



IMMUNOLOGICAL AND CLINICAL CHARACTERISTICS OF LATENT AUTOIMMUNE DIABETES IN THE ELDERLY

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/dmrr.3137

Abstract

LADA is determined by both a noninsulin-dependent clinical presentation and an autoimmune pathogenic process. GADA constitutes the most important marker, although IA-2A and ZnT8A also define LADA presentation. T2DM is the most prevalent type particularly over 65 years old. Studies about autoimmunity in this age group are scarce.

Objective: The aim of this work was to determine whether three autoantibodies for diabetes autoimmunity were present in elderly T2DM patients, and to assess the distinctive clinical features of autoantibody-positive patients.

Research Design and Methods: We recruited 153 patients with diabetes with onset of diabetes after 65 years of age and a BMI under 30 kg/m²

Results: The prevalence of at least one of the autoantibodies was 15.68% (24/153). The most prevalent autoantibody was GADA with 8.49% (13/153), followed by ZnT8A with 6.50% (10/153) and IA2A with 1.96% (3/153). The autoimmunity-positive group presented higher HbA1c (7.01±1.98 vs. 6.35±1.01; p=0.007) and more prevalent insulin therapy (25% vs. 10.85%; p=0.047). GADA-positive patients with diabetes presented higher FPG (7.79 ± 3.79 mmol/L vs. 6.43 ± 1.6 mmol/L ; p=0.014) and insulin therapy more frequently (46% vs. 10.71%; p=0.015). GADA titer levels in the individuals with BMI under 27 kg/m² were higher (35.00 ± 4.20) than those in the group with BMI over 27 kg/m² (8.83 ± 3.041; p=0.0005).

Conclusion: Autoantibodies GADA and Znt8A may be useful markers in identifying a subgroup of older patients with a clinical presentation of diabetes which could be characterized as latent autoimmune diabetes in the elderly .

Introduction

Latent autoimmune diabetes in adults (LADA) is a complex and multifactorial clinical presentation of noninsulin-dependent diabetes mellitus, or type 2 diabetes mellitus (T2DM), determined by the loss of self-tolerance against endocrine pancreas β cells, a well-known pathogenic component of type 1 diabetes mellitus (T1DM) (1). LADA thus combines features of both T1DM and T2DM. As a matter of fact, whether LADA represents a different group from T1DM or is merely the same entity presented later in life is still under debate, although it has been suggested that this group is genetically, immunologically and clinically different from T1DM (2).

Multiple factors are involved in the pathogenesis of LADA from genetics to clinical characteristics, including markers of autoimmune diabetes such as circulating autoantibodies, self-reactive T cells, evidence of lymphocytic infiltrates in β cells, non obesity, association with other autoimmune diseases and increasing insulin treatment, among others (3). Glutamic acid decarboxylase antibody (GADA) is a key marker of the autoimmune attack in LADA and by far the most common autoantibody in adult-onset diabetes (4). Several studies have determined other autoantibodies in LADA such as tyrosine phosphatase IA-2 antibodies (IA-2A) and Zinc T8 antibodies (ZnT8A), although these markers have not been found in patients with T2DM (5-6).

LADA patients are generally defined by onset >30 years of age, presence of circulating islet autoantibodies and no need of insulin treatment for 6 months after diagnosis (7). However, these patients need insulin earlier during diabetes progression than T2DM patients and are likely to respond poorly to oral antidiabetic drugs, although they may respond favorably to immunomodulatory therapy (8). To date, the largest cohort examined for LADA included over 6,000 adult-onset T2DM patients and revealed a prevalence of adult-onset autoimmune diabetes of 9.7% (9). As compared to T2DM patients, LADA patients generally present

earlier diabetes onset, lower body mass index (BMI) and a more pronounced loss of insulin secretion determined by lower C-peptide levels and hence increased insulin treatment (10).

