Diabetologia (1990) 33: 561–568

Diabetologia

Islet cell antibodies and fasting C-peptide predict insulin requirement at diagnosis of diabetes mellitus

M. Landin-Olsson[1], K. O. Nilsson[2], Å. Lernmark[1, 3] and G. Sundkvist[1]

Departments of [1] Medicine and [2] Paediatrics, Malmö General Hospital, University of Lund, Malmö, Sweden and [3] Department of Medicine, University of Washington, Seattle, USA

Summary. The differential diagnosis between Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes is complicated since no specific markers are available for either disease. In this study, 244 consecutive patients were diagnosed with diabetes mellitus during two years in Malmö (230000 inhabitants), corresponding to an incidence rate of $53 \cdot 100000^{-1} \cdot year^{-1}$. Age, body mass index, HbA_{1c}, C-peptide, and levels of islet cell antibodies were determined at the clinical onset, and related to the classification at diagnosis and at follow-up ($n = 233$) after a median time of 31 (range 1–49) months. After diagnosis, 42 of 244 (17%) were started on insulin while 202 of 244 (83%) were not. Islet cell antibodies were present in 25 of 42 (60%), and in 18 of 183 (10%), respectively. In the non-insulin treated group, patients with islet cell antibodies had lower body mass index ($p < 0.001$), higher HbA_{1c} ($p < 0.004$), and lower C-peptide ($p < 0.001$) than patients without. At follow-up, 11 of 18 (61%) islet cell positive patients were changed to insulin treatment, as were six other patients. Insulin was discontinued in five initially insulin-treated but islet cell antibody negative patients. The sensitivity, specificity and predictive value for insulin treatment at follow-up were for islet cell antibody positivity; 72%, 96% and 84%, respectively, and for low C-peptide value; 60%, 96%, and 80%, respectively. Islet cell antibodies and low C-peptide at diagnosis of diabetes mellitus are concluded to be useful markers to predict insulin dependence.

Key words: Type 1 (insulin-dependent) diabetes, Type 2 (non-insulin dependent) diabetes, incidence, age, body mass index, HbA_{1c}.

The classification of newly diagnosed diabetic patients is, in general, based on clinical observations. The Type 1 (insulin-dependent) diabetic patient is usually young, has rapidly developing symptoms of hyperglycaemia, weight loss and ketoacidosis, while the Type 2 (non-insulin-dependent) patient often is older and overweight with slowly progressive hyperglycaemia, and the majority of the patients are considered as Type 2. There are, however, less clearly classifiable patients, usually middle- or old-aged of normal weight who do not manage to maintain normoglycaemia on diet and oral hypoglycaemic agents (OHA). These patients may progress toward insulin-dependence, but cannot initially be clinically distinguished from other Type 2 diabetic patients. Other patients may present with severe hyperglycaemia requiring immediate treatment with insulin, but can later keep acceptable blood glucose levels on diet alone. A differential diagnosis between Type 1 and 2 diabetes is important and of interest to the individual patient. In elderly patients severe uncontrolled diabetes has a high rate of mortality [1], and insulin treatment in patients not needing insulin [2] may result in hyperinsulinaemia, weight gain and, perhaps, accelerated atherosclerosis [3]. Since the aetiologies and pathogeneses of Type 1 and 2 diabetes have yet to be determined, accurate laboratory tests are not available for a differential diagnosis. Low C-peptide [4], or islet cell antibodies (ICA) [5–9], have been suggested as markers for Type 1 diabetes among Type 2 diabetic patients failing on diet and/or OHA. We have tested this hypothesis by a different study design, since in contrast to other studies all consecutive patients with the diagnosis diabetes mellitus have been studied. A total of 244 consecutive patients of all ages with newly diagnosed diabetes mellitus in the city of Malmö, Sweden were followed prospectively with regard to type of treatment. The aims of the study were to determine the predictive values of age, heredity of diabetes, body mass index (BMI), HbA_{1c}, fasting serum C-peptide, and level of ICA at diagnosis for insulin treatment immediately or at follow-up.

Patients and methods

In 1986, the city of Malmö had a population of 230056 inhabitants, of which 14% were under 15 years of age and 39% were 50 years or older. Malmö General Hospital, affiliated with the University of Lund, is the only hospital in the city. A Diabetes Care Unit at the hospital takes care of the majority of all newly diagnosed diabetic adults (≥ 15 years of age). In Sweden Type 1 diabetes is considered a disease to be treated by specialists and these patients are all referred to the Diabetes Care Unit. Some non-insulin requiring patients may attend a limited number of general practitioners and specialists in private practice, who usually refer them to the Diabetes Care Unit for

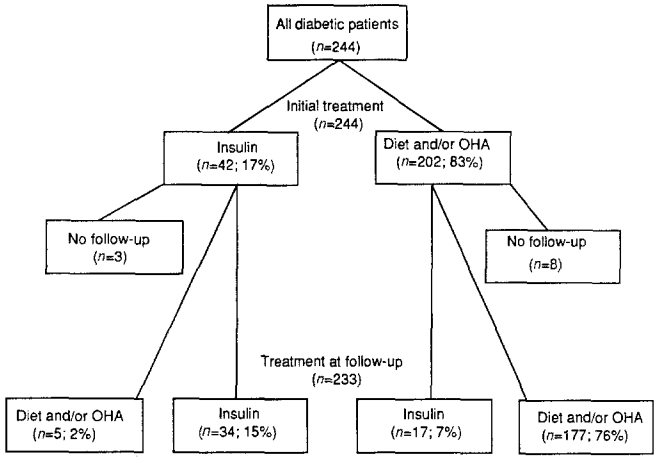

Fig. 1. Treatment based on clinical judgement at diagnosis of 244 consecutive patients and at follow-up (median 31 months) in 233 (95%). Eleven patients (5%) were not possible to follow up. The patients were diagnosed consecutively during two years, 1 September, 1985–31 August, 1987, in the city of Malmö

diet information, supporting a high ascertainment of Type 2 diabetic patients as well. Children (< 15 years) with newly diagnosed diabetes are all admitted to the Department of Paediatrics. During the study period information on patients with newly diagnosed diabetes mellitus in Malmö was collected from the Diabetes Care Unit, and from the departments of Medicine, Endocrinology and Paediatrics at Malmö General Hospital, and from the private physicians and general practitioners, who were informed about the study.

