A convenient diagnostic tool for discriminating adult-onset glutamic acid decarboxylase antibody-positive autoimmune diabetes from type 2 diabetes : a retrospective study

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Background. The glutamic acid decarboxylase antibody (GADA) test, commonly used to diagnose autoimmune diabetes, is not cost-effective in areas of low prevalence. The aim of this study was to develop a convenient tool to discriminate adult-onset GADA-positive autoimmune diabetes from type 2 diabetes (T2DM) in patients with newly diagnosed diabetes.

Methods. This retrospective cross-sectional study, conducted at Changhua Christian Hospital in Taiwan, collected electronic medical record data from January 2009 to January 2018. Patients were divided into a case group (GADA+, n = 152) and a reference group (T2DM, n = 358). Variables that differed significantly between the groups were subjected to receiver operator characteristic analysis to establish cutoff values. Discriminant function analysis was then employed to discriminate the two groups.

Results. At the onset of diabetes, the GADA+ group was younger, with lower body mass index (BMI), higher hemoglobin A1c (HbA1c), higher high-density lipoprotein cholesterol (HDL-C), and lower total cholesterol and triglycerides (TG). Five major factors were identified to form the linear discriminant functions: BMI, age at onset, TG, HDL-C, and HbA1c. BMI <23 kg/m^2 was the most important factor, followed by TG <98 mg/dL, HDL-C ≥46 mg/dL, age at onset <30 years, and HbA1c ≥ 8.6%. The overall accuracy of the linear discriminant functions was 87.1%, with 84.2% sensitivity and 88.3% specificity.

Conclusions. Routine tests in diabetes care were used to establish a convenient, low-cost tool that may assist in the early identification of adult-onset GAD+ autoimmune diabetes in clinical practice.

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2 antibody-positive autoimmune diabetes from type 2 diabetes : a retrospective study

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Abstract

21 **Abstract**

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24 study was to develop a convenient tool to discriminate adult-onset GADA-positive autoimmune

25 diabetes from type 2 diabetes (T2DM) in patients with newly diagnosed diabetes.

26 **Methods.** This retrospective cross-sectional study, conducted at Changhua Christian Hospital in

27 Taiwan, collected electronic medical record data from January 2009 to January 2018. Patients

28 were divided into a case group (GADA+, n = 152) and a reference group (T2DM, n = 358).

29 Variables that differed significantly between the groups were subjected to receiver operator

30 characteristic analysis to establish cutoff values. Discriminant function analysis was then

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32 **Results.** At the onset of diabetes, the GADA+ group was younger, with lower body mass index

33 (BMI), higher hemoglobin A1c (HbA1c), higher high-density lipoprotein cholesterol (HDL-C),

34 and lower total cholesterol and triglycerides (TG). Five major factors were identified to form the

35 linear discriminant functions: BMI, age at onset, TG, HDL-C, and HbA1c. BMI <23 kg/m^2 was

36 the most important factor, followed by TG <98 mg/dL, HDL-C ≥46 mg/dL, age at onset <30

37 years, and HbA1c ≥ 8.6%. The overall accuracy of the linear discriminant functions was 87.1%,

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39 **Conclusions.** Routine tests in diabetes care were used to establish a convenient, low-cost tool

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41 practice.

42

43 Introduction

44   In children and adolescents, type 1 diabetes (T1DM), also known as autoimmune diabetes,

45 usually presents with diabetic ketoacidosis (DKA) as the initial presentation and is relatively

46 straightforward to diagnose. However, the clinical features of adult-onset autoimmune diabetes

47   can be less typical. The period of insulin requirement after onset may allow differentiation

48   between patients with latent autoimmune diabetes in adults (LADA) and those with classic adult-

49   onset T1DM, who become insulin-dependent within 3 months of diagnosis [1]. LADA often

50   involves slow, progressive islet destruction. In the absence of autoantibody testing, it is not

51   uncommon for it to be diagnosed and treated as type 2 diabetes (T2DM) [1,2]. The lack of

52   optimal treatment can result in deterioration of the autoimmune process, the acceleration of beta

53   cell loss, faster progression to insulin dependence, and an increased risk of complications [2,3].

54   Testing of islet autoantibodies is essential to diagnose autoimmune diabetes. Islet cell

55   cytoplasmic autoantibodies (ICA), insulin autoantibodies (IAA), glutamic acid decarboxylase

56   antibodies (GADA), tyrosine phosphatase IA-2 autoantibodies (IA-2A), and zinc transporter 8

57   autoantibodies (ZnT8A) are the major autoantibodies of clinical and research interest [1,4]. The

58   reported incidence of T1DM-related autoantibodies in adult-onset diabetes is approximately 3%–

59   12%, with variations between countries and ethnicities [1-3]. GADA are persistent in patients

60   with long-standing diabetes and is not influenced by the age at disease onset. Therefore, the

61   presence of GADA represents the most sensitive marker in adult-onset autoimmune diabetes [1].