The LADA phenotype appears to vary in accordance with the GADA titer. GADA-positive patients with a high titer tend to have clinical characteristics resembling T1DM. These patients are younger and leaner at diagnosis, with a high risk of progression to insulin treatment. On the other hand, LADA patients with a low GADA titer are phenotypically more similar to those with T2DM. These differences are characterized by the presence of metabolic syndrome, which is more prevalent in T2DM than in T1DM or LADA (11-12-13).

The TODAY Study evaluated GADA and IA2A in 1206 subjects between 10 and 17 years, clinically considered T2DM patients. Among the 118 patients (9.8%) found to be antibody-positive, 71 (5.9%) were positive for a single antibody, while 47 were positive (3.9%) for both antibodies. This autoimmune process in Type 2 diabetes patients who are younger than Type 2 diabetes adults is referred to as latent autoimmune diabetes in the young (LADY).(14)

The incidence of diabetes increases an overall 23% every year, while prevalence increases 62% in patients 65 years and older (15). More than 20% of the population over 65 years of age suffer from diabetes; most of them have T2DM, while 0.3% women and 0.4% men between 60 and 80 years of age have T1DM (16). Pietropaolo *et al.* have reported GADA and IA-2A detection in 12% of 196 serum samples from phenotypically T2DM patients older than 65 years (17). However, no studies have been published determining ZnT8A in this T2DM patient age group.

In this context, the main goal of our study was to determine whether the autoantibodies currently regarded to have predictive value for autoimmunity, i.e. GADA, IA2A and ZnT8A, were present in T2DM patients with onset over 65 years of age and a BMI under 30 kg/m². The secondary aim was to compare the anthropometric, clinical and biochemical findings between patients with positive and negative autoantibodies.

Materials and Methods

Healthy Argentine control individuals

Control sera were obtained from 125 healthy Argentine individuals (52% female) without personal or family history of diabetes mellitus or autoimmune diseases and normal fasting glucose. The sample collection was approved by the Ethics Committee of the José de San Martín Clinical Hospital, University of Buenos Aires (UBA), Buenos Aires, Argentina. All subjects were informed about the purpose of the study, and a signed consent for study participation was obtained. GADA, IA2A, ZnT8A and IAA were assessed, and all of the individuals tested negative. Sera were stored at -20 °C until assayed.

Argentine patients with diabetes

This was a cross-sectional study which did not include a follow-up of the patients. The patients were recruited over one year, between July 2016 and August 2017, with the two inclusion criteria described below. All patients were receiving or had received treatment with oral drugs or insulin therapy targeting an A1c level under 7%, but received no further treatment as part of the study.

We randomly recruited 153 T2DM patients (58% women) with the following inclusion criteria: individuals with diabetes onset over 65 years of age, without insulin treatment until 12 months after diagnosis and BMI under 30 kg/m². The exclusion criteria were: patients with active systemic disorders and/or infections, individuals with a previous diagnosis of T1DM, liver or heart failure, surgery or hospitalization over the past year. The diagnosis of

diabetes was carried out in accordance with the guidelines of the American Diabetes Association (18). Written consent was obtained from all participants involved in this study.

Measurements

Anthropometric measurements (height, weight and waist circumference), systolic blood pressure and diastolic blood pressure were determined by standardized protocols. BMI was calculated as weight (kg)/[height(m)]². After a 12 h overnight fast, venous blood samples were obtained from every individual, centrifuged to obtain serum and analyzed immediately. Fasting plasma glucose (FPG), creatinine, total cholesterol, TG, LDL-C and HDL-C were determined in serum using standardized procedures by enzymatic methods. HbA1c was measured using high-performance liquid chromatography (HPLC) Variant II Turbo HbA1c Kit 2.0.

All subjects underwent a detailed standard evaluation to detect diabetic retinopathy (fundus photography), and nephropathy was defined by microalbuminuria (at least two of three consecutive visits with an albumin excretion rate of 20 µg/min or higher). Cardiovascular disease was established with respect patients' clinical records of coronary heart disease, stroke, or peripheral arterial disease.

