Metabolic and Immune Parameters at Clinical Onset of Insulin-Dependent Diabetes: A Population-Based Study

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The age at diagnosis of insulin-dependent diabetes mellitus (type I DM) varies between childhood and adulthood. The aim of this study was to define the immunologic and metabolic characteristics of the disease according to the age at which it is diagnosed. We evaluated the residual β-cell function (basal and stimulated C-peptide) and frequency of two major islet cell–related autoantibodies, glutamic acid decarboxylase (GAD) and tyrosine phosphatase-like molecule (IA-2ic), at the onset of type I DM. A population-based study was performed with 235 consecutive cases of recent-onset (<4 weeks) type I DM (ages 5 to 45 years) diagnosed in the Lazio region of central Italy. Five age groups were considered: patients diagnosed between ages 5 and 7 years (n = 10), 7 and 10 years (n = 38), 10 and 17 years (n = 94), 17 and 20 years (n = 17), and 20 and 45 years (n = 76). Patients diagnosed before puberty had significantly reduced C-peptide secretion compared with patients diagnosed at a later age ($P < .02$). Glycosylated hemoglobin (HbA_{1c}) did not differ at diagnosis between the different age groups. Patients diagnosed at puberty or after required significantly less insulin compared with younger patients ($P < .04$). GAD antibodies were found in 65% and IA-2ic antibodies in 59% of patients. GAD antibodies tended to be more frequent in patients diagnosed after age 17 compared with younger patients ($P = .05$), while IA-2ic antibodies were not age-related. These data suggest that (1) the extent of β-cell damage differs between patients diagnosed before and after puberty, the process being more destructive in children less than 7 years of age, when C-peptide levels are the lowest; and (2) residual β-cell function at diagnosis is not influenced by the presence or absence of islet cell–related antibodies. These findings have implications for trials in type I DM diagnosis aimed at protecting β cells from end-stage destruction and in attempts to prevent the disease in susceptible individuals.

THE TIME OF DIAGNOSIS of type I insulin-dependent diabetes mellitus (type I DM) represents the end stage of an autoimmune process leading to near-total β-cell destruction.[1] The introduction of immunotherapy at diagnosis may have the effect of controlling the immune response and possibly reducing the extent of β-cell damage.[2,3] Although it is too late for the disease to be cured, immunotherapeutic trials at clinical onset including the use of intensified insulin therapy[4,5] are performed with the aim of blocking further destruction of the remaining 10% to 20% of β cells and maintaining residual β-cell function as long as possible. This may beneficially influence the long-term metabolic control of type I DM patients and can be regarded as a protective factor against the development of late diabetic complications.[6]

Better characterization of the disease process as it now presents may allow a more logical approach for early therapy. Over 20 years ago, Hamilton et al[7] reported that in 66 consecutive patients, ketoacidosis, and coma in 10%, was a frequent mode of presentation. More recently, Pinkney et al[8] evaluated the presentation and progress of type I DM in a population-based study and showed that despite improved education, the likelihood of presentation with ketoacidosis remained high. However, it is possible that type I DM is diagnosed today at an earlier stage in the natural history of β-cell destruction,[9-11] but clinical data to confirm or negate this hypothesis are lacking.

Based on this rationale, the aim of this study was to investigate consecutive cases of recent-onset type I DM in patients diagnosed over the past 5 years in one Italian region, in whom the clinical presentation, parameters of metabolic control, and two major islet-related autoantibodies were correlated.