Patients of all ages with diabetes according to the diagnostic criteria established by The World Health Organisation (WHO) [10] were included in the study. Patients with the following disorders were excluded: gestational diabetes, diabetes secondary to steroid medication or to alcoholic pancreatitis, and impaired glucose tolerance determined with an oral glucose tolerance test using 75 g glucose. A total of 244 consecutive patients with newly diagnosed diabetes entered the study during the two-year period from September 1985 until August 1987. The family history of diabetes mellitus was obtained from patient records, and was available for 222 of 244 (91%) of the patients. Body mass index (BMI, kg/m^2) was calculated in 218 of 244 (89%) of the patients. Blood samples were taken after an overnight fast as soon as possible after diagnosis, but only accepted if taken within three months from diagnosis and within six months after the first hyperglycaemic symptoms. HbA_{1c} was determined in whole blood from 225 of 244 (92%) patients, C-peptide in serum from 212 of 244 (87%) and ICA in serum from 225 of 244 (92%) patients. Several experienced physicians were responsible for the clinical care of the patients, and decisions regarding therapy were in accordance to the criteria recommended by WHO [10], and based on clinical judgement without knowledge of the results of the biochemical tests except for the HbA_{1c} values. The therapy one week after diagnosis was defined as the initial therapy. The treatment at follow up, was obtained from the patient records. It was possible to follow 233 of 244 (95%) of the patients for a median time of 31 (range 1–49; quartile range 24–37) months.

The study was approved by the Ethical Committee at the University of Lund, Sweden.

Methods

HbA_{1c}. HbA_1c was determined by HPLC [11]. The reference values of healthy individuals ranged between 3.9 to 5.3%. The intra-assay coefficient of variation was 1% and the inter-assay 2%.

C-peptide analysis. Serum samples for fasting C-peptide were analysed consecutively to avoid long-term storage and possible degradation of C-peptide. All samples were analysed as duplicates. The lower detection limit in the radioimmunoassay used [12] was 0.10 nmol/l. Intra- and inter-assay coefficient of variation was 7% and 9%, respectively. Reference values were 0.25–0.75 nmol/l. The 10th percentile for fasting C-peptide values in the patients initially treated with diet and/or OHA (< 0.25 nmol/l) was considered as a cut-off limit for a low value. Serum creatinine was within normal range in all patients, except one who had increased values. This patient was excluded, since a decreased glomerular filtration could diminish renal excretion of peptides and give falsely high C-peptide values [13].

Islet cell antibodies. ICA were titrated in an indirect immunofluorescence assay, incubating sera with unfixed cryocut human pancreas as previously described [14]. Briefly, in a first incubation for 18 h at 4 °C, the serum samples mixed in phosphate buffered saline containing a monoclonal proinsulin antibody to mark pancreatic Beta cells, were applied to the dried pancreatic section. One pancreas was used for all end-point titrations of the samples in this study. After washing, the two fluorescent second antibodies, Texas red-conjugated antimouse IgG (N 2031, Amersham, Buckinghamshire, UK) to visualize the proinsulin antibodies, and FITC-conjugated antihuman IgG (Code F202, Dako, Copenhagen, Denmark) to visualize ICA, were added and incubated with the sections for 30 min at room temperature. After washing, the slides were mounted in TrisHCl buffered glycerol and evaluated in a fluorescence microscope by at least two independent observers. Quantitation of ICA was done by dilution of sera until ICA were no longer detectable. The interassay variation was 0.65 titration steps. Results were converted to Juvenile Diabetes Foundation (JDF) units [15], by a standard curve based on the international JDF reference serum sample.

Statistical analysis

All values are given in medians, percentiles or ranges. Comparisons between groups were done with the Mann-Whitney U-test and between frequencies with the chi-square test. Correlations were tested with the Spearman rank correlation test. All tests were two-tailed. A p-value less than 0.05 was considered significant. Sensitivity was defined as the number of patients who were positive for the tested marker at diagnosis expressed as a percent of all diabetic patients who later required insulin treatment. Specificity was defined as the number of patients who were negative for the tested marker as a percent of patients not requiring insulin at follow-up. Predictive value for a positive test was defined as the number of patients who needed insulin at follow-up as a percent of patients who were positive for the tested marker. Predictive value for a negative test was defined as the number of patients who did not need insulin at follow-up as percent of patients who were negative for the tested marker.

Results

All diabetic patients

During the study period of two years, 244 patients with diabetes mellitus were diagnosed, corresponding to an incidence rate of $53 \cdot 100000$ inhabitants$^{-1} \cdot$ year^{-1} in the city of Malmö. Insulin treatment was started within one week after diagnosis in 42 of 244 (17%) of the patients. In 202 of 244 (83%) patients, the attending physician considered the disease as non-insulin-requiring and these patients were treated with either diet alone ($n = 144$), or diet in combination with OHA ($n = 58$). Of the orginal 244 patients 233 were followed for 1–49 (median 31) months, 5 of 39 (13%) of the initially insulin treated patients were changed to diet and/or OHA, and 17 of 194 (9%) in the