Ethics statement

All procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (José de San Martín Clinical Hospital, University of Buenos Aires, UBA) and with the Helsinki Declaration of 1975, as revised in 2008.

Radioligand binding assay protocol for GADA, IA2A and ZnT8A

GADA, IA2A and ZnT8A were assessed by radioligand binding assay (RBA) as previously described (6). Briefly, 5 uL human sera were incubated overnight at 4°C with 10,000 cpm of [35S]-ZnT8-Arg-Trp325, [35S]-GAD65 or [35S]-IA2A, respectively, in a final volume of 60

uL in the RBA buffer. Afterwards, 50 uL protein A-Sepharose 4B FF (GE Healthcare Biosciences, Uppsala, Sweden) was added in the RBA buffer (2:3) to isolate immunocomplexes and incubated for 2 h at room temperature on an end-over-end shaker. Subsequently, samples were allowed to settle and the supernatants were discarded. Pellets were washed three times with 200uL RBA buffer and once with 200 uL of 0.2 M NaCl in RBA buffer. Finally, pellets were suspended in 100 uL of 1% w/v SDS and supernatants were carefully transferred to vials for scintillation counting (1 min per tube). Results for each sample were calculated as bound % (B%)=100 x (bound cpm/total cpm), and expressed as standard deviation scores (SDs)=(B%-Bc%)/SDc, where Bc% is the mean B% of control sera and SDc its standard deviation. Thirty normal control sera were included in each assay and Bc% was normally distributed. An assay was considered positive when SDs > 3. In the Diabetes Antibody Standardization Program (DASP) 2007, our GADA assay proved to have 79.6% sensitivity and 98.0% specificity. Likewise, in the Islet Autoantibody Standardization Program (IASP) 2015, our IA-2A assay had 66.0 % sensitivity and 97.8% specificity, while our ZnT8A assay showed 68.0% sensitivity and 97.8% specificity.

We established an arbitrarily high or low level of GADA considering whether the titer was above or under 20 SDs, respectively.

Statistical analysis

Data on continuous variables are shown as means \pm SDs. Categorical variables are reported as proportions (%). A two-tailed probability value of 0.05 was considered to be statistically significant. Odds ratios (ORs) are reported with their 95% confidence intervals (CIs). The D'Agostino and Pearson omnibus normality test was applied to assess whether data from normal human control sera were normally distributed. The Mann–Whitney U test was used to evaluate significant differences in SDs. For univariate analysis, differences between groups for qualitative variables were assessed by Fisher's exact test. One-way ANOVA was

conducted for comparisons of continuous variables and Levine's test was performed to study equality of variances. Variables that appeared to be associated in the univariate analysis were introduced into the multivariate analysis. Multiple linear regression and logistic regression analyses were used to adjust for possible confounding variables. Statistical analyses were conducted using Statistical Package for the Social Sciences software, version 12.0 (SPSS, Chicago, IL, USA) and Prism 4.0 (GraphPad Software, La Jolla, CA, USA).

Results

The prevalence of the three positive autoantibodies was 15.68%, including 24 out of 153 T2DM patients recruited. Among the 153 patients evaluated, GADA autoantibody exceeded the cutoff point in 8.49% cases (13/153), IA2A in 1.96% (3/153) and ZnT8A in 6.50% (10/153). Among the patients with autoimmune diabetes, GADA was the most prevalent autoantibody with 58.29% (13/24), ZnT8A was present in 41.66 % (10/24) and IA2A in 12.5% (3/24). Only two patients presented positivity for two antibodies simultaneously: one of them GADA and IA2A, and the other one GADA and ZnT8A (Figure 1).