SUBJECTS AND METHODS

Patients

Two hundred thirty-five consecutive type I DM patients were included in this study. At diagnosis, they had symptoms associated with a gross elevation of blood glucose and fasting blood glucose levels consistently greater than 7.8 mmol/L or postprandial blood glucose levels consistently greater than 11.1 mmol/L with unintentional concurrent weight loss. Exclusion factors included obesity and a strong family history of non–insulin-dependent diabetes. The diagnosis of type I DM was also confirmed, especially in older patients, by C-peptide values (<1 mmol/L fasting). Patients were first evaluated by physicians of the IMDIAB study group in Rome. This group was formed in 1988 in the Lazio region (which includes the capital of Italy, Rome) with the aim of intervening in recent-onset type I DM with adjuvant immunotherapy to protect β cells from end-stage destruction. Patients participating in the present study attended 10 outpatient diabetic clinics in the territory and were recruited as cases for several immunotherapy trials and the EURODIAB Registry.[12-17] The incidence of type I DM in the Lazio region is 7.8 per 100,000 under the age of 15 years.[13] A registry of all recent-onset type I DM patients has been available since 1989.

Characteristics of type I DM patients at presentation that were taken into account for this analysis included the presence or absence of ketonuria and the occurrence of coma.

The parameters of metabolic control measured at diagnosis included glycosylated hemoglobin (HbA_{1c}), residual β-cell function assessed by measurement of baseline and stimulated C-peptide levels, and the dose of insulin required by patients at diagnosis after blood glucose was

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normalized. Data from different centers were stored in the centralized office where statistical analysis was performed.

To evaluate whether the age at diagnosis could have influenced the parameters of metabolic control at presentation, patients were subdivided a priori into age groups reflecting a typical range of type I DM onset ages, namely patients clearly diagnosed before puberty (5 to 7 years), at the time when the initial phenomena of puberty, especially in females, might occur (7 to 10 years), during puberty (10 to 17 years), shortly after puberty (17 to 20 years), and in young adulthood (20 to 45 years). Furthermore, to analyze the immune parameters, patients were grouped as older or younger than 17 years, ie, according to the usual age at which puberty ceases.

The same therapeutic protocol was used for patients presenting to an adult or pediatric clinic, including intravenous insulin, if required, followed by adjustment of the subcutaneous insulin dose. Patients started on a 55% carbohydrate diet and received human insulin, short- and intermediate-acting as needed, to obtain near-normal blood glucose levels as quickly as possible in the week after diagnosis. If preprandial blood glucose values were less than 6.5 mmol/L, the insulin dose was decreased by 10%; if blood glucose levels were consistently less than 4.5 mmol/L, the insulin dose was decreased by 20%.

Metabolic Data

HbA_{1c} levels were measured by a column assay (BioRad, Milan, Italy) with normal values of less than 7%. C-peptide secretion (basal and stimulated with 1 mg intravenous glucagon) was evaluated in the 10 days after hyperglycemia was normalized with insulin therapy. The test was performed in the fasting state in the morning if blood glucose levels were less than 10 mmol/L, to avoid the effect of glucotoxicity on β-cell response. The last insulin injection was given the evening before the test. The C-peptide level was measured by radioimmunoassay using a commercially available kit (BioRad). The normal range for fasting C-peptide in our laboratory in 150 healthy subjects aged 5 to 40 years is 0.7 to 1.7 nmol/L, with intraassay and interassay coefficients of variation of 10% and 15%, respectively. For the purpose of this study, sera from 30 normal children aged 5 to 10 years (13 sera from age 5 to 7 years and 17 sera from age 7 to 10 years) were also evaluated.