62   However, the GADA test has not been widely used in primary care because of its cost and

63   limited availability. In areas with a low prevalence of autoimmune diabetes, GADA

64   measurement is not cost-effective as a routine test and can only be used in highly selective cases.

65   It would therefore be desirable to establish a practical tool that could be used to determine

66   whether to test GADA for suspected patients.

67   Fourlanos et al. proposed a LADA clinical risk score as a clinical screening tool to identify

68   patients with LADA [5]. The parameters included age at onset <50 years, acute symptoms, body

69   mass index (BMI) <25 kg/m^2, and a personal or family history of autoimmune disease.

70   According to a study by Shields, the two most important indicators for identifying T1DM and

71   T2DM are age at diagnosis <35 years and time to insulin <6 months [6]. These studies showed

72   that age at diagnosis and BMI were important discriminators. Conversely, Thunander et al.

73   demonstrated that C-peptide level at diagnosis was better than age or BMI in discriminating

74   between autoimmune and non-autoimmune diabetes [7]. However, C-peptide measurement is

75   also limited by its cost and availability.

76   The metabolic and clinical phenotypes of adult-onset autoimmune diabetes are

77   heterogeneous, ranging from patients who are lean and insulin sensitive to those who are obese

78   and insulin resistance [1]. There is evidence that the various disease processes observed in

79   subtypes of autoimmune diabetes can be detected by different autoantibodies. Diabetic patients

80   positive for the IA-2$_{(256-760)}$ autoantibody showed slow beta cell deterioration and clinical and

81   metabolic phenotype, similar to those with classic type 2 diabetes and obesity [1,3]. Conversely,

82   the A Diabetes Outcome Progression Trial (ADOPT) reported that GADA+ diabetic patients had

83   higher levels of serum high-density lipoprotein cholesterol (HDL-C) and lower levels of serum

84   triglycerides (TG) than those diagnosed T2DM, as well as a lower prevalence of metabolic

85   syndrome [8]. A Korean population study found that GADA levels were inversely associated

86   with age at onset, C-peptide levels, BMI, and total cholesterol and TG concentrations [9]. These

87   studies demonstrated that the lipid profile can help to distinguish autoimmune diabetes with

88   GADA + from T2DM.

89   Several related studies have used multiple islet autoantibodies to detect autoimmune

90   diabetes rather than focusing on GADA [1-3]. Subtype studies pointing to GADA+ diabetes are

91   therefore valuable in providing additive information for managing adult-onset autoimmune

92   diabetes. In addition, there is still some debate on the definition of LADA as well as on the

93   classification of type 1 diabetes mellitus [2]. Using GADA+ diabetes in the present study would

94   help in defining the research subjects more distinctly and precisely.

95   The aim of the present study was to develop a convenient, cost-effective tool that can be used

96   to discriminate adult-onset GADA+ autoimmune diabetes from T2DM. Such a tool could help

97   physicians decide whether to perform a GADA test. To avoid additional costs, we based the tool

98   on parameters obtained from diabetes routine tests, such as lipid profile and hemoglobin A1c

99   (HbA1c).

100

101   **Materials & Methods**

102   **Subjects**

103   This retrospective cross-sectional study was conducted at Changhua Christian Hospital

104   (CCH), a medical center in Taiwan. A total of 636 patients with diabetes who received the

105   GADA test between January 1, 2009 and December 31, 2018 were screened for eligibility based

106   on data from the hospital's electronic medical record system. Among these, 484 patients were

107   excluded for the following reasons: with GADA < 1.0 U/mL, incomplete lipid and A1c data,

108   diabetes not diagnosed within the previous six months, age at onset < 20 years, and excessive

109   alcohol consumption. Finally, 152 patients were enrolled into the case group (GADA+ group).

110   Excessive alcohol consumption was defined as consuming 7 drinks, amounting to approximately

111   70 grams of alcohol, or more per week for both men and women.

112   The reference group (T2DM group) included 358 type 2 diabetic patients, newly diagnosed

113   between January 1, 2010 and December 31, 2013, with age at onset ≥ 20 years. GADA was not a

114   routine test in the reference group. However, GADA testing was selectively performed for

115   patients suspected of having autoimmune diabetes in follow-up visits. Two diabetologists

116   reviewed the medical records from January 1, 2010 to December 31, 2018, for each patient of the

117   reference group to confirm the diagnosis and to reduce the possibility of misclassification to the

118   maximum extent. Thus, 15 were excluded from the 406 patients identified for assessment (Figure

119   1).

