Islet Cell and Glutamic Acid Decarboxylase Antibodies Present at Diagnosis of Diabetes Predict the Need for Insulin Treatment

A cohort study in young adults whose disease was initially labeled as type 2 or unclassifiable diabetes

BENGT LITTORIN, MD
GÖRAN SUNDKVIST, MD, PHD
WILLIAM HAGOPIAN, MD, PHD
MONA LANDIN-OLSSON, MD, PHD
ÅKE LERNMARK, PHD
JAN ÖSTMAN, MD, PHD
HANS J. ARNQVIST, MD, PHD
GÖRAN BLOHMÉ, MD, PHD
JAN BOLINDER, MD, PHD
JAN W. ERIKSSON, MD, PHD
FOLKE LITHNER, MD, PHD
BENGT SCHERSTÉN, MD, PHD
LARS WIBELL, MD, PHD

OBJECTIVE — To clarify the predictive value of islet cell antibody (ICA) and GAD65 antibody (GADA) present at diagnosis with respect to the need for insulin treatment 6 years after diagnosis in young adults initially considered to have type 2 or unclassifiable diabetes.

RESEARCH DESIGN AND METHODS — The patient material was representative of the entire Swedish population, consisting of patients who were 15–34 years old at diagnosis of diabetes in 1987–1988 but were not considered to have type 1 diabetes at onset. At follow-up, 6 years after the diagnosis, it was noted whether the patient was treated with insulin. The presence of ICA was determined by an immunofluorescence assay, and GADAs were measured by a radioligand assay.

RESULTS — Six years after diagnosis, 70 of 97 patients were treated with insulin, and 27 of 97 patients were treated with oral drugs or diet alone. At diagnosis, ICAs and GADAs were present in 41 (59%) of 70 patients and 41 (60%) of 68 patients, respectively, of those now treated with insulin, compared with only 1 (4%) of 26 patients and 2 (7%) of 27 patients who were still not treated with insulin. For either ICA or GADA, the corresponding frequencies were 50 (74%) of 68 for patients who were later treated with insulin and 3 (12%) of 26 for those who were still not treated with insulin, respectively. The sensitivity for later insulin treatment was highest (74%) for the presence of ICA or GADA, and the specificity was highest (100%) for ICA and GADA. The positive predictive value was 100% for the combination of ICA and GADA, 98% for ICA alone, and ~95% for GADA alone.

CONCLUSIONS — Determination of the presence of ICA and GADA at diagnosis of diabetes improves the classification of diabetes and predicts the future need of insulin in young adults.

Diabetes Care 22:409–412, 1999

From the Department of Community Health Sciences (B.L., B.S.), University of Malmö/Lund, Malmö/Lund; Department of Endocrinology (G.S.), Malmö University Hospital, Malmö; Department of Internal Medicine (J.Ö., J.B.), Huddinge Hospital, Huddinge; Department of Internal Medicine (M.L.-O.), University Hospital of Lund, Lund; Department of Internal Medicine (H.J.A.), Faculty of Health Sciences, University of Linköping, Linköping; Department of Internal Medicine (G.B.), Sahlgrenska, University Hospital, University of Göteborg, Gothenburg; Department of Medicine (J.W.E., F.L.), University Hospital, Umeå; Department of Medicine (L.W.), University Hospital, Uppsala, Sweden; and R.H. Williams Laboratory (W.H., Å.L.), University of Washington, Seattle, Washington.

Address correspondence and reprint requests to Bengt Littorin, MD, Södervärn Primary Health Care Center, Ahlmansgatan 12, S-214 27 Malmö, Sweden.

Received for publication 9 July 1998 and accepted in revised form 5 November 1998.

Abbreviations: DISS, Diabetes Incidence Study in Sweden; GADA, GAD65 antibody; ICA, islet cell antibody; NPV, negative predictive value; PPV, positive predictive value; UKPDS, U.K. Prospective Diabetes Study.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

The clinical classification of diabetes (type 1 versus type 2) has been based on the clinical presentation at the onset of disease (1,2). Recently, however, it has been recognized that measurement of islet cell antibodies (ICAs) and GAD65 antibodies (GADAs) are useful in the classification of diabetes in adult patients. The presence of ICA and GADA in patients clinically considered to have type 2 diabetes at diagnosis is associated with β-cell failure and overt type 1 diabetes occurring within 3 years (3,4). In 1992, we reported a high frequency (23–50%) of ICA found in young adult subjects with a diagnosis of type 2 or unclassifiable diabetes (5). We have now followed these incident cases to 6 years after diagnosis of diabetes. The aim of this study was to clarify the predictive value of ICA and GADA with regard to future insulin therapy in these patients.