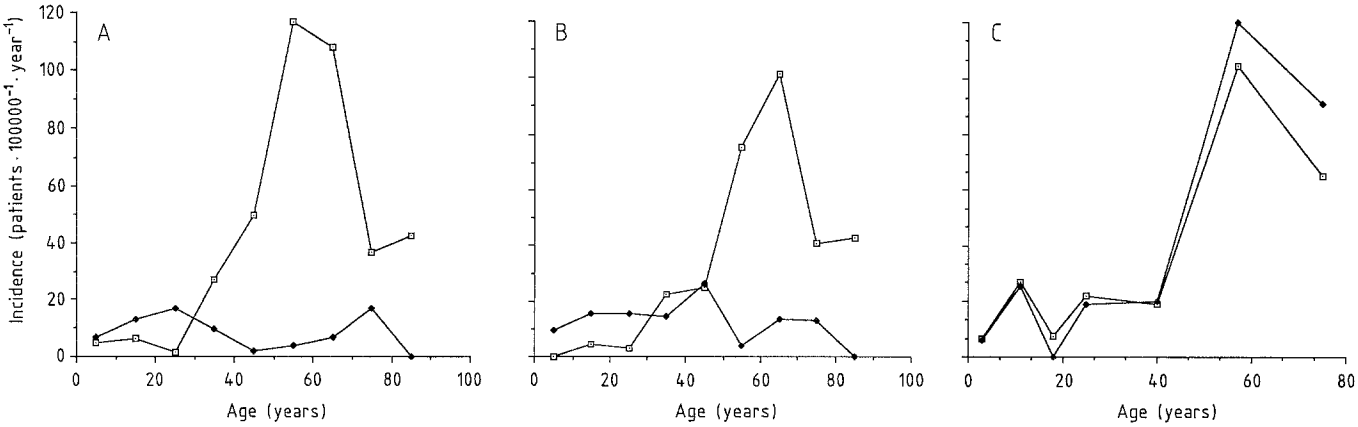

Fig. 2 A–C. **A** Age related annual incidence rate of insulin treated Type 1 (insulin-dependent) diabetes (■) and non-insulin-treated Type 2 diabetes (□) per 100000 inhabitants in the city of Malmö September 1985–August 1987. The classification of diabetes was defined as insulin or non-insulin-treatment within a week from diagnosis. **B** Age related annual incidence rate of diabetes in relation to mode of therapy at follow-up; insulin-treated Type 1 (insulin-dependent) diabetes (■) and non-insulin-treated Type 2 diabetes (□) per 100000 inhabitants in the city of Malmö September 1985–August 1987. **C** Age related annual incidence rate of diabetes mellitus in Malmö during September 1985–August 1987 in male (■) and in female (□) patients

initially diet and/or OHA treated group were changed to insulin (Fig 1). A total of 17 patients in the original cohort died during the observation period, 13 non-insulin-treated patients, three insulin-treated, and one initially insulin-treated and later on diet.

The ages of all patients ranged from 3 to 88 years (median 56 years). The incidence rate for all ages was $53 \cdot 100000^{-1} \cdot \text{year}^{-1}$ and increased with increasing age, among the 0–14 year olds the incidence rate was $17 \cdot 100000 \text{ children}^{-1} \cdot \text{year}^{-1}$, and among the 50 year and older $92 \cdot 100000^{-1} \cdot \text{year}^{-1}$. Age related incidence rates for insulin- and non-insulin-treated diabetes at diagnosis are shown in Figure 2 A. After reclassification according to mode of therapy at follow-up the incidence rates of insulin- and non-insulin-treated diabetes are as in Figure 2 B. The male/female ratio was 1.1 (128/116) for all patients. The annual incidence rates per 100000 age related inhabitants was 53 for males in all ages, 18 among 0–14 year olds, and 66 among 15–100 year olds. The corresponding incidence rates for females were 48, 16, and $53 \cdot 100000^{-1} \cdot \text{year}^{-1}$, respectively. There was no significant difference in incidence rates between the sexes in any age group (Fig 2 C).

Initially insulin treated patients

In the 42 patients treated with insulin within one week from diagnosis, the sex ratio was 0.6 (16 males and 26 females). The age ranged from 3–78 (median 30; quartile range 21–61) years. The age related incidence (Fig. 2 A) for insulin treated patients was bimodal with a first peak in the age group, 10–30 years, and another in the age group 65–80 years. Family history of diabetes was known in 38 of 42 (90%) of the patients, 6 of 38 (16%) had a first degree relative treated with insulin, 6 of 38 (16%) had a diabetic first degree relative not treated with insulin, while 12 of 38 (32%) had a more distant relative and 15 of 38 (39%) did not know of any relative with diabetes mellitus. The frequency distributions for BMI, HbA_{1c}, fasting C-peptide and ICA levels for positive patients are shown in Figure 3. BMI ranged from 15–35 (median 20; quartile range 17–24) kg/m^2, HbA_{1c} from 6.2–15.8 (median 10.6; quartile range 8.2–11.1) %, and fasting C-peptide from 0.10–1.54 (median 0.24; quartile range 0.14–0.36) nmol/l. ICA were present in 25 of 42 (60%), ranging from 3 to 980 (median 30) JDF units.

Initially non-insulin-treated patients

In the 202 patients initially treated with diet and/or OHA, the sex ratio was 1.2 (112 males and 90 females). The age ranged from 6–88 (median 58; quartile range 50–66) years, and the age at diagnosis was significantly higher compared with the initially insulin-treated patients ($p < 0.001$). The age related incidence was unimodal with peak in the age group 55–69 years (Fig. 2 A). Heredity of diabetes was known in 184 of 202 (91%) of the patients, insulin treatment in a first degree relative was present in 25 of 184 (14%), 50 of 184 (27%) had a first degree relative with non-insulin-treated diabetes, while 24 of 185 (13%) had a more distant relative and 46% (85 of 184) did not know of any heredity of diabetes. Family history of different types of diabetes did not differ significantly between initially insulin- and non-insulin treated patients. The frequency distributions for BMI, HbA_{1c}, fasting C-peptide and ICA levels are shown in Figure 3. BMI ranged from 14–44 (median 28; quartile range 25–32) kg/m^2, HbA_{1c} from 4.2–14.8 (median 7.8; quartile range 6.1–9.5) %, and fasting C-peptide from 0.10–4.10 (median 0.73; quartile range 0.43–1.16) nmol/l. All three parameters differed significantly ($p < 0.001$) from the initially insulin-treated patients. ICA were present in 18 of 183 (9.8%) of the patients, and ranged from 20 to 5520 (median 73) JDF units. The ICA levels in the 18 ICA positive patients were significantly higher ($p < 0.02$) compared with the 25 ICA positive patients in the insulin-treated group.