120

121   **Data collection**

122   The data for both groups were obtained from the hospital's electronic medical record system.

123   Basic data (age at the onset of diabetes, and gender), personal habits (smoking and drinking),

124   height, and body weight were those collected at the initial visit for the diagnosis of diabetes. The

125   laboratory data used in the analysis, including HbA1c, creatinine (Cr), and glutamic pyruvic

126   transaminase (GPT) levels, were those measured closest to the first visit. The lipid profile

127   selected, including total cholesterol, HDL-C and TG levels, was that measured 2–12 weeks after

128   the first visit, documenting the use of any lipid-lowering agents (fibrates or statins) before the

129   lipid profile test.

130

131   **Measurements**

132   The diagnosis of diabetes mellitus was made according to the American Diabetes Association

133   criteria of 2019 [10]. Two diabetologists with more than five years' clinical experience reviewed

134   the medical records to validate the diagnosis. In cases of disagreement, the patient was excluded.

135   The total amount of alcohol consumption in grams per week was calculated from weekly alcohol

136   consumption (in millimeter) and the alcoholic concentration of each kind of beverage.

137   GADA was measured with an anti-GAD radioimmunoassay kit (RSR Ltd, Cardiff, UK).

138   According to the manufacturer, a serum concentration of GADA $\geq$1 u/ml was considered to be

139   positive for GADA. The specificity of the GADA test for diagnosing adult autoimmune diabetes

140   can be as high as 98%, with a sensitivity of about 70%–80% [11].

141   Serum biochemistry, including glucose, Cr, and GPT levels and the lipid profile, was

142   performed using a UniCel DxC 800 SynchronTM Clinical System (Beckman Coulter, Brea, CA,

143   USA). Serum HbA1c was measured by ion-exchange high-performance liquid chromatography

144   using a VARIANTTM II Turbo system.

145   BMI was calculated as the body weight in kilograms divided by height in meters squared. A

146   BMI of 23.0–24.9 kg/m² was classified as overweight and ≥25 kg/m² as indicating obesity,

147   according to the Regional Office for the Western Pacific standard [12].

148

149   **Statistical analyzes**

150   To establish a convenient diagnostic tool for discriminating the case group (GADA+) from

151   the reference group (T2DM), the major discriminating factors were first identified and then used

152   to form discriminant functions by discriminant function analysis.

153   Step 1: Differences between the two groups for selected variables were evaluated by

154   Student's t-test for continuous variables and chi-square test for categorical variables. The

155   continuous variables with statistical significance were tested for normal distribution using the

156   Kolmogorov–Smirnov test, and data with P-values of <0.05 were regarded as non-normally

157   distributed. The non-normally distributed variables were transformed to ordinary variables for

158   further analysis. A cutoff value for each of these variables was determined from the point on the

159   receiver operating characteristic (ROC) curve with the minimum distance to the upper left

160   corner, calculated as the square root of $[(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2]$.

161   Step 2: Discriminant function analysis using the enter method was then applied to establish

162   the major discriminating variables according to the discriminant loadings (with absolute values >

163   0.3) in the structure matrix. These major discriminating variables were then analyzed, again with

164   the enter method, to determine the standardized canonical discriminant function.

165   All the tests were two-tailed with a significance level of 0.05. SPSS version 25 software

166   (IBM Corp., Armonk, NY, USA) was used for the analysis.

167

168   **Ethics statement**

169   The study was approved by the Institutional Review Board of Changhua Christian Hospital

170   (CCH IRB No: 190702). Informed consent was waived.

171

172   ## Results

173   This study included a total of 510 Chinese patients who were ethnically homogeneous. In the

174   GADA+ group, 84 patients (55.3%) had higher GADA titers (≥32 U/mL) than the others. Table

175   1 summarizes the clinical features of the patients at the time of diagnosis of diabetes. The

176   GADA+ group was significantly younger, with lower BMI, higher HbA1c and HDL-C levels,

177   and lower total cholesterol, TG and GPT levels at onset of DM. There were no differences

178   between the two groups in the gender ratio, Cr levels, prevalence of smoking, or the use of lipid-

179   lowering agents (fibrates or statins).