RESEARCH DESIGN AND METHODS

— Since 1 January 1983, a population-based nationwide prospective registration of all newly diagnosed cases of diabetes in patients aged 15–34 years in Sweden, the Diabetes Incidence Study in Sweden (DISS), has been ongoing (6,7). On 31 December 1987, the Swedish population comprised 8,414,083 individuals, 2,317,044 of whom were in the age-group 15–34 years. The Swedish racial mix is considered to be <10%. The prevalence of diabetes in Sweden among individuals 15–34 years old is considered to be 0.7% (8), which indicates a prevalence of type 1 diabetes in this age-group of 0.5%, based on the annual incidence rates of diabetes (6). The ascertainment of the DISS registry is 85% with regard to type 1 diabetes (9). All hospitals and primary health care centers in Sweden report all incident cases of diabetes (except gestational diabetes) in the age-group 15–34 years on a standardized

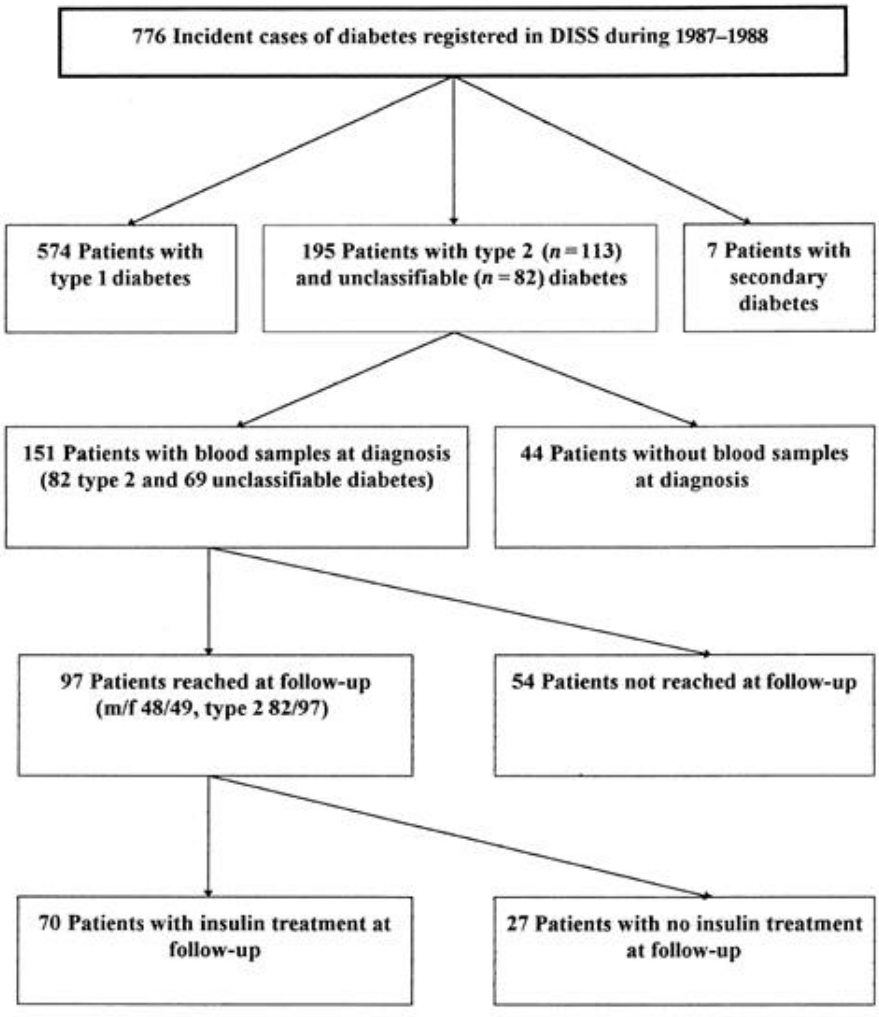

Figure 1—*Classification at diagnosis and treatment 6 years thereafter in patients considered at-diagnosis to have type 2 or unclassifiable diabetes.*

form that lists civic number, name and address, reporting department and physician, circumstances at diagnosis (screening or clinical symptoms), concomitant pregnancy, diagnostic criteria, maximum blood glucose during the first 2 weeks after diagnosis of diabetes, and height and body weight at diagnosis. Based on the clinical impression, the diabetes is classified by the reporting physician as type 1, type 2, secondary to other diseases, or unclassifiable, according to the World Health Organization's 1985 criteria (1).