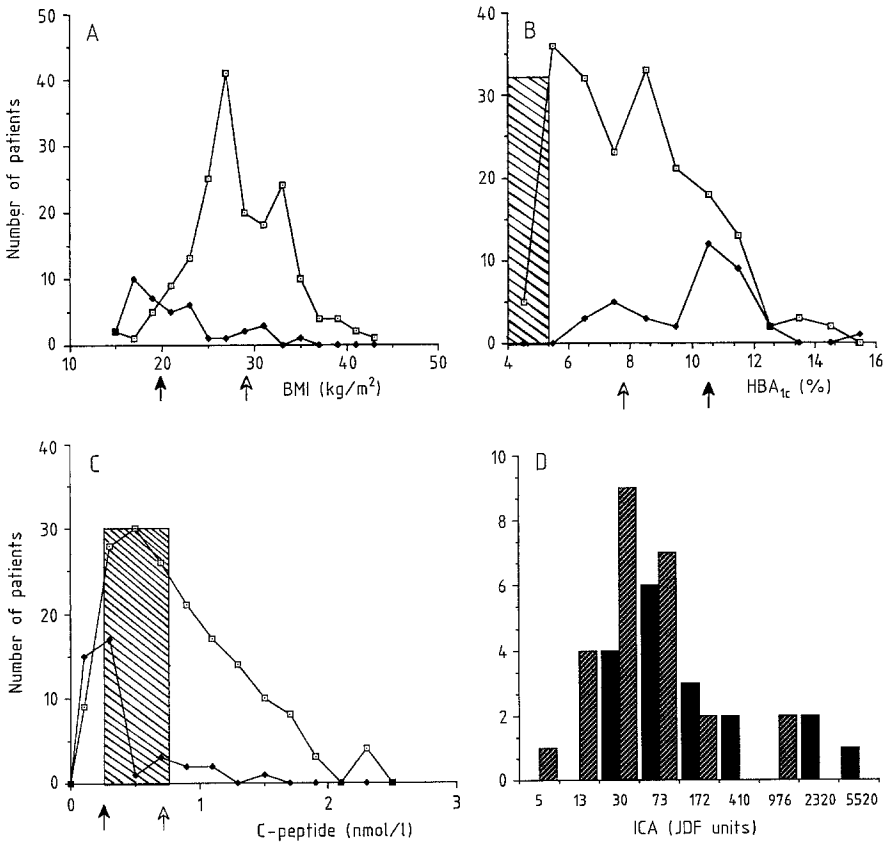

Fig. 3 A–D. Frequency distributions of body mass index (BMI) (panel **A**), HbA_{1c} (panel **B**), fasting C-peptide (panel **C**), and levels of islet cell antibodies (ICA) (panel **D**) at diagnosis of diabetes mellitus in patients ($n = 42$) initially treated with insulin (■ in panel **A–C** and hatched bar in panel **D**) and in patients ($n = 202$) initially treated with diet and/or oral hypoglycaemic agents (□ in panel **A–C** and filled bar in panel **D**). The values between insulin and non-insulin treated patients differed for BMI ($p < 0.001$), HbA_{1c} ($p < 0.001$), C-peptide ($p < 0.001$), and ICA levels ($p < 0.02$) (Mann-Whitney U-test), and median values are indicated by arrows. The reference limits for HbA_{1c} and C-peptide are indicated by hatched areas

ICA positive patients

Compared with the ICA negative non-insulin-treated patients ($n = 165$) the ICA positive initially non-insulin treated patients ($n = 18$) had lower BMI (median 25 vs 28 kg/m^2; $p < 0.001$), lower C-peptide (median 0.25 vs 0.81 nmol/l; $p < 0.001$), and higher HbA_{1c} (median 9.4 vs 7.6%; $p < 0.004$). Age (median 58 vs 58 years) was not different ($p = 0.95$). Compared with all the initially insulin treated patients ($n = 42$) the ICA positive initially non-insulin-treated patients ($n = 18$) were older (median 58 vs 30 years; $p < 0.007$) and had higher BMI (median 25 vs 20 kg/m^2 $p < 0.008$). Neither C-peptide values (median 0.24 vs 0.24 nmol/l; $p = 1.0$) nor HbA_{1c} (median 9.4 vs 10.6%; $p = 0.1$) differed between the groups.

When the ICA levels of all 43 ICA positive patients irrespective of treatment were tested against age, BMI, HbA_{1c} and C-peptide, there was a significant though weak correlation to BMI ($r_s = 0.3$; $p < 0.05$) but not to the other parameters.

Clinical and laboratory parameters at diagnosis in relation to insulin treatment at follow-up

At follow-up 51 of 233 (22%) patients were treated with insulin and 182 of 233 (78%) patients were treated with diet and/or OHA. Compared with the patients remaining on diet and/or OHA (Group D; Table 1), the patients who were changed to insulin treatment (Group C) were already at diagnosis slightly younger, had lower BMI and C-peptide values, while levels of HbA_{1c} were higher and the frequency of ICA was higher. Compared to the initially insulin-treated patients (Group B), the patients who were changed to insulin therapy (Group C) were older, had slightly higher BMI, while levels of HbA_{1c}, C-peptide and frequency of ICA did not differ (Table 1).

A total of 18 of 183 (10%) non-insulin-treated patients were ICA positive at the time of clinical diagnosis (patients 1–18; Table 2) and 11 of 18 (61%) were changed to insulin treatment during the follow-up period (Table 2). The remaining seven ICA positive patients were after 25–43 months of observation still treated with diet and/or OHA. An additional six initially non-insulin-treated patients (patients 19–24) were changed to insulin therapy at follow-up; five were ICA negative and one not tested (Table 2).

