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The natural history of pre-Type 1 (insulin-dependent) diabetes mellitus in patients with autoimmune endocrine diseases

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Summary An 11-year prospective study was carried out in 180 non-diabetic patients with organ-specific autoimmune diseases to evaluate islet cell antibodies in predicting Type 1 (insulin-dependent) diabetes mellitus. Islet cell antibodies were characterised according to titres, persistence, complement-fixing ability, and pattern. During follow-up, 14 of 46 patients with islet cell antibodies persistently greater than 5 Juvenile Diabetes Foundation Units (JDF-U) (30.4%), none of 23 with islet cell antibodies between 2.5 and 5 JDF-U or fluctuating, and 3 of 109 without islet cell antibodies (2.7%), developed diabetes. The cumulative risk of developing diabetes was 70%, 0%, and 4%, respectively. All the patients who developed diabetes were females. Eight progressed to insulin-dependence acutely, four showed a transient period of non-insulin-dependence, while two were still insulin-free. No difference was found in titres of islet cell antibodies for the risk of diabetes. Complement-fixing islet cell antibodies enhanced the cumulative risk for the disease in patients with conventional islet cell antibodies at low-middle ($\geq$ 2.5–40 JDF-U), but not at high ($\geq$ 80 JDF-U) titres. Forty-two patients with islet cell antibodies were investigated for the *whole* or the *selective* pattern. In the presence of the *whole* pattern the cumulative risk for diabetes rose to 100%, while with the *selective* pattern it declined to 34%. The *whole* pattern was found in 83% of patients who developed Type 1 diabetes acutely. In patients with organ-specific autoimmune diseases, the *whole* islet cell antibody pattern greatly enhances the prediction for diabetes. [Diabetologia (1994) 37: 95–103]

Key words Autoimmune disease, Type 1 (insulin-dependent) diabetes mellitus, islet cell antibodies, autoimmune polyendocrinopathy, HLA-DR.

Type 1 (insulin-dependent) diabetes mellitus is considered an organ-specific autoimmune disease affecting individuals with a genetic susceptibility [1, 2]. It has also been observed that Type 1 diabetes is associated with other endocrine autoimmune disorders, mainly thyro-gastric and adrenal, more frequently than would be expected in a background population [3, 4]. Similar to other organ-specific autoimmune diseases, where the relevant circulating autoantibodies may be found many years before they become manifest [5, 6], Type 1 diabetes has a long silent prodromal period. In genetically susceptible individuals, before the clinical onset of the disease the immune system begins to produce antibodies against a variety of pancreatic islet cell autoantigens [7]. Islet cell antibodies (ICA), originally identified in patients with autoimmune polyglandular failure [8], have been extensively investigated, and their detection has become common in most laboratories. Furthermore, efforts have made to standardise their measurement [9, 10]. Previous investigations have shown that ICA, especially when fixing complement or at high titres, confer elevated risk for developing diabetes in unaffected individuals, such as in identical twins and triplets [11, 12], first-degree relatives (FDR) [13–16], schoolchildren [17–19], and patients with organ-specific autoimmunity [20, 21]. Although ICA have been reported to be strong markers of potential diabetes, many ICA-positive subjects remain disease-free after

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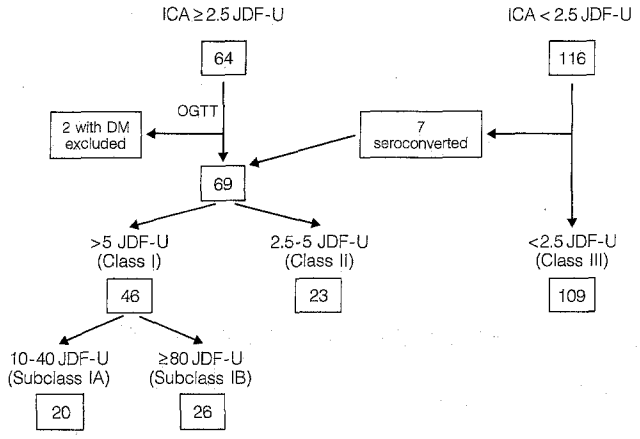

Fig. 1. Organ-specific autoimmune patients with different classes of islet cell antibodies (ICA) who entered into the study. DM, diabetes mellitus; OGTT, oral glucose tolerance test; JDF-U, Juvenile Diabetes Foundation Units

several years of follow-up. It has recently been shown that ICA are heterogeneous [22, 23], and this may account for the different rates of progression to diabetes reported in susceptible individuals [22, 23].