Subjects

During 1987–1988, a case-referent study that included a blood sample at time of diagnosis was conducted (5). During the study period, a total of 776 cases were diagnosed; of these cases, 113 were diagnosed as type 2 diabetes and 82 as unclassifiable diabetes. A blood sample was available at diagnosis for a total of 151 (80%) of these 195 patients with type 2 and unclassifiable diabetes: 82 (40 female patients) with type 2 diabetes and 69 (34 female patients) with unclassifiable diabetes (Fig. 1).

At least 6 years after diagnosis, patients initially classified as type 2 diabetes or unclassified diabetes were contacted. At this follow-up, each patient received a letter in which he or she was asked to fill in a questionnaire regarding current treatment. If the questionnaire was not returned within 2 weeks, the patients were reminded by a postcard. If the patient still did not report within 1 month, those with a telephone number were called by a research nurse, who completed the questionnaire by interviewing the patient. The study was approved by the ethics committee at the Karolinska Institute as well as by the Swedish Data Inspection Board.

ICA assay

The presence of ICA was determined by a prolonged immunofluorescence assay as previously described in detail (10). The detection limit (cutoff value for abnormality) was 2 Juvenile Diabetes Foundation units for used pancreas. In the last (13th) International Diabetes Workshop proficiency test for ICA, our assay showed 100% sensitivity and 100% specificity.

GADA assay

GADAs were measured by a radioligand binding assay using in vitro translated [^{35}S]methionine-labeled human GAD65 (4). GADAs were expressed as an index related to a positive and negative internal standard (counts by antibody in the test serum minus counts by antibody in the negative standard divided by counts by antibody in positive standard minus counts by antibody in negative standard). The cutoff value for GADA (mean + 3 SD of 115 control sera) was set at 0.03; equal and higher values were considered positive. Intra-assay and interassay coefficients of variation were <16%. The assay was 100% sensitive and 100% specific in the international GADA workshop (11).

Statistical analysis

Differences in frequencies were evaluated by χ^2 test, and differences between groups were evaluated by nonparametric Mann-Whitney *U* test. Data are presented as means ± SD. $P < 0.05$ was considered significant. Sensitivity was defined as positive patients divided by all insulin-treated patients, specificity as negative patients divided by all non–insulin-treated patients, positive predictive value (PPV) as all positive insulin-treated patients divided by all positive patients, and negative predictive value (NPV) as all negative non–insulin treated patients divided by all negative patients.

RESULTS — At follow-up, at least 6 years after diagnosis (Fig. 1), information regarding current treatment was available from a total of 97 (64%) of those 151 patients with a blood sample available at diagnosis. Of these, 82 (40 female patients) were determined at diagnosis to have type 2 diabetes, and 15 (9 female patients) were determined at diagnosis to have unclassifiable diabetes. Among all patients, 42 of 96 (44%), 43 of 95 (45%), 53 of 94 (56%), and 31 of 94 (33%) were found at diagnosis to be ICA$^+$, GADA$^+$, ICA$^+$ or GADA$^+$, and both ICA$^+$ and GADA$^+$, respectively (Table

Table 1—*Clinical characteristics and antibody status at diagnosis versus treatment at follow-up in patients considered at diagnosis to have type 2 or unclassifiable diabetes*

Characteristics at diagnosis	All patients	Insulin treatment at follow-up	No insulin treatment at follow-up
n	97	70	27
M/F	50 (48/49)	50 (35/35)	48 (13/14)
Age (years)	28.5 ± 5	28.4 ± 5	28.6 ± 5
BMI	26.4 ± 4	25.8 ± 4	27.8 ± 5
ICA*	44 (42/96)	59 (41/70)	4 (1/26)
GADA*	45 (43/95)	60 (41/68)	7 (2/27)
ICA or GADA	56 (53/94)	74 (50/68)	12 (3/26)
ICA and GADA	33 (31/94)	46 (31/68)	0 (0/26)

Data are means ± SD or % (proportion). *ICA was not analyzed in one patient, and GADA was not analyzed in two other patients.