The sensitivity, specificity, and predictive value for insulin treatment at follow-up were calculated for age, BMI, HbA_{1c}, C-peptide and ICA. These calculations were done to assess to what extent an analysis of one or several of these parameters at the time of diagnosis would be useful to predict later insulin treatment. The sensitivity of the ICA test was 72% since 36 of 50 of the patients treated with insulin at follow-up were ICA positive at clinical onset. The specificity was 96% since 158 of 165 of the non-insulin-treated patients at follow-up were ICA negative at diagnosis (Table 3). The data in Table 3 show that presence of ICA at diagnosis predicted later insulin treatment in 84% of the patients. The corresponding values for sensitivity, specificity, and predictive value for low fasting C-peptide were 60%, 96% and 80%, respectively. The sensitivity was 83% when ICA were positive or C-peptide was low, corresponding to a specificity of 92%. The sensitivity decreased to 49% if the simultaneous presence of

Table 1. Clinical and laboratory parameters in relation to follow-up treatment of 233 consecutive diabetic patients

Group		A	B	C	D
Initial treatment		Insulin	Insulin oral hypoglycemic agents (OHA)	Diet and/or OHA	Diet and/or OHA
Follow up treatment	Total number investigated	Diet and/or OHA	Insulin	Insulin	Diet and/or OHA
Number	233	5	34	17	177
Sex (male/female)	233	3/2	11/23	8/9	98/79
Age (years) median quartile range	233	65 43–72	24[b] 15–39	46 34–62	58[a] 50–66
BMI (kg/m^2) median quartile range	214	28 23–31	19[a] 17–23	24 20–26	28[c] 26–32
HbA_{1c} (%) median quartile range	221	10.9 8.6–11.1	10.6 8.1–11.1	9.4 8.8–10.1	7.6[b] 6.1–9.4
C-peptide (nmol/l) median quartile range	204	0.98 0.73–1.18	0.23 0.14–0.28	0.22 0.16–0.38	0.79[c] 0.54–1.17
Islet cell antibody frequency (JDF units) median range	215	0/5 (0%) – –	25/34 (74%) 30[a] 5–980	11/16 (69%) 73 20–5520	7/160 (4%)[c] 73 20–1500

OHA – oral hypoglycaemic agents
Differences compared with group C: [a] $p < 0.05$, [b] $p \leq 0.01$, [c] $p < 0.001$

Table 2. Clinical and laboratory parameters in the 24 patients initially treated with diet and/or oral hypoglycaemic agents (OHA), who were islet cell antibody positive at diagnosis ($n = 18$) and/or later changed to insulin treatment ($n = 17$)

Patient ($n = 24$)	Age (years)	Body mass index (kg/m^2)	HbA_{1c} (%)	C-peptide (nmol/l)	Islet cell antibodies (JDF units)	Treatment at follow-up (Time to insulin treatment or time of observation; months)
1	59	31.2	9.50	0.27	5520	insulin (0.5)
2	65	23.6	8.83	0.10	2320	insulin (19)
3	75	–	11.55	0.22	1500	diet and/or OHA (28)
4	41	23.5	11.52	0.24	270	diet and/or OHA (36)
5	58	26.9	6.29	0.25	270	diet and/or OHA (25)
6	58	27.8	10.28	0.10	170	insulin (24)
7	69	22.0	9.93	0.16	170	insulin (25)
8	44	–	–	0.38	170	insulin (11)
9	42	23.9	8.50	–	73	insulin (9)
10	57	26.6	10.14	0.16	73	insulin (18)
11	79	19.2	9.10	0.42	73	diet and/or OHA (38)
12	34	21.2	8.93	0.23	47	insulin (14)
13	67	26.1	9.69	0.62	47	diet and/or OHA (32)
14	53	26.4	8.70	0.74	47	diet and/or OHA (43)
15	30	20.3	10.52	0.21	30	insulin (26)
16	61	25.1	9.42	0.44	30	insulin (1)
17	29	27.5	5.53	0.41	20	diet and/or OHA (30)
18	69	25.3	8.79	0.21	18	insulin (20)
19	46	18.4	–	–	–	insulin (10)
20	34	19.6	–	–	neg	insulin (12)
21	6	15.7	6.42	0.12	neg	insulin (9)
22	11	–	6.44	0.25	neg	insulin (26)
23	66	–	8.92	1.47	neg	insulin (31)
24	41	26.1	14.75	1.48	neg	insulin (17)

ICA and low C-peptide were taken into account. The frequency of falsely insulin requiring patients was, however, as low as 1%, since the specificity increased to 99% (Table 3). The probability of being non-insulin requiring at follow-up if the marker was absent is indicated by the predictive value of negative test, which was 92% for ICA alone and 95% when ICA were combined with C-peptide values (Table 3).

Discussion

The design of our study was to include all patients given the diagnosis diabetes mellitus irrespective of age and classification of type of diabetes based on clinical judgement. Clinical and laboratory parameters were first determined at diagnosis, and by follow-up of the patients it was tested if these parameters predicted insulin treatment. As

Table 3. The sensitivity, specificity and predictive value for insulin treatment at follow-up, of clinical and laboratory parameters obtained at diagnosis of diabetes mellitus in consecutive patients ($n = 233$).

Parameter	Cut-off limit[a]	Sensitivity	Specificity	Predictive value of positive test	Predictive value of negative test
Age	< 39 years	59% (30/51)	91% (166/182)	65% (30/46)	89% (166/187)
Body mass index	< 22 kg/m^2	63% (29/46)	93% (157/168)	73% (29/40)	90% (157/174)
HbA_{1c}	> 11.1%	19% (9/47)	90% (156/174)	33% (9/27)	80% (156/194)
C-peptide	< 0.25 nmol/l	60% (28/47)	96% (150/157)	80% (28/35)	89% (150/169)
ICA	positivity	72% (36/50)	96% (158/165)	84% (36/43)	92% (158/172)
ICA positivity and/or C-peptide < 0.25 nmol/l		83% (39/47)	92% (145/157)	76% (39/51)	95% (145/153)
ICA positivity and C-peptide < 0.25 nmol/l		49% (23/47)	99% (155/157)	92% (23/25)	87% (155/179)

[a] 10th (age, body mass index, C-peptide) or 90th (HbA_{1c}) percentile for values recorded at diagnosis in initially diet and/or oral hypoglycaemic agents treated patients.
ICA – islet cell antibodies

expected, patients initially treated with insulin were younger, had lower BMI, more advanced metabolic dysregulation measured as HbA_{1c}, lower fasting C-peptide, and higher frequency of ICA compared with non-insulin treated patients. At follow-up the insulin treated group had increased in number, mainly due to the addition of patients who already at diagnosis had lower BMI and fasting C-peptide, but higher HbA_{1c} and frequency of ICA compared with patients remaining on diet and/or OHA.