The comparison between autoimmunity-positive and negative patients revealed statistically significant differences with respect to sex, as women accounted for 75% of autoantibody-positive subjects but only 54.2% of the antibody-negative group ($p=0.046$). In contrast, no significant differences were detected in terms of BMI, age, age at diagnosis or duration of disease regarding the presence or absence of islet autoantibodies. The analysis of HbA1c levels also rendered significant differences, as 56.52% (13/23) autoimmunity-positive subjects exceeded HbA1c of 7% (53.01 mmol/mol) regarded as a metabolic control cutoff, as compared to 22.83% (29/127) subjects in the autoimmunity-negative group ($p=0.038$). In this

way, the autoimmunity-positive group presented worse metabolic control measured by HbA1c than the autoimmunity-negative group (7.01 ± 1.98 vs. 6.35 ± 1.01 , $p=0.007$). On the other hand, FPG in the autoimmunity-positive group was higher, though not statistically different from that observed in the autoimmunity-negative group (Table 1).

In agreement with worse metabolic conditions, more autoimmunity-positive patients received insulin treatment (25%) as compared to autoimmunity-negative subjects (10.85%; $p=0.047$). These results were sex-adjusted with a considerably elevated OR of 3.097 (1.01-9.42% CI), which reinforced the relevance of insulin treatment for autoimmunity-positive patients with the evolution of diabetes. No statistical differences were found between groups with respect to treatment with antidiabetic oral drugs (Table 2).

No differences were detected between groups regarding T2DM comorbidities such as obesity, hypertension and metabolic syndrome, or microangiopathic complications such as nephropathy and retinopathy (Table 3).

GADA-positive T2DM patients presented higher levels of FPG (7.79 ± 3.79 mmol/L) as compared to GADA-negative ones (6.43 ± 1.6 mmol/L; $p=0.014$). Also, in accordance with the worse metabolic control, the GADA-positive T2DM group rendered a larger proportion of insulin treatment cases (5/13, 38.46%) than the GADA-negative T2DM group (15/140, 10.71%; $p=0.015$) with a remarkably high OR of 8.208 (1.509-17.981; 95% CI).

The analysis of GADA levels revealed statistical differences regarding the high-GADA titer group, which accounted for a large proportion of the BMI < 27 kg/m² group (6/7 patients, 85.7%) but no cases in the BMI > 27 kg/m² group (0/6, 0%; $p=0.004$). Finally, assays on GADA levels as a continuous variable rendered a GADA titer of 35.00 SDs \pm 4.20 in BMI < 27 kg/m² individuals, which was significantly different from the GADA titer of 8.83 ± 3.041 in the BMI > 27 kg/m² group ($p=0.0005$) (Figure 2).

Discussion

Diabetes is known to have major impact on the health of elderly patients, which makes it essential to implement appropriate treatment based on its clinical and pathophysiological presentation (19). Still today, clinical practice with over 65 patients with diabetes involves the pathophysiological interpretation, the metabolic changes and the treatment strategies used in younger patients.

This study constitutes a first report on the presence of the three antibodies against β cells currently used in diagnosis, i.e. GADA, IA2A and ZnT8A, in a population of elderly T2DM or non-insulin-dependent diabetes mellitus patients, diagnosed over 65 years of age and exhibiting normal weight or slight overweight. To the best of our knowledge, no previous studies have been conducted on ZnT8 in elderly T2DM patients, and most trials about the prevalence of autoimmunity in adult diabetes range from 30- to 70-year-old patients.

Moreover, this study provides evidence of autoimmunity in a scarcely documented population, i.e. non-obese patients with diabetes. In 1999, an Italian population composed of lean, BMI < 25%, newly diagnosed patients between 30-54 years old was studied in order to determine the prevalence of islet β cell antibodies (ICA) and GADA. Among 130 patients recruited, 34.6 % were ICA-positive, 22.3% were GADA-positive, and almost 50% presented slowly progressive autoimmune diabetes (20). In addition, previous studies by our group have analyzed the presence of proinsulin autoantibody (PAA), GADA, IA2A and ZnT8 in 271 patients diagnosed with diabetes at mean age 53.4 ± 10.9 , BMI ≤ 30 , without insulin treatment for the first year of disease. Among them, 22.1% presented at least one humoral marker, 2.6% were PAA+, 12.5% were GADA+, 3.3% were IA-2A+, and 10.7% were ZnT8A+ (6).