Immunological Data

Two major type I DM–associated autoantibodies, glutamic acid decarboxylase (GAD) and tyrosine phosphatase-like molecule (IA-2ic), were measured in a total of 144 serum samples available for analysis.[18-21] Measurement of these antibodies rather than islet-cell antibody (ICA) was used on the basis of their antigen specificity and the availability of sensitive assays for their measurement. Furthermore, no data are yet available on GAD and IA-2 antibodies in relation to C-peptide levels in different age groups of type I DM patients. Patients from each age group were represented in the 144 samples obtained. The radioimmunoprecipitation assays for IA-2ic (intracellular region, amino acids 603 to 980) and GAD65 use in vitro transcription and translation systems. Human IA-2ic cDNA in pGEM4Z[19] was transcribed and translated in vitro, and human islet GAD65 cDNA in vector pB 1882 (donated by Dr Thomas Dyrberg, Novo Nordisk, Copenhagen, Denmark) was used according to the manufacturer's instructions (Promega, Madison, WI). For the two antibody assays, between 0.8 and 1.0 mg DNA was transcribed and translated with SP6 (IA-2ic) and T7 (GAD65) RBA polymerase in a TNT-coupled rabbit reticulocyte lysate system (Promega) in the presence of 35S methionine (0.8 mCi/mL) (Amersham, Amersham, UK). Incorporated radioactivity was determined by precipitation with 10% trichloroacetic acid and scintillation counting. For immunoprecipitation in each assay, 50-μL aliquots of 35S methionine (50,000 to 75,000 cpm)–labeled antigen were incubated overnight with 5 μL serum (final dilution, 1:25) in Tris-buffered saline. The immunocomplexes were isolated by adding 1 mg protein A-Sepharose and counted on a multiwell Wallac counter (Milton Keynes, UK). Values greater than 3 SD above a control population were taken as positive.

Our laboratory participated in the first and second GAD proficiency workshops, with sensitivity, specificity, validity, and consistency of 100%.[22]

Statistics

Data were analyzed after stratification of patients according to age at presentation. Parameters tested in different age groups were compared using ANOVA and the Mann-Whitney *U* test.

RESULTS

Eighty-one percent of the patients were hospitalized when first diagnosed. No differences were observed between the different age groups with respect to hospitalization. Clinical parameters at presentation are shown in Table 1. Elevated blood glucose together with ketonuria were observed in 69% to 86% of all patients, the remaining showing hyperglycemia only, and 3% presented with coma. There were no statistically significant differences between the different age groups for these parameters.

Age distribution was similar between males and females, but for the age group 20 to 45 years, in which there were more males (53 *v* 23 females), this gender difference was unexpected, and an investigation with a larger number of patients diagnosed in this age group is required before drawing any conclusion on this finding. Overall, the mode peak time of onset of type I DM was age 9 years for males and 12 years for females.

Parameters of Metabolic Control

Overall mean fasting C-peptide 1 week after diagnosis was 0.27 ± 0.21 nmol/L (range, 0 to 1.3), and the stimulated value was 0.49 ± 0.37 nmol/L (range, 0.02 to 2.5). No significant gender differences were found with regard to fasting C-peptide at onset in any of the age groups studied. However, basal C-peptide was significantly higher as patients became older ($P < .02$ by ANOVA; Fig 1). C-peptide values in normal children were similar between the age groups 5 to 7 and 7 to 10 (data not shown). For stimulated C-peptide only, patients aged 5 to 7 years showed a significant reduction compared only with the pubertal age group 10 to 17 years ($P < .03$; Fig 2).

HbA_{1c} levels were not statistically different between age groups. It is of interest that the mean HbA_{1c} level was less than 10% in all age groups (Fig 3), well below that reported in previous studies.[8-10]

The insulin dose required initially to control the disease (1

Table 1. Clinical Characteristics of Type I DM at Presentation

Characteristic	Age at Diagnosis (yr)				
	5-7	7-10	10-17	17-20	>20 < 45
No. of patients (female/male)	6/4	21/17	45/49	11/6	23/53
Hyperglycemia and ketonuria	8 (80)	33 (87)	81 (86)	13 (76)	51 (68)
Hyperglycemia only	2 (20)	5 (13)	10 (11)	4 (24)	19 (25)
Coma	—	—	3 (3)	—	6 (6)

NOTE. Values in parentheses are the percentage of patients.