Representative study populations are important to enable an accurate estimate of sensitivity, specificity and predictive value. This was possible in the city of Malmö since it has only one hospital and a limited number of general and private physicians. Furthermore, the incidence rate in younger age groups in this study is in agreement with previous incidence studies of 0–14 year [16], and 15–34 year [17] olds in Sweden. The sex difference in the 15–34 year olds with a dominance of males [17], was however, not reproduced in this study, possibly due to the few patients in this age group. Compared to an incidence study in Oslo, Norway [18], our incidence rates were comparable in ages up to 60 years but lower thereafter. This difference could be explained by different approaches to estimate the incidence rate, or to the possibility that some elderly patients were missed, or to a geographic difference in incidence. The possible missed patients would only influence the results in the unlikely event that ICA were present at a high frequency.

Among the parameters tested, young age was a feature of patients needing insulin at diagnosis, but not for patients changing therapy to insulin-treatment during follow-up. In a Finnish population-based study it was also demonstrated that nearly all patients diagnosed before the age of 19 years were insulin treated, but 37% of all insulin-treated patients were diagnosed after that age [19]. The family history of insulin- or non-insulin treatment in diabetic relatives was found to be common, but not helpful in the classification of the patients. Our data on family history was similar to that reported for Type 1 and 2 diabetic probands [20]. BMI was significantly lower for insulin-treated patients both at diagnosis and at follow-up compared with non-insulin-treated patients. It is clinically well known that insulin-dependent patients tend to be lean. This can partly be explained by age since BMI was positively correlated with age ($r_s = 0.71$; $p < 0.001$) in insulin-treated patients at follow-up. BMI is age related in children, but this cannot explain the difference between BMI between group C and D (Table 1), and may therefore in fact reflect a disturbed Beta-cell function. In accordance to this, the HbA_{1c} values were also higher both in patients needing insulin at diagnosis (Group B) and also in those who were later insulin treated (Group C). Moreover the C-peptide were also lower in these patients.

In previous studies fasting C-peptide [21, 22], as well as C-peptide stimulation by glucagon have been shown to mark insulin-therapy during follow-up of Type 2 patients [4, 23]. C-peptide is secreted from the pancreatic Beta-cells equimolar to insulin [24], and there is a close correlation between fasting C-peptide and C-peptide in response to glucagon [25]. This study lends support to previous investigations, since a low C-peptide would identify 60% of insulin-treated patients. There would be only 4% false positive tests. As shown in Table 3, insulin treatment could be predicted in 80% of patients with low C-peptide.

Analysis of ICA at diagnosis was the best predictor (84%) and showed the highest sensitivity (72%) for insulin treatment. The high specificity for ICA (96%) was equal to that of fasting C-peptide. The possibility to predict insulin treatment with ICA already at the time of diagnosis was not possible in previous studies of non-insulin-dependent diabetic patients who either failed to control their blood glucose by OHA [5–9], or were cross-sectional studies [26, 27]. Our finding of a high predictive value of ICA extends studies on consecutively diagnosed non-insulin-treated patients who were either selected [28] or only older than 65 years of age [29]. The high predictive value of ICA among diabetic patients contrasts sharply to the low predictive value in ICA positive first degree relatives [30–32] as well as in control children [33]. It is, in fact, possible that the predictive value may increase during our prolonged follow-up since it cannot be excluded that the remaining non-insulin-treated ICA positive patients may also develop insulin dependency. The higher ICA levels in the initially non-insulin-treated patients may therefore re-

flect an ongoing Beta-cell destruction [34]. It should be noted that all ICA positive patients who were changed to insulin treatment showed low C-peptide values except in two patients (Table 2). These two ICA negative patients (patients 23 and 24) had an extremely bad compliance to dietery recommendations and their metabolic regulation was not improved by insulin. It is of interest that ICA negativity was used to predict non-insulin-dependence in diabetic patients [2]. This issue was also elucidated in this study demonstrating a predictive value of 92% for a negative ICA test and 89% for high C-peptide for no need of insulin.

The diagnostic difficulties with classification of diabetes increase with patient age. Type 1 diabetes may occur at any age, and in adults the progression of symptoms may evolve gradually to insulin-dependence [35]. In children almost all receive insulin at diagnosis [19], but in the middle-aged and elderly patients the development toward insulin-dependency can take years. The frequency of ICA among newly diagnosed children with Type 1 diabetes is about 80% [33, 36], but appears lower, 60–70% in adults with Type 1 diabetes [34, 37]. Our analysis demonstrates the value of follow up since the frequency of ICA among insulin-treated changed due to altered therapy (Table 1). It has been speculated that genetic heterogeneity [38] may explain possible clinical differences. We are currently studying whether the addition of genetic markers for HLA-DR and HLA-DQ [39], determinations of insulin- [40] as well as other autoantibodies [33, 41] may further improve the prediction for insulin treatment in patients with diabetes mellitus.