In the current study, we observed a high prevalence of autoimmunity in older patients with diabetes, as 15.68% of them tested positive for at least one antibody. The most prevalent

autoantibody was GADA with 8.49%, followed by ZnT8A with 6.50%, in agreement with previous reports on autoimmunity in T2DM patients. In this sense, it has been demonstrated that ZnT8A is, in fact, a helpful humoral marker that should be included in diagnostic tests for the screening of LADA. Previous reports have proven that patients with adult-onset diabetes mellitus, initially identified as marker-negative based on GADA and IA-2A screening, were reclassified as autoantibody-positive when tested for ZnT8A. On the other hand, in non-obese, adult-onset patients with diabetes the use of a dimeric construct (C-terminal domain of ZnT8 -amino acids 268–369- carrying Arg325–Trp325) showed the highest percentage of autoimmunity detection. It is reasonable to think that the increase in assay sensitivity may be due to an increased functional avidity of specific antibodies for these best structured antigenic variant (6, 21).

Only 2 patients proved positive for two simultaneous antibodies, GADA and IA2A in one case, and GADA and ZnT8A in the other one. As expected in cases of late autoimmunity presentation, no patients presented three antibodies. Our study also shows women to be more affected by autoimmunity than men, in agreement with several studies showing women susceptibility to autoimmune diseases (7, 22).

The largest cohort studied so far involved a European population of 6,156 T2DM patients in which 9.75% patients were found to be autoimmunity-positive. Regarding specific antibody expression, 541 (8.8%) were GADA-positive and only 57 (0.9%) were IA-2A or ZnT8A-positive (7). In turn, the U.K. Prospective Diabetes Study (UKPDS), which included mainly overweight patients, focused on ICA and GADA in a group of newly diagnosed patients ranging between 35 and 54 years of age. In this case, 12.5% patients were positive for at least one antibody, with 6.5% ICA-positive and 10.7% GADA- positive patients, and only 4.6% two-antibody-positive (23).

On the other hand, a report from Pittsburgh about the prevalence of GAD 65AA analyzed a sample of 3,318 adults 65 years or older included in the Cardiovascular Health Study. The individuals were classified into five groups: 1) known diabetes, 2) newly diagnosed diabetes, 3) impaired glucose tolerance only, 4) impaired fasting glucose and 5) controls. The prevalence of GADA was 5.3%, 2.2%, 1.9%, 2% and 1.8%, respectively. The prevalence of GADA was statistically higher in participants diagnosed with diabetes compared to normoglycemic controls and increased with decreasing glucose tolerance.. GADA positivity in this elderly population was associated with diagnosed diabetes and the use of insulin and oral drugs combined. In this work, the authors studied the overall population of T2DM patients over 65 years old and found, in agreement with our results, that GADA-positive T2DM patients used insulin treatment more frequently (24).

A Swedish study on newly diagnosed patients over 65 showed a high 15% prevalence of GADA among 231 patients. Of 20 patients, 5 (25%) with two or three antibodies were 65 years of age or older at diagnosis, and 4 of 5 (80%) had developed complete β -cell failure after 12 years. (25)

Recently, the LADA China Study published research on 529 non-insulin-requiring patients with diabetes over 30 years old with positive autoantibodies GADA or IA2A. Those LADA patients were separated in two groups in accordance with the onset of diabetes over or under 60 years of age, LADA-Elderly and LADA-Young, respectively. LADA-E compared with LADA-Y had better residual β -cell function, higher insulin resistance, more serious metabolic syndrome characteristics, similar proportion of islet autoantibody positivity and, strikingly, different HLA-DQ genetic background (26).

On the other hand, it is worth pointing out that the discrepancy observed in the prevalence of GADA across different studies may be due to differences in recruitment criteria, genetic background, ethnic origin and BMI, among other factors. However, GADA is unequivocally the most prevalent antibody generally observed.

