($p<0.0001$), and in both, lower than in type 2 diabetes 55.1 (10.1) years ($p<0.0001$). Clinical and biochemical features of each group are shown in Table 2.

The prevalence of the Metabolic Syndrome, including hyperglycemia as a component, was 75.5% in all diabetes patients and 26.5% in control subjects ($p<0.0001$). Metabolic Syndrome was detected in significantly more type 1 diabetes (31.9%) and LADA patients (41.9%) than controls ($p=0.006$). Referring to controls the Odds Ratios (OR) for Metabolic Syndrome are: for type 2 diabetes 22.5 (CI 15-33.7, $p<0.0001$); for LADA 2.2 (CI 1.2-3.6, $p<0.004$); for type 1 diabetes 1.1 (CI 0.7-1.9, $p=0.6$). There was a significant difference in the OR between type 1 diabetes and LADA for Metabolic Syndrome, even after correction for age, duration of disease, sex and ethnicity (OR=3.2 CI 1.2-8.3, $p=0.015$). Prevalence of Metabolic Syndrome was higher in type 2 diabetes (88.8%) than in either LADA (41.9%) or type 1 diabetes (31.6%) ($p<0.0001$ for both comparisons). No differences were seen in gender for Metabolic Syndrome (males 50% versus males 50%). The risk of Metabolic Syndrome increases with age (OR= 1.05 per year, CI: 1.04- 1.06) ($p<0.0001$).

When glucose was excluded as a variable, Metabolic Syndrome was not more prevalent in autoimmune diabetes (type 1 diabetes and LADA) (17.3%) than in controls (23.7%), but remained more prevalent in type 2 diabetes (47.8%) ($p=0.001$ for all groups). Metabolic Syndrome was less prevalent

in type 1 diabetes (16.9%) than LADA (25.0%) or controls (23.7%) ($p=0.04$), and was similar in the two latter ($p=0.7$). Referring to controls, the Odds Ratios (OR) were: for type 2 diabetes 2.4 (CI 1.6-3.6, $p<0.0001$); for LADA 0.9 (CI 0.50-1.60, $p=0.69$); for type 1 diabetes 1.1 (CI 0.39-1.12, $p=0.60$). The frequency of Metabolic Syndrome, considering all groups, was still similar in males (53.1%) and females (46.9%) ($p=0.45$). The risk of Metabolic Syndrome still increased with age (OR=1.02 per year, CI: 1.01- 1.03) ($p<0.0001$). It follows that factors associated with Metabolic Syndrome include: diabetes and older age, and, when glucose is excluded as a trait, type 2 diabetes and older age.

Of the individual components of the Metabolic Syndrome, after adjusting for age of onset, duration of disease, sex and ethnicity: waist circumference was similar in type 1 diabetes and LADA ($p=0.44$), but lower in each than in patients with type 2 diabetes ($p<0.0001$ for both comparisons); systolic and diastolic blood pressure were similar in type 1 diabetes and LADA ($p=0.28$, $p=0.49$, respectively), but lower in each than in patients with type 2 diabetes ($p<0.0001$); TG were similar in type 1 diabetes and LADA ($p=0.63$), but lower in each than in patients with type 2 diabetes ($p<0.0001$); HDL cholesterol was similar in type 1 diabetes and LADA ($p=0.40$) and, in both, higher than in type 2 diabetes ($p<0.0001$). Waist circumference in the combined diabetes cases ($n=1652$) was directly associated with age at sampling ($r = 0.28$; $p <0.0001$) and inversely related with disease duration ($r = -0.28$; $p <0.0001$). Of the individual components, waist circumference ($r = 0.16$; $p <0.0001$) and systolic blood pressure ($r = 0.29$; $p <0.0001$), elevated TG ($r = 0.13$; $p <0.0001$) but not diastolic blood pressure

($r = 0.03$; $p= 0.26$), were more common with increasing age, whereas HDL cholesterol ($r = -0.09$; $p <0.008$) decreased with age.

A Q-Q plot of GADA positive patients was performed to seek distinct populations and we identified an inflexion point corresponding to 200 WHO IU consisting with two modes (data not shown). Further, a plot of GADA titre according to patient frequency revealed a possible bimodality, and the lowest value between the two modes was at GADA titre 200 WHO IU (data not shown). Therefore, we arbitrarily analysed the Metabolic Syndrome according to GADA positivity in those above or below 200 WHO units of GADA and found it similar in LADA patients with high (>200 IU) ($n=39$) as low (70-200 IU) ($n=78$) GADA titre (47.3% and 40.3% respectively) even after correction for age, sex, duration of disease and ethnicity ($p= 0.37$, CI 0.29-1.6).