Determinations of fasting C-peptide and ICA at diagnosis of diabetes seem to be of value in identification of Type 1 diabetic patients. Early insulin treatment may be beneficial to the patient, since insulin therapy may decrease the autoimmune activity directed toward the Beta-cells. Beta-cells in vitro in a hyperglycaemic environment may be more antigenic, exemplified by glucose-stimulated expression of the M_r 64000 Beta-cell autoantigen [42, 43]. Prophylactic insulin therapy in the spontaneously diabetic BB rat [44] and NOD mouse [45] decreased the frequency of both diabetes and insulitis. Similarly, intensified insulin therapy in patients with new onset Type 1 diabetes seems to preserve residual Beta-cell function in Type 1 diabetic patients [46, 47]. The present study allows us to conclude that ICA and low C-peptide in newly diagnosed patients with diabetes mellitus are useful to predict insulin-dependence. It would therefore be of interest to test whether early and intense insulin therapy in such patients will preserve the residual Beta-cell function.

Acknowledgements. We are in debt to Drs. P. Fernlund and J.-O. Jeppsson, Department of Clinical Chemistry, Malmö General Hospital, for analysing C-peptide and HbA_{1c}. Mrs. B. Lörnstam is thanked for excellent technical assistance. Thanks are also due to the team at the Diabetes Care Unit, in particular the nurses R. Persson, U. Persson, M. Österlin, B. Nordström and the secretary E. Malmsten, to nurse R. Bergesen at the Department of Pediatrics, Malmö General Hospital, and to our colleagues who critically read the manuscript. Dr. M. Landin-Olsson is a recipient of a Juvenile Diabetes Foundation International Fellowship (file # 388199). The study was supported by the Swedish Medical Research Council (project 07507), Nordisk Insulin Foundation, The Foundations of Malmö General Hospital, Hoescht Foundation, Research Foundation of the Swedish Assurance Companies and in part by the National Institutes of Health (DK 26190).

Addendum

After the manuscript was submitted five additional initially non-insulin treated ICA positive patients, nos 3, 4, 13, 14 and 17 in Table 2, have been changed to insulin therapy.

References

1. Gale EAM, Dornan TL, Tattersall RB (1981) Severely uncontrolled diabetes in the over-fifties. Diabetologia 21: 25–28
2. Grant PJ, Barlow E, Miles DW (1984) Plasma C-peptide levels identify insulin-treated patients suitable for oral hypoglycaemic therapy. Diab Med 1: 284–286
3. Stout RW (1979) Diabetes and atherosclerosis – the role of insulin. Diabetologia 16: 141–150
4. Madsbad S, Krarup T, McNair P, Christiansen C, Faber OK, Transböl I, Binder C (1981) Practical clinical value of the C-peptide response to glucagon stimulation in the choice of treatment in diabetes mellitus. Acta Med Scand 210: 153–156
5. Irvine WJ, Sawers JSA, Feek CM, Prescott RJ, Duncanc JP (1979) The value of islet cell antibody in predicting secondary failure of oral hypoglycemic agent therapy in diabetes mellitus. J Clin Immunol 2: 23–26
6. Gleichmann H, Zörcher B, Greulich B, Gries FA, Henrichs HR, Bertrams J, Kolb H (1984) Correlation of islet cell antibodies and HLA-DR phenotypes with diabetes mellitus in adults. Diabetologia 27: 90–92
7. Wilson RM, Van der Minne P, Deverill I, Heller SR, Gelsthorpe K, Reeves WG, Tattersall RB (1985) Insulin dependence: problems with the classification of 100 consecutive patients. Diab Med 2: 167–172
8. Koskinen P, Viikari J, Irjala K, Kaihola H, Seepälä P (1986) Plasma and urinary C-peptide in the classification of adult diabetes. Scand J Clin Lab Invest 46: 655–663
9. Groop LC, Bottazzo GF, Doniach D (1986) Islet cell antibodies identify latent type 1 diabetes in patients aged 35–75 years at diagnosis. Diabetes 35: 237–241
10. WHO Study Group (1985) Report of diabetes mellitus technical report series No 727. WHO, Geneva
11. Jeppsson J, Jerntorp P, Sundkvist G, Englund H, Nylund V (1986) Measurement of hemoglobin A1c by a new liquid-chromatographic assay: Metodology, clinical utility, and relation to glucose tolerance evaluated. Clin Chem 32: 1867–1872
12. Heding LS (1975) Radioimmunological determination of C-peptide in serum. Diabetologia 11: 541–548
13. Kajinuma H, Tanabashi S, Ishiwata K, Kuzuya N (1979) Urinary excretion of C-peptide in relation to renal function. In: Baba S, Koneko T, Yanihara N (eds) Pro-insulin, insulin, and C-peptide. Excerpta Media, Amsterdam-Oxford, pp 183–189
14. Landin-Olsson M, Sundkvist G, Lernmark Å (1987) Prolonged incubation on the two-colour immunofluorescent test increases the prevalence and titres of islet cell antibodies in Type 1 (insulin-dependent) diabetes mellitus. Diabetologia 30: 327–332
15. Bonifacio E, Lernmark Å, Dawkins RL and co-authors (1988) Serum exchange and use of dilutions have improved precision of measurement of islet cell antibodies. J Immunol Methods 106: 83–88
16. Dahlquist G, Blom L, Tuvemo T, Nyström L, Sandström A, Wall S (1989) The Swedish childhood diabetes study – Results from a nine year case register and a one year case-referent study indicating that Type 1 (insulin-dependent) diabetes mellitus is associated with both Type 2 (non-insulin-dependent) diabetes mellitus and autoimmune disorders. Diabetologia 32: 2–6