The present study shows ZnT8A as the second most prevalent antibody, with a high 6.50% in this particular population of autoimmune diabetes over 65 years of age. Our group had previously determined ZnT8A positivity in another cohort of Argentine patients, which rendered 29 (10.3%) ZnT8-positive cases out of 282 non-obese adult patients with diabetes (27). Furthermore, the NIRAD study analyzed ZnT8A in 196 patients with adult onset autoimmune diabetes (ADA) previously known to be GADA or IA-2A-positive, with results showing 18.6% ZnT8A detection (21). This recently discovered antibody may thus be regarded as extremely useful in the diagnosis of ADA, being second to GADA in prevalence among both adult and elderly patients with diabetes.

Zampetti et al. found that the presence of ZnT8 antibodies was more frequent in LADA patients with high GADA titer compared with LADA patients with low GADA titer and type 2 diabetes patients. These authors also found a high prevalence of other autoantibodies in LADA patients with high LADA titer such as thyroid peroxidase, steroid 21-hydroxylase, tissue transglutaminase and antiparietal cell antibodies.(28)

The present work also shows worse metabolic conditions among autoimmunity-positive patients with diabetes considering $< 7\%$ HbA1c levels a good metabolic control, as established by the American Diabetes Association (19). In this regard, GADA-positive T2DM patients presented higher levels of both FPG and HbA1c as compared to GADA-negative patients, which confirms the metabolic alterations associated to autoimmunity. Along the same line, a recent retrospective study in a Swedish population showing a 107-

month follow-up of 372 GADA-positive LADA patients revealed worse metabolic conditions than those of early diagnosed T2DM patients (29).

The current study further found that patients with autoimmunity had higher insulin requirements than those without autoimmunity. Multiple studies have found that LADA patients require insulin treatment more frequently and earlier than those with antibody-negative T2DM. In the HUNT study, 40% GADA-positive patients vs. 22% GADA-negative patients received insulin treatment 14 years after diagnosis. (30).

In the CARDS report, 56% and 17% of GADA-positive and negative patients, respectively, required insulin at the beginning of the study. In addition, during a 45-month follow-up, 16% LADA patients needed insulin treatment, as opposed to 5% among those with non-autoimmune T2DM (31). Taken together, these findings suggest that the proper identification of autoimmunity in elderly T2DM patients may facilitate the implementation of earlier insulin treatment.

The present work also shows that, among patients with autoimmunity, those with a high GADA titer were leaner than those with a low GADA titer. As expected, no differences were found regarding metabolic syndrome between low and high-GADA-titer patients, although it should be considered that our population was recruited with a BMI below 30%. In line with these results, several reports have shown high-GADA-titer patients to share some clinical characteristics to T1DM, such as earlier diagnosis, lower BMI and lower risk of progression to insulin treatment. In contrast, low-GADA-titer patients are phenotypically more similar to those suffering T2DM, with clinical characteristics resembling metabolic syndrome and insulin resistance (31, 32, 33).

Even if the seemingly small number of T2DM patients with positive autoimmunity may be thought of as a weakness of the present study, proper considerations should be made as to the specific characteristics of the cohort and the fact that the prevalence of autoimmunity in the

entire population of elderly T2DM patients remains unknown. In addition, it is important to recognize the presence of autoimmunity as a means to attain optimal metabolic control and preserve β -cell function to decrease the risk of long-term diabetes complications. In this regard, recent progress has been made through specific treatment with dipeptidyl peptidase 4 inhibitors to protect C-peptide levels and improve glycemic control in LADA patients (34, 35).

In summary, the findings reported here support the use of GADA and Znt8A as two key markers in identifying a subgroup of elderly patients whose condition may be referred to as latent autoimmune diabetes in the elderly, with a clinical presentation resembling that of T2DM, albeit with worse metabolic control and a higher need of insulin treatment. In this regard, GADA and Znt8A determination may shed light on the adequate classification and treatment of autoimmunity in elderly patients and the mechanisms involved in autoimmune diabetes. The present work thus contributes to a better understanding of diabetes in the elderly and to the implementation of healthcare practices which better match their recognized age-related weakness.