1). Mean level of GADA in 43 GADA$^+$ patients was 1.04 ± 0.83 index values. At follow-up, 70 of these 97 patients now obtained insulin treatment, 12 were treated with oral antidiabetic drugs, and 15 had only nonpharmacological treatment. Of the 27 patients without insulin treatment at follow-up, only one was ICA$^+$ and two were GADA$^+$. In agreement, insulin treatment 6 years after diagnosis was significantly more frequent in those with ICA and/or GADA at diagnosis compared with those without these autoantibodies: for ICA, 41 of 42 (98%) vs. 29 of 54 (54%) ($P < 0.0001$); for GADA, 41 of 43 (95%) vs. 27 of 52 (52%) ($P < 0.0001$); for ICA or GADA, 50 of 53 (94%) vs. 18 of 41 (43%) ($P < 0.0001$); and for ICA and GADA, 31 of 31 (100%) vs. 37 of 63 (59%) ($P < 0.0001$). There were no significant differences in age or BMI between the groups with regard to later insulin treatment.

The sensitivity for insulin treatment at follow-up was highest (74%) for the presence of ICA or GADA, and the specificity was highest (100%) for the combination of ICA and GADA (Table 2). The PPV for ICA alone was also high (98%), whereas it was slightly lower (95%) for GADA alone.

Compared with patients reached at follow-up, nonresponders (n = 54) did not significantly differ with regard to sex (the ratio of male to female patients was 29:25 vs. 48:49). The frequency of ICA and GADA at diagnosis, however, was lower in the nonresponders than in the responders (11 of 53 vs. 42 of 96, and 20 of 53 vs. 43 of 95, respectively) (P = 0.006). There were no significant differences between the initial cohort of 195 patients and the 97 patients at the end of the study with regard to age (27.5 ± 4.9 vs. 27.5 ± 5.4 years), BMI (27.5 ± 6.9 vs. 26.4 ± 5.1), or sex (ratio of male to female patients: 1.16 vs. 0.90). Similarly, among patients treated with insulin at follow-up, there were no significant differences regarding age or BMI between those with or without GADA or ICA.

CONCLUSIONS — In agreement with our previous study (12), this prospective study clearly demonstrates that in a classification of diabetes based on the clinical impression, age, and BMI, it is difficult to distinguish type 1 diabetes from other types of diabetes. The detection of ICA and/or GADA at the time of diagnosis of diabetes in people considered to have type 2 diabetes or unclassifiable diabetes predicts a future need for insulin treatment and therefore most likely identifies these patients as having type 1 diabetes. In the classification of diabetes, ICA and GADA assessment has clear advantages over clinical judgment alone, as can be inferred from the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (13).

In type 2 diabetes, GADA has been suggested to be a feature of latent autoimmune diabetes in adults (LADA) (14). In our previous prospective study, GADA also predicted the type of diabetes better than clinical judgment (4). In that study, however, the concordance between ICA and GADA was high (93%) and therefore it was difficult to separate the impact of GADA from that of ICA. Moreover, in a recent study of incident cases of diabetes in elderly diabetic Swedish patients, only the combination of ICA and GADA was associated with low C-peptide values and endogenous insulin production, as evidenced by the fact that C-peptide was not clearly disturbed in patients with only GADA (15). Hence, further studies are needed to clarify the association between β-cell failure and presence of ICA or GADA.

It is of interest that the U.K. Prospective Diabetes Study (UKPDS) (16) also evaluated the presence of ICA and/or GADA in relation to insulin treatment 6 years after diagnosis of diabetes, i.e., the same duration of follow-up used in our study. Compared with the patients <34 years of age in the UKPDS, the PPVs for ICA and/or GADA versus future insulin treatment were higher in our study (98 vs. 94% for ICA, 95 vs. 84% for GADA, and 100 vs. 94% for ICA and GADA, respectively). This could be due to differences in assay methodologies (we used recombinant GAD65, whereas porcine brain-extract GAD antigen was used in the UKPDS) and/or to population differences (Sweden has a population at high risk for type 1 diabetes, whereas the U.K. has a population at intermediate risk for type 1 diabetes).

In conclusion, the clinical classification of diabetes in young adults is unreliable. Determination of the presence of ICA and GADA, alone or in combination, improves the classification of diabetes. ICA or GADA present at diagnosis in patients considered to have type 2 diabetes or unclassifiable diabetes strongly predicts further insulin treatment or dependence.