180   ROC curves were used to determine the cutoff values of these variables for discriminating

181   the patients in the GADA+ group, as follows: HbA1c ≥8.6%, HDL-C ≥46 mg/dL, total

182   cholesterol <183 mg/dL, TG <98 mg/dL, and GPT ≥22 U/L (Table 2). ROC curve figures are

183   shown in supplement file 1 (Figure S1).

184   The discriminant function analysis identified five major discriminating variables from the

185   structure matrix: BMI, age at onset, TG, HDL-C, and HbA1c. Conversely, use of statins, use of

186   fibrates, GPT and total cholesterol were excluded because their discriminant loadings were <0.3.

187   The remaining five major variables (BMI, age at onset, TG, HDL-C, and HbA1c) were analyzed,

188   again by using the enter method, to form linear discriminant functions (Table 3). In order of the

189   absolute magnitude of the correlation within the function, BMI <23 kg/m^2 was the most

190   important factor, followed by TG <98 mg/dL, HDL-C ≥46 mg/dL, age at onset <30 years, and

191   HbA1c ≥8.6%. The overall accuracy of the linear discriminant functions in discriminating

192   GADA+ diabetes was 87.1% (Table 4). The sensitivity was 84.2% and the specificity reached

193   88.3%.

194

195   **Discussion**

196   This study demonstrated that measuring serum TG and HDL-C levels at the onset of disease

197   can be used to help discriminate GADA+ autoimmune diabetes from T2DM in adults. The

198   findings were consistent with those of previous studies that reported lower TG and higher HDL-

199   C levels in patients with autoimmune diabetes [8,9]. To the best of our knowledge, the present

200   study is the first to focus on TG and HDL-C combined with BMI, age at onset, and HbA1c to

201   develop a practical discrimination tool to discriminate adult-onset GADA+ autoimmune diabetes

202   from T2DM in ethnic Chinese patients. The LADA clinical risk score proposed by Fourlanos et

203   al. showed a sensitivity of 90% and specificity of 71% for detecting LADA diagnosed by GADA

204   [5]. A Swedish study used fasting C-peptide level <0.7 nmol/L to identify autoimmune diabetes;

205   this showed a sensitivity of 89% and specificity of 66% [7]. In comparison with these previous

206   studies, the tool proposed in the present study focused on discriminating GADA+ rather than

207   LADA from T2DM. In other words, the target population of GADA+ patients in our study

208 included patients with LADA and classic T1DM. The latter are usually leaner and younger at the

209 onset of diabetes.

210   Similar to those reported previously, age at diabetes onset and BMI were still major

211 discriminators of autoimmune diabetes. In contrast to the LADA clinical risk score of Fourlanos

212 et al., which uses BMI <25 kg/m^2 as a cutoff value and does not include patients younger than 30

213 years [5], the present study classified both age and BMI into three levels to make the scoring

214 more precise and showed that BMI <23 kg/m^2 and age at onset <30 would be better

215 discriminating features of GADA+ autoimmune diabetes in Chinese patients than BMI 23–25

216 kg/m^2 and age 30–50 years. The possible cause of the lower cutoff of BMI in our study may be

217 due to the different characteristics of study subjects. First, our study subjects included patients

218 with LADA and classic T1DM. Many studies have reported that LADA patients could often be

219 overweight or obese and that being overweight or obese is associated with an increased risk of

220 LADA [13,14]. Second, we enrolled subjects with age at onset ≥ 20 years. This was different

221 from the studies that enrolled patients with LADA; the latter are often defined as age at onset >

222 30 years [1]. Third, the BMI values in our study were obtained from the initial weight data of

223 newly diagnosed diabetic patients who just received short-term treatment and did not regain their

224 weight fully. Finally, the risk of diabetes starts to increase at a BMI of approximately 23 kg/m^2

225 in Chinese, which is lower than the WHO BMI cutoff value used to define an increase in the

226 incidence among Europeans [12,15]. Nevertheless, such a low cutoff may harbor the risk of not

227 being able to identify some patients with LADA, particularly in non-Asians and in areas of high

228 prevalence.

229   Autoimmune diabetes differs from T2DM in several respects. Its pathogenesis is primarily

230 based on insulin deficiency rather than insulin resistance. The present study showed that patients

231   with GADA+ autoimmune diabetes exhibited higher serum HDL-C and lower TG levels, which

232   can be used to discriminate the condition from T2DM. Although hypertriglyceridemia may occur

233   in undertreated individuals with autoimmune diabetes due to hyperglycemia, intensive glucose

234   control normalizes the lipid abnormalities [16-17]. To reduce any interference from

235   hyperglycemia, the lipid data in the current study were collected about 2–12 weeks after

236   commencing treatment for acute hyperglycemia.