The frequency of features of Metabolic Syndrome formed a hierarchy which was similar in all the groups, irrespective of the presence or type of diabetes, such that elevated waist circumference $>$ high blood pressure $>$ low HDL cholesterol $>$ high TG. Among those patients with the Metabolic Syndrome, the most frequent features of its components are shown in Table 3. The cluster of hyperglycemia, increased waist and high blood pressure was seen in 62.1% of those with the Metabolic Syndrome; whilst hyperglycemia, hypertension, and abdominal obesity were seen in 32%, and hyperglycemia, hypertension, high triglycerides, and low HDL cholesterol were seen in 17.9%.

DISCUSSION

These observations indicate that the Metabolic Syndrome is a frequent finding

in autoimmune diabetes but is not more frequent in autoimmune diabetes than in normal subjects when glucose is excluded as a risk factor. In contrast, Metabolic Syndrome is far more prevalent in type 2 diabetes, even when glucose is excluded as a variable. Whether glucose was, or was not, used as a variable, we found that individual components of the Metabolic Syndrome in both type 1 diabetes and LADA, were similar, but in each less than in type 2 diabetes patients. It follows that there is no evidence from this data set that autoimmune diabetes is distinct in terms of prevalence of Metabolic Syndrome from normal subjects and the hypothesis, that it would be distinct, is rejected. Nevertheless, Metabolic Syndrome was more prevalent in LADA than type 1 diabetes when glucose was included as a variable.

Glucose is a debateable component of the Metabolic Syndrome and was introduced for assessing type 2 diabetes, not type 1 autoimmune diabetes (14). When glucose was excluded, the prevalence of Metabolic Syndrome in autoimmune diabetes was not greater than in normal subjects. Therefore there is no evidence to suggest that autoimmune diabetes is due to decreased insulin sensitivity, instead decreased insulin sensitivity might predispose to an earlier time of diagnosis. Such decreased insulin sensitivity could explain why age, overweight and physical inactivity are strong predictors of LADA as they are of type 2 diabetes (15, 16). These present observations indicate that Metabolic Syndrome is common in adult autoimmune diabetes as it is in normal subjects. Thus, agents such as metformin and other insulin sensitizers may be beneficial in autoimmune diabetes, as they are in type 2 diabetes, in line with

recent studies indicating that metformin is efficacious in type 1 diabetes (17).

Of the three current criteria for Metabolic Syndrome (IDF, NCEP and WHO), we used the NCEP criteria because they are more appropriate to apply here to populations of variable geographical origin, in preference to IDF criteria, for simplicity, and the WHO criteria, which include insulin resistance and microalbuminuria (6). Whilst it is possible that Metabolic Syndrome should be defined differently in autoimmune diabetes, including type 1 diabetes and LADA, the hierarchy of features associated with Metabolic Syndrome was similar to that found in type 2 diabetes and controls. Nevertheless, the Metabolic Syndrome was not originally introduced to identify a feature of autoimmune diabetes, but to capture the clustering of a group of continuous variables associated with cardiovascular risk. That cluster has subsequently been extended to include measures of endothelial dysfunction and low-grade inflammation, but it remains unclear whether these new parameters are also features of autoimmune diabetes, for example, type 2 diabetes is associated with increased levels of pro-inflammatory serum cytokines and acute-phase proteins especially in association with obesity, while in type 1 diabetes such inflammatory changes are mild or non-existent (18). LADA patients have not been consistently found to exhibit the systemic low-grade inflammation previously identified in type 2 diabetes (18). Thus, it is reasonable to conclude that autoimmune diabetes, whether type 1 diabetes or LADA, differs from type 2 diabetes with respect to systemic low grade inflammation, as it does with Metabolic Syndrome.

Previous studies of Metabolic

Syndrome in LADA have confirmed that, whilst it is prevalent, it is less prevalent than in type 2 diabetes (19,20). There are only two previous studies comparing LADA with type 1 diabetes, each suggesting a tendency for LADA to have more features of the Metabolic Syndrome but without including control subjects; each study was very small with a combined total of 94 LADA and 112 type 1 diabetes patients, and one of them used highly selected LADA patients (9, 21). Large studies of type 1 diabetes have identified Metabolic Syndrome in Finnish patients (39%) which was approximately three times the separately observed prevalence in non-diabetic Finnish subjects (22) and higher than the frequency in American patients with type 1 diabetes (22%) (24). However, in the latter study that frequency of Metabolic Syndrome increased from 14.6% to 36.1% after 9 years diabetes duration (23). It follows that there is a dynamic in the frequency of Metabolic Syndrome in autoimmune diabetes, whether type 1 diabetes or LADA, which must be considered in comparing the groups. Therefore, the disparity in the prevalence of Metabolic Syndrome between the Finnish, and American studies, and our own European study probably reflects differences in population characteristics including duration of diabetes, renal function, intensive insulin therapy, male gender and age, all of which have been independently associated with Metabolic Syndrome.