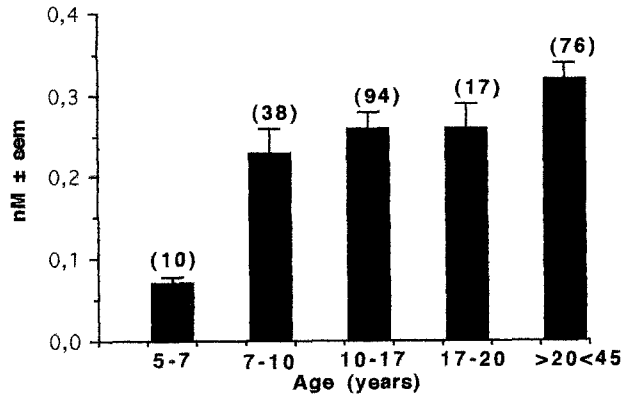

Fig 1. Baseline C-peptide at onset of type I DM. Significantly higher C-peptide secretion is associated with older age at diagnosis ($P < .02$, 1-way ANOVA). Number of patients is in parentheses.

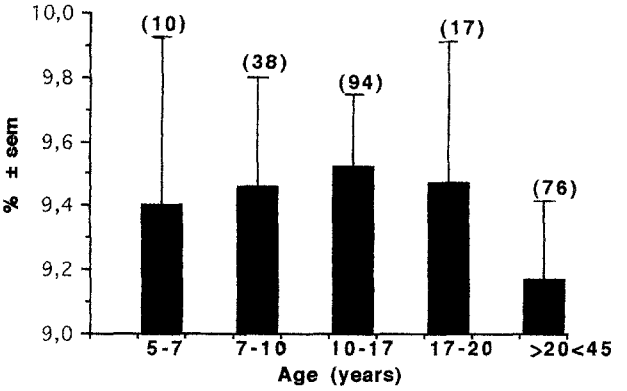

Fig 3. HbA_{1c} at disease presentation. No significant differences were observed in different age groups (Mann-Whitney *U* test). Number of patients is in parentheses.

week after presentation) was significantly different among age groups. Thus, there was an inverse correlation with age in older patients, who require significantly less insulin compared with younger patients to obtain near-normal blood glucose levels ($P < .04$ by ANOVA; Fig 4). Thus, type I DM patients diagnosed at a later age possess higher residual β-cell function and require less insulin to obtain optimal metabolic control.

Immunological Data

Autoantibodies to GAD and IA-2ic were measured in 144 serum samples available for testing and were representative of the five age groups. Overall, GAD antibodies were found in 65% and IA-2ic antibodies in 59% of the patients; antibodies to at least one of these antibodies were detected in 79% and both antibodies were present in 40% of the patients. Autoantibody frequency in different age groups is shown in Table 2. Although there were no significant differences in the frequency of GAD and/or IA-2ic antibodies in the different age groups, GAD antibodies were more frequent in patients diagnosed after age 17 years compared with younger patients ($P = .05$, 2×2 contingency table). There was no relationship between autoantibody frequency and C-peptide secretion (Table 3). The presence of GAD and/or IA-2ic antibodies alone or in combination does not affect residual β-cell function at diagnosis.

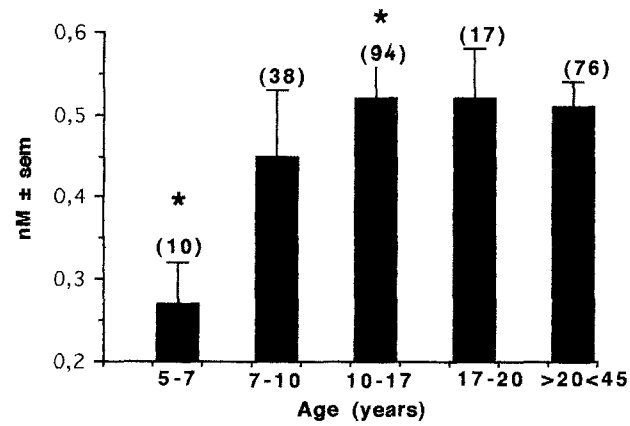

Fig 2. Stimulated C-peptide at onset of type I DM (no significant differences by ANOVA). Only patients diagnosed at ages 5 to 7 years have a significant reduction of stimulated C-peptide compared with patients diagnosed at pubertal age (Mann-Whitney *U* test, $P < .03$). Number of patients is in parentheses.