Acknowledgments — This study was supported by grants from the Swedish Medical Research Council (7507), the Swedish Diabetes

Table 2—*Sensitivity specificity, PPV, and NPV for autoantibodies at diagnosis of diabetes with respect to insulin treatment at follow-up 6 years after diagnosis*

	ICA	GADA	ICA or GADA	ICA and GADA
Sensitivity	41/70 (59)	41/68 (60)	50/68 (74)	31/68 (46)
Specificity	25/26 (96)	25/27 (93)	23/26 (88)	26/26 (100)
PPV	41/42 (98)	41/43 (95)	50/53 (94)	31/31 (100)
NPV	25/54 (46)	25/52 (48)	24/42 (57)	26/63 (41)

Data are proportions (%).

Association, Novo Nordisk Foundation, the National Institutes of Health (DK-26190, DK-42654), and the Juvenile Diabetes Foundation-Wallenberg Diabetes Research Program (K98–99JD-128B).

We thank Lennarth Nyström, Department of Epidemiology and Public Health, Umeå University, Umeå, and Jan-Åke Nilsson, Department of Statistics and Information Processing, Malmö University Hospital, for expert statistical assistance; Ann Radelius for interviewing the patients; and Christina Rosborn for skillful technical assistance.

References

1. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
2. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039–1057, 1979
3. Gottsäter A, Landin-Olsson M, Fernlund P, Lernmark Å, Sundkvist G: β-Cell function in relation to islet cell antibodies during the first 3 yr after the clinical diagnosis of diabetes in type II diabetic patients. *Diabetes Care* 16:902–910, 1993
4. Hagopian WA, Sanjeevi CB, Kockum I, Landin-Olsson M, Karlsen AE, Sundkvist G, Dahlquist G, Palmer J, Lernmark Å: Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. *J Clin Invest* 95:1505–1511, 1995
5. Landin-Olsson M, Karlsson FA, Lernmark Å, Sundkvist G, and the Diabetes Incidence Study in Sweden Group: Islet cell and thyrogastric antibodies in 633 consecutive 15- to 34-yr-old patients in the Diabetes Incidence Study in Sweden. *Diabetes* 41:1022–1027, 1992
6. Östman J, Arnquist H, Blohmé G, Lithner F, Littorin B, Nyström L, Sandström A, Scherstén B, Wall S, Wibell L: Epidemiology of diabetes mellitus in Sweden: results of the first year of a prospective study in the population age group 15–34 years. *Acta Med Scand* 220:437–445, 1986
7. Blohmé G, Nyström L, Arnquist H, Lithner F, Littorin B, Olsson PO, Scherstén B, Wibell L, Östman J: Male predominance of type 1 (insulin-dependent) diabetes mellitus in young adults: results from a 5-year prospective nationwide study of the 15–34 year age group in Sweden. *Diabetologia* 35:56–62, 1992
8. Berger B, Stenström G, Yue-Fang C, Sundkvist G: The prevalence of diabetes in a Swedish population of 280,411 inhabitants: a report from the Skaraborg Diabetes Registry. *Diabetes Care* 21:546–548, 1998
9. Littorin B, Sundkvist G, Scherstén B, Nyström L, Arnqvist HJ, Blohmé G, Lithner F, Wibell L, Östman J: Patient administrative system as a tool to validate the ascertainment in the Diabetes Incidence Study in Sweden (DISS). *Diabetes Res Clin Pract* 33:129–133, 1996
10. Landin-Olsson M, Sundkvist G, Lernmark Å: Prolonged incubation in the two-colour immunofluorescence test increases the prevalence and titres of islet cell antibodies in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 30:327–332, 1987
11. Schmidli RS, Colman PG, Bonifacio E, Bottazzo GF, Harrison LC, and participating laboratories: High level of concordance between assays for glutamic acid decarboxylase antibodies: the first international glutamic acid decarboxylase antibody workshop. *Diabetes* 43:1005–1009, 1994
12. Arnqvist HJ, Littorin B, Scherstén B, Nyström L, Blohmé G, Lithner F, Wibell L, Östman J: Difficulties in classifying diabetes at presentation in young adults. *Diabet Med* 10:606–613, 1993
13. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
14. Zimmet PZ, Tuomi T, Mackay IR, Rowley MJ, Knowles W, Cohen M, Lang DA: Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. *Diabet Med* 11:299–303, 1994
15. Wroblewski M, Gottsäter A, Lingärde F, Fernlund P, Sundkvist G: Gender, autoantibodies, and obesity in newly diagnosed diabetic patients aged 40–75 years. *Diabetes Care* 21:250–255, 1998
16. Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R: UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. *Lancet* 350:1288–1293, 1997