There is some limited evidence, other than the clinical phenotype, to support the contention that LADA is an intermediate form of diabetes between type 1 diabetes and type 2 diabetes. LADA, albeit defined as GADA positive adults irrespective of therapy, was reported to be associated with the CT and

TT genotypes of the TCFL2 gene and therefore apparently shares genetic features with type 2 diabetes (24). However, the high false positive rate of the GADA assay used in that study raises issues regarding the validity of the observation. We limited this potential error by repeating GADA assays in GADA positive subjects thereby reducing the risk of false positivity to less than 0.2%, much less than the proportion of LADA patients. The error from not testing other diabetes-associated autoantibodies is probably small given that 94% LADA patients sampled from this cohort were detected by GADA testing alone (manuscript in preparation). The proposal that the Metabolic Syndrome is more prevalent in those LADA patients with low titer GADA as compared with those with an arbitrary selected high titer of GADA was not confirmed in this present study using a Q-Q plot to identify two apparently distinct GADA positive distributions (21). However, there is substantial variation between laboratories with respect to threshold GADA titers defining a positive result, and caution should be exercised in using such thresholds.

So metabolic differences between LADA and type 1 diabetes may not be categorical, but part of a continuum, implying that LADA is one end of a rainbow of a pathophysiological variation encompassing autoimmune diabetes (1). If we accept that Metabolic Syndrome is a surrogate marker for insulin resistance, there is now evidence that adult LADA patients are more insulin resistant than adult type 1 diabetes patients, but not when glucose is excluded as a variable, when both are similar to normal subjects. This conclusion is important, as it implies that autoimmune diabetes, whether type 1 diabetes or LADA, can be identified by diabetes-associated autoantibodies, e.g.

GADA, as a categorical trait, irrespective of individual components of the Metabolic Syndrome or the need for insulin treatment. Indeed, we have previously suggested that insulin treatment is an insubstantial feature to diagnose LADA since it is subject to local issues such as medical practise and context, including availability of GADA results (25). Thus, the role of insulin resistance in autoimmune diabetes may be limited, and there is no characteristic clinical phenotype of adult-onset autoimmune diabetes, specifically neither forms of autoimmune diabetes showed an increased frequency of Metabolic Syndrome, in striking contrast to type 2 diabetes.

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Table 1. Demographics of the groups

	Normal subjects	Type 1 diabetes	LADA	Type 2 diabetes
Numbers	359	288	117	1247
Female (%)	176 (49%)	156 (54.2%)	56 (47.8%)	665 (53.3%)
Mean age (years) (SD)	53.6 (10.7) [*]	43.8 (9.8) [*]	49.4 (10.2) [*]	55.1 (9.1) [§]
Mean age of onset (years) (SD)	--	25.7 (11.7) [*]	47.1 (10.4) [*]	52.7 (9.2) [*]
Mean disease duration (years) (SD)	--	18.2 (11.7) [°]	2.7 (1.8)	2.4 (1.8) [°]

^{*}p < 0.0001: Type 1 diabetes vs LADA; Type 1 diabetes and LADA vs Type 2 diabetes and controls.

[§]p = 0.043: Type 2 diabetes vs controls.

[°]p < 0.0001: Type 1 diabetes vs LADA and Type 2 diabetes.

Table 2. Mean (SD) clinical and biochemical characteristics of each group of subjects.

	Normal subjects	Type 1 diabetes	LADA	Type 2 diabetes
Waist (cm)	93.7(17)	89.6 (14.4)	93 (15.5)	107.2 (13.8)
Systolic BP (mmHg)	130.6 (14.6)	127.1(14.8)	126.9 (16.9)	141.3 (17.4)
TG (mmol/L)	1.7 (0.8)	1.3 (0.7)	1.6 (1.4)	2 (1.5)
HDL-cholesterol (mmol/L)	1.5 (0.4)	1.6 (0.5)	1.5 (0.5)	1.3 (0.4)

Table 3. Percentage of subjects with values over cut-off for each component of Metabolic Syndrome. Patients with type 2 diabetes have a higher frequency of increased waist and systolic blood pressure. Patients with autoimmune diabetes (type 1 diabetes and LADA) tend to resemble normal subjects for all features apart from increased blood glucose (though a fraction of normal subjects had impaired fasting glucose).

	Normal subjects (359)	Type 1 diabetes 288)	LADA (117)	Type 2 diabetes (1247)
Glucose	6.7%	100%	100%	100%
Waist	43.7%	25.7%	35.9%	82.8%
Systolic BP	65.9%	50.7%	49.6%	86.7%
TG	39.6%	23.6%	35.8%	49.8%
HDL-cholesterol	21.2%	17%	29%	36.1%