DISCUSSION

To our knowledge, this is the largest single population-based study of recent-onset type I DM patients conducted with respect to the characteristics at disease presentation including both metabolic and immune parameters. We analyzed the presentation and parameters of metabolic control in consecutive cases of newly diagnosed type I DM to establish the relation of residual β-cell function according to the age at diagnosis. A striking finding is that very young patients (7 years of age at diagnosis) show the lowest baseline residual β-cell function and require the highest insulin dose to obtain optimal blood glucose control. These observations support the concept of a different disease progression in the prediabetic period according to age: that is, the disease progresses more rapidly and more aggressively in early childhood. Since the destruction of insulin-producing cells in younger patients may lead to a rapid decline of β-cell function, these patients are probably the least amenable to treatment with immune intervention aimed at maintaining and preserving β-cell function. Moreover, this concept could be

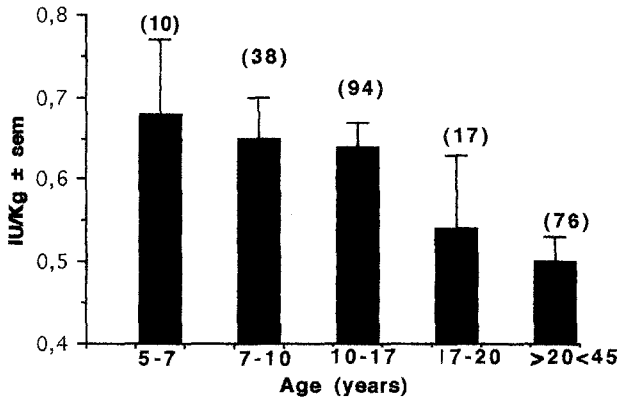

Fig 4. Insulin dose 1 week after type I DM diagnosis. A significantly higher insulin dose is required in younger patients ($P < .04$, ANOVA) to achieve metabolic control. Number of patients is in parentheses.

Table 2. Immunological Parameters in Different Age Groups and in Patients Subdivided Into Two Groups Before (<17 years) and After (>17 years) Puberty

	Age Group (yr)				
Autoantibodies	5-7	7-10	10-17	17-20	>20 > 45
GAD-positive	2 (50)	18 (72)	23 (50)	13 (100)	38 (68)
IA-2-positive	2 (50)	15 (60)	27 (58.5)	10 (77)	31 (55)
	Subdivided by Puberty				
	<17 years	>17 years			
GAD-positive	43	51	$P = .05$, 2 × 2 contingency table		
GAD-negative	32	18			
IA-2-positive	44	41	P = NS, 2 × 2 contingency table		
IA-2-negative	31	28			

NOTE. The percentage of positive subjects is in parentheses.

extended to the prediabetic period in which a young age also predicts a more rapid progression to diabetes in children with type I DM–associated antibodies.[23]

Our study is also the first population-based study in which IA-2ic antibodies have been estimated at the onset of type I DM. We determined antibody reactivity to part of the whole molecule of IA-2 (amino acids 1 to 979) representing the intracellular portion of IA-2, designated IA-2ic (amino acids 603 to 979). The evidence is that this carboxyl-terminal fragment of the intracellular molecule contains the immunodominant epitope in patients with type I DM.[20,23,24] Our data demonstrate that these novel IA-2ic antibodies are indeed prevalent in type I DM at clinical onset, being found in 59%, while GAD65 antibodies are found in 65%. It remains to be determined whether the absence of either antibody identifies a group of type I DM patients with different characteristics versus antibody-positive subjects.