Acknowledgments

SY: Data research

APS: Data analysis/manuscript writing

CM: Data research

NF: Laboratory analysis

GC: Manuscript revision/edition

SL: Data research

ER: Manuscript writing/revision

SV: Manuscript revision/edition

GF: Manuscript writing/revision/edition

Financial support: Argentine Diabetes Association

University Institute of Health Sciences, Barcelo Foundation

The authors have no relevant conflict of interest to disclose.

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Table 1 Clinical characteristics of the sample

| Variable | Without autoimmunity (n=129) | With autoimmunity (n=24) | Total (n=153) | p value |
|--------------------------|---------------------------------|-----------------------------|------------------|---------|
| Sex (women/men) | 70/59 | 18/6 | 88/65 | 0.046 |
| Age (yr) | 77.48±6.25 | 76.83±5.30 | 77.32±6.16 | 0.653 |
| Weight (kg) | 66.47±12.26 | 64.14±8.38 | 66.11±11.75 | 0.375 |
| BMI (kg/m ²) | 25.62±2.60 | 25.88±2.30 | 25.68±2.54 | 0.636 |
| WC (cm) | 92.50±10.41 | 93.95±12.85 | 92.83±10.76 | 0.545 |
| WC (cm) (women) | 88.91±9.61 | 91.63±12.37 | 89.49±10.24 | 0.307 |
| WC (cm) (men) | 96.76±9.76 | 101.00±11.42 | 97.15±9.90 | 0.322 |
| FPG (mmol/L) | 6.45 ± 1.63 | 7.04 ± 3.03 | 6.54 ± 1.90 | 0.172 |
| HbA1c (%) | 6.35 ± 1.01 | 7.01 ± 1.98 | 6.47 ± 1.22 | 0.007* |
| mmol/mol) | 45±11 | 53± 2 | 47± 3 | |
| TC (mmol/L) | 4.80 ± 0.96 | 5.13 ± 1.11 | 4.85 ± 0.99 | 0.158 |
| HDL-C (mmol/L) | 1.28 ± 0.28 | 1.36 ± 0.38 | 1.29 ± 0.30 | 0.222 |
| LDL-C (mmol/L) | 2.94 ± 0.82 | 3.10 ± 1.05 | 2.96 ± 0.85 | 0.421 |
| TG (mmol/L) | 1.35 ± 0.57 | 1.55 ± 0.75 | 1.38 ± 0.6 | 0.155 |
| Creatinine (µmol/L) | 97.24 ± 37.12 | 91.93 ± 23.86 | 96.35 ± 35.36 | 0.52 |
| C-peptide (ng/mL) | 0.52 ± 0.25 | 0.48 ± 0.24 | 0.51 ± 0.25 | 0.515 |

BMI, Body mass index; WC, waist circumference; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides. *statistically significant

Table 2. Treatment with insulin and antidiabetic oral drugs

| | | Without autoimmunity n (%) | With autoimmunity n (%) | OR (95% CI) | p ^a |
|--------------|-----|----------------------------------|-------------------------------|---------------------|----------------|
| MTF | NO | 51 (39.53) | 10 (41.66) | 0.915 (0.378-2.218) | 0.601 |
| | YES | 78 (60.46) | 14 (58.33) | | |
| Sulph | NO | 100 (77.5) | 19 (79.16) | 0.907 (0.312-2.641) | 0.883 |
| | YES | 29 (22.5) | 5 (20.83) | | |
| INS | NO | 115 (89.14) | 18 (75.0) | 2.738 (0.932-8.044) | 0.047* |
| | YES | 14 (10.85) | 6 (25.0) | | |
| | | (n =129) | (n = 24) | | |

Treatment: MTF, metformin; Sulph, sulphonylurea; INS, insulin. p^a adjusted by sex