237   Patients with autoimmune diabetes have worse glycemic control than those with T2DM,

238   possibly due to the limited production of endogenous insulin [2]. The present study showed that

239   patients with GADA+ autoimmune diabetes had higher baseline HbA1c values at disease onset

240   than those with T2DM. Paradoxically, the GADA+ patients had healthier lipid levels despite the

241   higher baseline HbA1c values. Hence, baseline HbA1c was another significant discriminator in

242   our linear discriminant functions, in combination with age, BMI, TG, and HDL-C.

243   This study had several strengths. All the patients were newly diagnosed diabetes, thus their

244   blood lipid and HbA1c levels were less affected by lipid-lowering and anti-diabetic agents. We

245   also excluded patients who had a habit of drinking alcohol. Using the parameters identified in

246   this study within 3 months of the diagnosis of diabetes may help physicians to decide earlier

247   whether to seek a GADA test for a patient.

248   The study also had several limitations. First, GADA was not measured for the reference

249   group, so it cannot be ruled out that some of the patients in this group had LADA; however, the

250   possibility of misclassification was reduced by the confirmation from two diabetologists that all of the

251   patients in this group had T2DM. Second, this hospital-based study would inevitably exhibit

252   selection bias. The patients who did receive GADA testing were not representatives for the

253   overall diabetes population. Third, we did not exclude smokers from study participation.

254   Although there was no significant difference in the distributions of smokers between the two

255   groups, smoking might reduce HDL levels and hence increase the probability of false negative

256   results [18]. Fourth, the incidence and clinical features of autoimmune diabetes may vary

257   between regions and ethnicities [1,3]. In this study, all the research subjects were Chinese; it

258   remains to be verified whether the findings can be applied to other ethnic groups. Fifth, although

259   87.1% of original grouped cases could be correctly classified by the linear discriminant functions

260   developed in this study, these functions were obtained from limited samples; they should be

261   tested in an independent population to verify the validity of the proposed method. Finally,

262   although patients with LADA than those with T2DM showed a higher prevalence of family

263   history or personal history of related autoimmune diseases [5,19], we did not have complete data

264   to include them into our model. From another perspective, the tool requires less information than

265   that needed for calculating Fourlanos et al.'s LADA clinical risk score, as well as a lesser

266   requirement for judgment about acute symptoms at onset and a history of related autoimmune

267   diseases; therefore, our tool may be more efficient in clinical practice.

268   However, to date, due to the broad heterogeneity of LADA, islet-cell antibodies

269   measurement remains essential to decrease the number of misdiagnosis of diabetes. Moreover, to

270   detect GADA+ diabetic patients is not the final goal. The clinical problem concerned should be

271   whether insulin-dependency can be detected early by all means.

272

273   Conclusions

274   An early diagnosis of adult-onset autoimmune diabetes would avoid patients receiving

275   unnecessary oral medication, as well as helping to predict the disease course and reducing acute

276   events such as DKA. The present study established a convenient diagnostic tool based on data

277 obtained from existing routine tests for diabetes care, including BMI, TG, HDL-C, age at onset,

278 and HbA1c, which may assist with the early identification of adult-onset GADA+ autoimmune

279 diabetes, avoiding extra medical costs and reducing the burden to patients.

280

281 **References**

282 [1] Buzzetti R, Zampetti S, Maddaloni E. Adult-onset autoimmune diabetes: current knowledge

283 and implications for management. Nat Rev Endocrinol 2017;13:674–86.

284 doi:10.1038/nrendo.2017.99

285 [2] Carlsson S. Etiology and pathogenesis of latent autoimmune diabetes in adults (LADA)

286 compared to type 2 diabetes. Front Physiol 2019 doi: 10.3389/fphys.2019.00320

287 [3] Pieralice S, Pozzilli P. Latent autoimmune diabetes in adults: A review on clinical

288 implications and management. Diabetes Metab J 2018;42:451-64.

289 [4] Winter WE, Schatz DA. Autoimmune markers in diabetes. Clinical Chemistry 2011;57:168-

290 75.

291 [5] Fourlanos S, Perry C, Stein MS, Stankovich J, Harrison LC, Colman PG. A clinical screening

292 tool identifies autoimmune diabetes in adults. Diabetes Care 2006;29:970-5.