In this study, 180 non-diabetic patients with organ-specific autoimmune diseases were followed-up for a maximum of 11 years to investigate the role of ICA according to their titres, behaviour, complement-fixing ability, and pattern, as serological markers in predicting Type 1 diabetes. Sex, genetics, and other factors have been also considered.

Subjects and methods

Subjects

Between September 1976 and December 1991 autoantibody screening was performed on 3,042 patients with one or more organ-specific autoimmune diseases (OSAD), but without overt diabetes (mean age 40.1 years, range 5–74; 2,541 females and 501 males, female/male ratio = 5.1). All were living in north-eastern Italy, mostly in the Veneto region. Patients with ascertained primary endocrine deficiencies were receiving hormone replacement therapy (l-thyroxine and/or cortisone acetate), those with active Graves' disease were treated with antithyroid drugs.

Of those patients with ICA of 2.5 Juvenile Diabetes Foundation Units (JDF-U) or more, 64 were enrolled for the prospective survey (Fig. 1). They were investigated for ICA-IgG titres and complement-fixing (CF)-ICA. ICA-positive patients were re-evaluated for ICA-IgG, ICA titres, and CF-ICA every 2–14 months (mean 7 months). The mean duration of their follow-up was 43.1 months (range 2–132), for a total observation period of 2,974 months. The average number of serum determinations was 5.4 per patient (range 2–12), for a total of 346 tests. Forty-two subjects with persistent ICA of more than 5 JDF-U have also been retrospectively investigated to define their ICA pattern.

Another 116 randomly selected OSAD patients without detectable ICA (< 2.5 JDF-U) were also followed-up. They were subsequently investigated by repeated tests for ICA and basal glycaemia every 12–36 months (mean 22 months). The mean time of their follow-up was 52.1 months (range 2–144), for a total observation period of 5,675 months. The average number of sera samples tested was 2.2 per patient, for a total of 255 determinations. All the 180 subjects were questioned concerning family history of diabetes. Prior informed consent was obtained from all patients where appropriate. The investigation was performed in accordance with the principles of the Declaration of Helsinki.

A further 180 unselected patients with newly-diagnosed Type 1 diabetes were also interviewed to establish family history of diabetes, and to compare it with that of OSAD patients.

Islet cell antibodies of the IgG class (ICA-IgG)

ICA-IgG were determined using the standard indirect immunofluorescence technique, according to the protocol proposed by the International Committee on Cytoplasmic Islet cell Antibody Standardisation [9, 10].

All 3,042 sera were tested undiluted, at 1/2 and 1/4 dilution in the same session. If the positivity exceeded 1/4, sera were further tested at 1/8, 1/16, 1/32, and so on, up to the end-point. ICA titres were therefore converted to JDF-U by comparison with the standard curve, obtained by diluting the Reference International Standard Serum supplied by the Immunology and Diabetes Workshops (IDW) Committee, which had a titre of 80 JDF-U. Accordingly, sera with ICA titres 1/1, 1/2, 1/4, 1/8, 1/16, 1/32, and more than 1/32 were regarded as having values of 2.5, 5, 10, 20, 40, 80, and more than 80 JDF-U, respectively. Undiluted sera that gave negative reactions were conventionally considered to have ICA less than 2.5 JDF-U. In our laboratory, 54 of the 57 negative control sera, supplied blindly in 1991 by the IDW Committee, were found to have ICA values less than 2.5 JDF-U, while 3 were found ICA positive with a titre of 5 JDF-U. In the periodical Proficiency Tests performed from 1988 to 1991, our laboratory revealed a validity ranging from 93 to 100%, a consistency from 87 to 100%, a sensitivity from 81 to 100%, and a specificity of 100%.