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Table 3. Comorbidities and chronic complications with and without autoimmunity

| | | Without autoimmunity n (%) | With autoimmunity n (%) | OR (95% CI) | p |
|--------------------|-----|----------------------------------|-------------------------------|---------------------|---------------|
| MS | NO | 49 (37.98) | 8 (33.330) | 1.225 (0.488-3.074) | 0.425 |
| | YES | 80 (62.02) | 16(66.66) | | |
| HT | NO | 27 (18.00) | 4(37.5) | 1.235 (0.377-4.041) | 0.491 |
| | YES | 82(82.00) | 15(62.5) | | |
| Retinopathy | NO | 118 (91.47) | 19(79.16) | 2.823 (0.882-9.031) | 0.081 |
| | YES | 11(8.52) | 5(20.83) | | |
| Nephropathy | NO | 102(79.06) | 20(83.34) | 0.756 (0.238-2.396) | 0.436 |
| | YES | 27(20.93) | 4(16.66) | | |
| CV | NO | 94(72.87) | 22(91.67) | 0.244 (0.055-1.093) | 0.036* |
| | YES | 35 (27.13) | 2(8.33) | | |
| HyT | NO | 108 (83.72) | 17 (70.83) | 2.118 (0.782-5.737) | 0.115 |
| | YES | 21 (16.27) | 7 (29.16) | | |
| | | (n =129) | (n = 24) | | |

MS = metabolic syndrome; HT, hypertension; CV, cardiovascular disease; HyT, hypothyroidism

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Figure 1. Prevalence of GADA, IA2A and ZnT8A

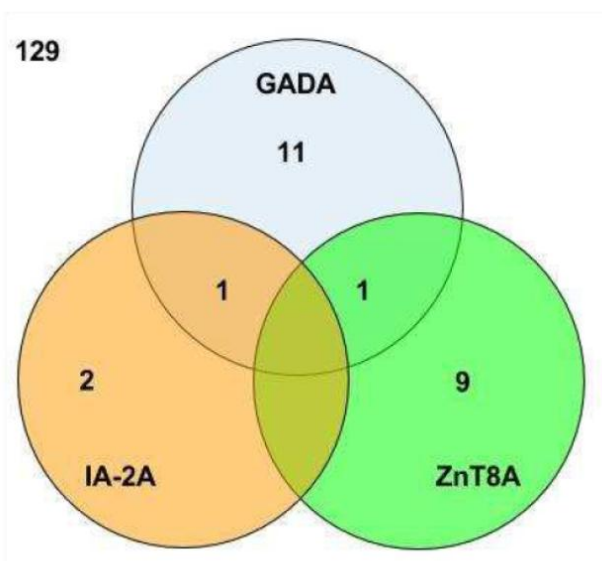
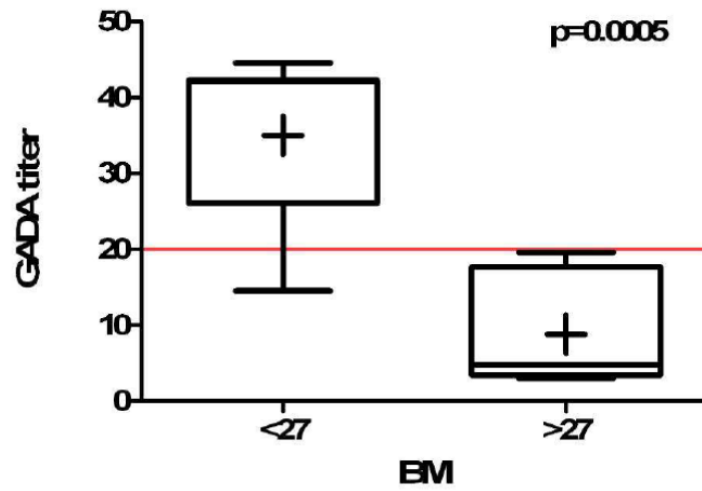


Figure 2: High and low GADA titer considering a cutoff point of 20 U with respect to BMI over or under 27 kg/m²



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