293 [6] Shields BM, Peters JL, Cooper C, Lowe J, Knight BA, Powell RJ, Jones A, Hyde CJ,

294 Hattersley AT. Can clinical features be used to differentiate type 1 from type 2 diabetes? A

295 systematic review of the literature. BMJ Open 2015;5:e009088. doi:10.1136/bmjopen-2015-

296 009088

297 [7] Thunander M, Torn C, Petersson C, Ossiansson B, Fornander J, Landin-Olsson M. Levels of

298 C-peptide, body mass index and age, and their usefulness in classification of diabetes in relation

299 to autoimmunity, in adults with newly diagnosed diabetes in Kronoberg, Sweden. Eur J

300 Endocrinol 2012;166:1021-9. doi: org/10.1530/EJE-11-0797

301 [8] ADOPT Study Group. Phenotypic characteristics of GAD (+) recently diagnosed patients

302 with type 2 diabetes in North America and Europe. Diabetes 2004;53:3193-200. doi:

303 org/10.2337/diabetes.53.12.3193

304 [9] Roh MO, Jung CH, Kim BY, Mok JO, Kim CH. The prevalence and characteristics of latent

305 autoimmune diabetes in adults (LADA) and its relation with chronic complications in a clinical

306 department of a university hospital in Korea. Acta Diabetol 2013;50:129-34. doi:

307 10.1007/s00592-010-0228-y

308 [10] American Diabetes Association. Classification and diagnosis of diabetes: standards of

309 medical care in diabetes-2019. Diabetes Care 2019;42 Suppl 1:S13-28. doi: org/10.2337/dc19-

310 S002

311 [11] Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, Rewers M, Eisenbarth GS,

312 Jensen J, Davidson HW, Hutton JC. The cation efflux transporter ZnT8 (Slc30A8) is a major

313 autoantigen in human type 1 diabetes. Proc Natl Acad Sci USA 2007;104:17040–5.

314 [12] World Health Organization, Regional Office for the Western Pacific (WPRO),

315 International Association for the Study of Obesity, International Obesity Task Force. The Asia-

316 Pacific Perspective: redefining obesity and its treatment. Sydney, Health Communications

317 Australia Pty Ltd, 2000. https://apps.who.int/iris/handle/10665/206936

318 [13] Hjort R, Ahlqvist E, Carlsson PO, Grill V, Groop L, Martinell M, Rasouli B, Rosengren A,

319 Tuomi T, Åsvold BO, Carlsson1 S. Overweight, obesity and the risk of LADA: results from a

320 Swedish case-control study and the Norwegian HUNT Study. Diabetologia 2018;61(6):1333–43.

321 doi: 10.1007/s00125-018-4596-0

322 [14] Luk AOY, Lau ESH, Lim C, Kong APS, Chow E, Ma RCW, Chan JCN. Diabetes-related

323 complications and mortality in patients with young-onset latent autoimmune diabetes: a 14-year

324 analysis of the prospective Hong Kong Diabetes Register. Diabetes care 2019;42:1042-50.

325 [15] Ko G, Chan J, Cockram C, Woo J. Prediction of hypertension, diabetes, dyslipidaemia or

326 albuminuria using simple anthropometric indexes in Hong Kong Chinese. Int J Obes

327 1999;23:1136–42. doi: 10.1038/sj.ijo.0801043

328 [16] Subramanian S, Chait A. Hypertriglyceridemia secondary to obesity and diabetes. Biochim

329 Biophys Acta 2012;1821(5):819-25.

330 [17] Guy J, Ogden L, Wadwa RP, Hamman RF, Mayer-Davis EJ, Liese AD, D'Agostino R,

331 Marcovina S, Dabelea D. Lipid and lipoprotein profiles in youth with and without type 1

332 diabetes: the SEARCH for Diabetes in Youth case-control study. Diabetes Care 2009;32(3):416-

333 20.

334 [18] Jain RB, Ducatman A. Associations between smoking and lipid/lipoprotein concentrations

335 among US adults aged ≥20 years. J Circ Biomark 2018;7:1849454418779310.

336 [19] Zampetti S, Capizzi M, Spoletini M, Campagna G, Leto G, Cipolloni L, Tiberti C, Bosi E,