Antibodies associated with type I DM, including GAD and IA-2ic antibodies, could reflect a more severe form of the disease with greater β-cell destruction at diagnosis. Factors associated with an increase in IA-2ic antibody frequency in previous studies of type I DM patients include a young age at diagnosis and the presence of HLA-DR4.[25] We found no relationship between antibody positivity and age at onset of type I DM. Prior to the present investigation, no other study had assessed metabolic control, including residual insulin secretion, in IA-2ic antibody–positive subjects at diagnosis of type I DM. We found no difference in C-peptide levels according to IA-2ic or GAD antibodies in these patients; therefore, there is no evidence from this study that antibody-positive subjects can be distinguished from antibody-negative subjects at the onset of type I DM by assessing residual β-cell function. Since there is evidence that these two antibodies can be detected in the prediabetic period[26-28] and since either GAD or IA-2ic antibodies could be detected in 79% of our type I DM patients, significantly more than any one antibody alone, it is likely that a combination of the two could increase the sensitivity of population screening for disease prediction and enhance the capacity for predicting type I DM by using recombinant antigens in screening assays rather than ICAs.[29,30]

The ICA assay is technically difficult, requiring human pancreatic tissue, and therefore is not readily applicable to large-scale analysis.[31] The tendency for subjects with ICA to be positive for IA-2ic or GAD antibodies reported elsewhere is consistent with the proposal that ICA is composed, to a degree, of antibodies to these antigens. Our present study is consistent with the hypothetical potential for prediction of type I DM using combinations of antibodies to specific islet antigens.[26,27,29,30]

Puberty represents a major hazard for individuals in whom the autoimmune process of β-cell damage is already ongoing due to insulin resistance associated with growth. It is well known that during puberty, insulin resistance may play a major role in precipitating the clinical onset of the disease,[32] and indeed, many patients are diagnosed at or near puberty. It is therefore particularly interesting in our study that the indices of β-cell function were similar in peripubertal patients compared with those diagnosed at a later age, and in both there was a degree of residual function, suggesting that immune intervention to protect β cells might be worthwhile even during the pubertal years.

When our data are compared with similar studies,[33-38] the characteristics of type I DM patients appear to have changed. Thus, in our study, the rates of ketoacidosis and levels of HbA_{1c} and C-peptide were lower and higher, respectively, than in previous studies, consistent with an earlier time of diagnosis in the natural history of β-cell destruction. The large series of patients studied by Snorgaard et al[10] and diagnosed in the early 1980s contrasts with our results. They studied 204 recent-onset type I DM patients with an age range of 18 to 36 years and therefore primarily diagnosed after puberty. In that study, C-peptide levels were much lower and HbA_1 levels were higher than in our study. Similarly, data reported by Drash[9] showed worse parameters of metabolic control in children diagnosed before puberty versus our prepubertal children. In summary, it appears that type I DM is now diagnosed at an earlier stage and the presentation is therefore milder, at least in the Western world. In light of the present attempts at immune intervention at diagnosis of type I DM,[2] any therapy adjuvant to insulin to protect residual β cells may be more beneficial in patients diagnosed at puberty or after. Indeed, the results of residual β-cell function in the age group 5 to 7 years, where both basal and stimulated C-peptide levels were extremely low, suggest that destruction of β cells has been massive in this age group,

Table 3. Basal C-Peptide in Patients Grouped According to GAD and/or IA-2 Positivity

	GAD-Positive	GAD-Negative	IA-2-Positive	IA-2-Negative	GAD-Positive + IA-2-Positive (n = 58)	GAD-Negative + IA-2-Negative (n = 27)
Basal C-peptide	0.28 ± 0.22 (78)	0.29 ± 0.20 (41)	0.29 ± 0.24 (72)	0.29 ± 0.17 (47)	0.29 ± 0.24	0.30 ± 0.17

NOTE. Differences between groups are not significant. Number of sera tested is in parentheses.

indicating (but not excluding) that immune intervention would be ineffective in young patients.

Today, considerable attention is paid to identification of subjects before they become overtly diabetic, to conduct immunointervention when β-cell function is still appreciable. Age is likely to be an important variable when considering trials aimed at type I DM prevention. Subjects with immune markers who are young are likely to progress more rapidly to type I DM than older subjects, and intervention may be less likely to succeed. Trials of therapy aimed at prevention should consider stratification of patients according to age at entry into the study.

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