17. Östman J, Arnqvist H, Blohmé G, Lithner F, Littorin B, Nyström L, Sandström A, Scherstén B, Wall S, Wibell L (1986) 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
18. Uvstedt HJ, Olsen E (1977) Incidence of diabetes mellitus in Oslo, Norway. Br J Prev Soc Med 31: 251–257
19. Laakso M, Pyörälä K (1985) Age of onset and type of diabetes. Diabetes Care 8: 114–117
20. Gottlieb MS (1979) Diabetes in offspring and siblings of juvenile and maturity-onset-diabetics. J Chronic Dis 33: 331–339
21. Katzeff HL, Savage PJ, Barclay-White B, Nagulesparan M, Bennet PH (1985) C-peptide measurement in the differentiation of Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 28: 264–268
22. Nielsen NV, Tronier B (1987) C-peptide and insulin secretion in diabetes mellitus treated with oral hypoglycemic agents or diet alone. A 3 years epidemiological cohort study on the island of Falster, Denmark. Diabetes Research 4: 135–139
23. Faber OK, Binder C (1977) C-peptide response to glucagon. A test for the residual B-cell function in diabetes mellitus. Diabetes 26: 605–610
24. Horwitz DL, Starr JJ, Mako ME, Blackard W, Rubenstein AH (1975) Proinsulin, insulin and C-peptide concentrations in human portal and peripheral blood. J Clin Invest 55: 1278–1283
25. Gjessing HJ, Matzen LE, Fröland A, Faber OK (1987) Correlations between fasting plasma C-peptide, glucagon-stimulated plasma C-peptide, and urinary C-peptide in insulin-treated diabetics. Diabetes Care 10: 487–490
26. Kobayashi T, Sugimoto T, Itoh T, Kosaka K, Tanaka T, Suwa S, Sato K, Tsuji K (1986) The prevalence of islet cell antibodies in Japanese insulin-dependent and non-insulin-dependent diabetic patients studied by indirect immunofluorescence and by a new method. Diabetes 35: 335–340
27. Groop L, Miettinen A, Groop P, Meri S, Koskimies S, Bottazzo GF (1988) Organ-specific autoimmunity and HLA-DR antigens as markers for β-cell destruction in patients with type II diabetes. Diabetes 37: 99–103
28. Di Mario U, Irvine WJ, Borsey DQ, Kyner JL, Weston J, Galfo C (1983) Immune abnormalities in diabetic patients not requiring insulin at diagnosis. Diabetologia 25: 392–395
29. Kilvert A, Fitzgerald MG, Wright AD, Nattrass M (1986) Clinical characteristics and aetiological classification of insulin-dependent diabetes in the elderly. Quart J Med, New Series 60: 865–872
30. Nordén G, Jensen E, Stilbo I, Bottazzo GF, Lernmark Å (1983) B-cell function and islet cell and other organ-specific autoantibodies in relatives to insulin-dependent diabetic patients. Acta Med Scand 213: 199–203
31. Srikanta S, Ganda OP, Rabizadeh A, Soeldner JS, Eisenbarth GS (1985) First-degree relatives of patients with Type 1 diabetes mellitus. Islet-cell antibodies and abnormal insulin secretion. N Engl J Med 313: 462–464
32. Tarn AC, Thomas JN, Dean BM, Ingram D, Schwarz G, Bottazzo GF, Gale EAM (1988) Predicting insulin-dependent diabetes. Lancet I: 845–850
33. Landin-Olsson M, Karlsson A, Dahlquist G, Blom L, Lernmark Å, Sundkvist G (1989) Islet cell and other organ-specific autoantibodies in all children developing Type 1 (insulin-independent) diabetes mellitus in Sweden during one year and in matched control children. Diabetologia 32: 387–395
34. Marner B, Agner T, Binder C, Lernmark Å, Nerup J, Mandrup-Poulsen T, Walldorff S (1985) Increased reduction in fasting C-peptide is associated with islet cell antibodies in Type 1 (insulin-dependent) diabetic patients. Diabetologia 28: 875–880
35. Tarn AC, Smith CP, Spencer KM, Bottazzo GF, Gale EAM (1987) Type 1 (insulin-dependent) diabetes: a disease of slow clinical onset? Br Med J 294: 342–345
36. Kolb H, Dannehl K, Grüneklee D, Zielasek J, Bertrams J, Hübinger A, Gries FA (1988) Prospective analysis of islet cell antibodies in children with Type 1 (insulin-dependent) diabetes. Diabetologia 31: 189–194
37. Landin-Olsson M, Sundkvist G, Lernmark Å, Nyström L, Arnqvist H, Blohmé G, Lithner F, Littorin B, Schersten B, Wibell L, Östman J (1989) Islet cell antibodies in relation to the type of diabetes at the clinical diagnosis in 464 recent onset 15–34 years old diabetic patients. Diabetologia 32: 508 Abstract
38. Karjalainen J, Salmela P, Ilonen J, Surcel H, Knip M (1989) A comparison of childhood and adult Type 1 diabetes mellitus. N Engl J Med 320: 881–886
39. Michelsen B, Lernmark Å (1987) Molecular cloning of a polymorphic DNA endonuclease fragment associates insulin-dependent diabetes mellitus with HLA-DQ. J Clin Invest 79: 1144–1152
40. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paguette TL (1983) Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 222: 1337–1339
41. Drell DW, Notkins AL (1987) Multiple immunological abnormalities in patients with Type 1 (insulin-dependent) diabetes mellitus. Diabetologia 30: 132–143
42. Bækkeskov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark Å (1982) Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. Nature (Lond) 298: 167–169
43. Kämpe O, Andersson A, Björk E, Hallberg A, Karlsson FA (1989) High-glucose simulation of 64000-M_r islet cell autoantigen expression. Diabetes 38: 1326–1328
44. Gotfredsen CF, Buschard K, Frandsen EK (1985) Reduction of diabetes incidence of BB Wistar rats by early prophylactic insulin treatment of diabetes-prone animals. Diabetologia 28: 933–935
45. Atkinson MA, Maclaren NK, Luchetta R, Burr I (1989) Prophylactic insulin therapy prevents insulitis and insulin dependent diabetes (IDD) in NOD mice. Diabetes 38 [Suppl 2]: 87 Abstract
46. Mirouze J, Selam JL, Pham TC, Mendoza E, Orsetti A (1978) Sustained insulin-induced remissions of juvenile diabetes by means of an external artificial pancreas. Diabetologia 14: 223–227
47. Shah SC, Malone JI, Simpson NE (1989) A randomized trail of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. N Engl J Med 320: 550–554

Received: 29 December 1989
and in revised form: 11 April 1990

Dr. M. Landin-Olsson
Department of Medicine
University of Lund
Malmö General Hospital
S-214 01 Malmö
Sweden