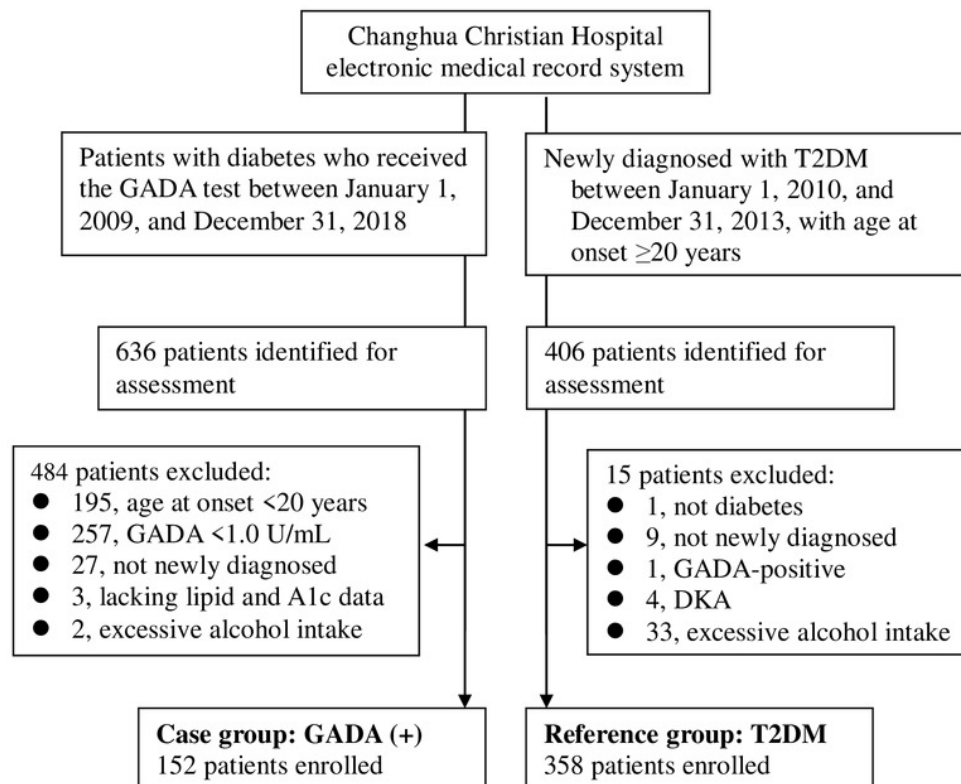
337 Falorni A, Buzzetti R. GADA titer-related risk for organ-specific autoimmunity in LADA

338 subjects subdivided according to gender (NIRAD study 6). J Clin Endocrinol Metab

339 2012;97:3759–65. doi: 10.1210/jc.2012-2037

Figure 1

Enrollment of participants in the study

Abbreviations: GADA: glutamic acid decarboxylase antibody; T2DM: type 2 diabetes mellitus; DKA, diabetic ketoacidosis.

Clinical features of the patients at the diagnosis of diabetes mellitus

[a] Chi-square test. [b] Fisher's exact test. Other variables were examined by t-test.

Abbreviations: GADA+: Glutamic acid decarboxylase antibody-positive group; T2DM: type 2 diabetes mellitus group; BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; HbA1c: hemoglobin A1c; Cr: creatinine; GPT: glutamic pyruvic transaminase.

1   Table 1. Clinical features of the patients at the diagnosis of diabetes mellitus

	GADA+ (n = 152)	T2DM (n = 358)	
	Mean ± SD	Mean ± SD	p
Gender: Men, n (%)	74 (48.7)	202 (56.4)	0.109[a]
Age at onset (years)	37.6 ± 12.6	50.4 ± 11.7	<0.001
20–29, n (%)	48 (31.6)	18 (5.0)	
30–49, n (%)	75 (49.3)	133 (37.2)	
≥50, n (%)	29 (19.1)	207 (57.8)	
BMI (kg/m^2)	21.7 ± 3.8	26.8 ± 4.5	<0.001
<23, n (%)	114 (75.0)	62 (17.3)	
23–24.9, n (%)	16 (10.5)	70 (19.6)	
≥25, n (%)	22 (14.5)	226 (63.1)	
Lipid profile			
Total cholesterol (mg/dL)	184.0 ± 46.9	194.4 ± 41.9	0.014
TG (mg/dL)	82.9 ± 65.2	162.2 ± 119.9	<0.001
HDL-C (mg/dL)	58.0 ± 18.7	42.7 ± 10.5	<0.001
HbA1c (%)	10.5 ± 3.2	8.2 ± 2.3	<0.001
GPT (U/L)	25.1 ± 18.7	35.2 ± 29.2	<0.001
Creatinine (mg/dL)	0.8 ± 0.2	0.8 ± 0.2	0.734
Smoking, n (%)	33 (21.7)	64 (17.9)	0.313[a]
Use of statins, n (%)	5 (3.3)	6 (1.7)	0.317[b]

| Use of fibrates, n (%) | 1 (0.7) | 5 (1.4) | 0.675[b] |

2   [a] Chi-square test. [b] Fisher's exact test. Other variables were examined by t-test.

3   Abbreviations: GADA+: Glutamic acid decarboxylase antibody-positive group; T2DM: type 2 diabetes

4   mellitus group; BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides;

5   HbA1c: hemoglobin A1c; Cr: creatinine; GPT: glutamic pyruvic transaminase.

Receiver operating characteristic analysis of variables for differentiating the GADA+ from the T2DM patients

[a] The minimum distance between a point on the receiver operating characteristic curve and the upper left corner, calculated as the square root of [(1–sensitivity)² + (1–specificity)²]. The point with the minimum distance was used to define the cutoff value.

Abbreviations: GADA: Glutamic acid decarboxylase antibody; T2DM: type 2 diabetes mellitus; AUC: area under the receiver operating characteristic curve; CI: confidence interval; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; HbA1c: hemoglobin A1c; GPT: glutamic pyruvic transaminase.

1 Table 2. Receiver operating characteristic analysis of variables for differentiating the GADA+

2 from the T2DM patients

Variables	Cut-off value	Sensitivity	Specificity	Minimum distance [a]	AUC	95% CI
HbA1c (%)	8.6	67.1%	65.6%	0.226	0.72	0.67-0.77
Total cholesterol (mg/dL)	183	57.2%	58.9%	0.351	0.59	0.53-0.65
HDL-C (mg/dL)	46	74.3%	70.1%	0.155	0.78	0.73-0.83
TG (mg/dL)	98	77.6%	70.7%	0.136	0.80	0.76-0.85
GPT (U/L)	22	55.3%	65.6%	0.318	0.66	0.60-0.71

3 [a] The minimum distance between a point on the receiver operating characteristic curve and the upper left

4 corner, calculated as the square root of $[(1-\text{sensitivity})^2 + (1-\text{specificity})^2]$. The point with the minimum

5 distance was used to define the cutoff value.

6 Abbreviations: GADA: Glutamic acid decarboxylase antibody; T2DM: type 2 diabetes mellitus; AUC:

7 area under the receiver operating characteristic curve; CI: confidence interval; HDL-C: high-density

8 lipoprotein cholesterol; TG: triglycerides; HbA1c: hemoglobin A1c; GPT: glutamic pyruvic transaminase.

Linear discriminant functions constructed from five major variables for discriminating GADA+ from T2DM patients

[a] Standardized canonical discriminant function: Wilks' lambda = 0.475, p < 0.001; eigenvalue 1.106; canonical correlation = 0.725. Abbreviations: GADA+: Glutamic acid decarboxylase antibody-positive group; T2DM: type 2 diabetes mellitus group; BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; HbA1c: hemoglobin A1c.

1 Table 3. Linear discriminant functions constructed from five major variables for discriminating

2 GADA+ from T2DM patients

Variables	Standardized canonical discriminant function coefficient[a]	Discriminant loading (Structure matrix)	Classification function coefficients (Fisher's linear discriminant functions)	
			GADA+	T2DM
Age at onset <30 years	0.46	0.37	5.556	2.208
Age at onset: 30–50 years	0.36	0.11	4.274	2.576
BMI <23 kg/m^2	0.63	0.63	6.030	2.371
BMI 23–25 kg/m^2	0.22	-0.11	3.756	2.421
TG ≥98 mg/dL	-0.34	-0.47	2.525	4.298
HbA1c ≥8.6%	0.32	0.30	2.442	0.911
HDL-C ≥46 mg/dL	0.36	0.43	4.658	2.808
(Constant)			−7.917	−3.764

3 [a] Standardized canonical discriminant function: Wilks' lambda = 0.475, $p < 0.001$; eigenvalue 1.106;

4 canonical correlation = 0.725.

5 Abbreviations: GADA+: Glutamic acid decarboxylase antibody-positive group; T2DM: type 2 diabetes

6 mellitus group; BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides;

7 HbA1c: hemoglobin A1c.

Accuracy of the linear discriminant functions for detecting GADA+ patients

a. 87.1% of the original grouped cases were correctly classified Abbreviations: GADA+: Glutamic acid decarboxylase antibody-positive group; T2DM: type 2 diabetes mellitus group.

1 Table 4. Accuracy of the linear discriminant functions for detecting GADA+ patients

	Predicted group membership		
	GADA+	T2DM	Total
GADA+	128 (84.2%)	24 (15.8%)	152 (100%)
T2DM	42 (11.7%)	316 (88.3%)	358 (100%)

3 a. 87.1% of the original grouped cases were correctly classified

4 Abbreviations: GADA+: Glutamic acid decarboxylase antibody-positive group; T2DM: type 2 diabetes

5 mellitus group.