Complement-fixing (CF) ICA

In the 69 ICA-positive patients followed-up, CF-ICA were detected by standard indirect immunofluorescence technique on normal human pancreas of blood group 0. Normal human serum was used as the source of complement followed by rabbit anti-human serum C3 fluorescein isothiocyanate-conjugated (FITC) (Behringwerke, Marburg, Germany), as previously described [20].

Classes of ICA

According to titres and behaviour of ICA during follow-up, patients were arbitrarily ranked into three classes.

Class I included patients who, during follow-up, had ICA values mainly 5 JDF-U or more, or who persistently seroconverted for ICA beyond this level. This class was further split in two subclasses, if ICA were detectable mainly at middle (10–40 JDF-U, *Subclass IA*) or high (≥ 80 JDF-U, *Subclass IB*) titres.

Class II included patients with ICA mainly between 2.5 and 5 JDF-U. Fluctuating ICA were considered as those with values moving repeatedly above and below the threshold of 2.5 JDF-U.

Class III included patients who permanently remained ICA-negative (< 2.5 JDF-U). Patients who developed a seropositivity for ICA during follow-up, i.e. those moving from ICA values less than 2.5 JDF-U to more than 5 JDF-U, and who persistently held such a positivity, were transferred from ICA *Class III* into *Class I*.

ICA pattern

Forty-two patients with persistent ICA of 5 JDF-U or more were further investigated to identify their ICA pattern by a four-layer immunofluorescence technique according to Genovese et al. [22]. All serum samples were retested by the same technique after incubation with rat brain homogenates [22].

Metabolic study

ICA-positive patients underwent an oral glucose tolerance test (OGTT) at the start of the study, and periodically during ICA evaluations, by plasma glucose assessment at 0 and 120 min, according to the criteria of the National Diabetes Data Group [24]. ICA-negative patients underwent OGTT at the beginning of their investigation, and were retested for basal glycaemia at the time of their ICA determination.

Genetic study

Fifty-nine OSAD patients, 35 ICA-positive and 24 ICA-negative, were typed for HLA-DR locus by a standard microlymphocytotoxicity technique.

Statistical analysis

Differences between the various groups and subgroups of subjects were evaluated by contingency tables (chi-square test) with Yates' correction for continuity if the number in any expected class was five or less. Fisher's exact test was employed if any class was zero. Actuarial survival rates, according to the Cutler-Ederer method [25], were adopted to estimate the likelihood of progression toward diabetes. All patients entered the life-table when ICA were first investigated in our laboratory. The follow-up ended when diabetes developed, which was referred to as the event of interest, or when ICA were last detected for non-diabetic individuals. The results of survival analysis were plotted drawing curves of "cumulative risk of morbidity" (CR), at 11 years, regarding ICA status. The log-rank statistic was used to compare the estimates between the selected categories [26]. If two groups did not show the same length of observation time when compared, the significance level was calculated at the last time-interval common to both the groups considered. The non-parametric Mann-Whitney U test was additionally employed to evaluate differences between curves, and a two-tailed p value of 0.05 was considered to indicate statistical significance. The annual incidence of diabetes in the cohorts of interest was calculated by dividing the number of diabetic patients by the patient years of follow-up. Each patient contributed to the sum of patient years a period (in months) from the beginning of their observation until diabetes was diagnosed or the observation ended. Sampling errors and 95% confidence intervals (CI) were calculated.

Results

ICA in OSAD patients

Of 3,042 subjects with OSAD, 110 (3.6%) had detectable ICA ($\geq$ 2.5 JDF-U) in their sera. OGTT, initially performed on 64 ICA-positive non-selected patients, revealed a normal glucose tolerance (NGT) in 57, an impaired glucose tolerance (IGT) in 3, a previous abnormal glucose tolerance (Prev-AGT) in 2, and diabetes in 2 individuals who were then excluded from the study. The 7 patients who seroconverted during follow-up, and arising from *Class III*, were added to the initial 62 cases. So, the final group with persistent ICA included 69 subjects (Fig. 1).

Fifty-eight were females and 11 males (female/male ratio (F/M) = 5.3), with a mean age of 40.6 years (range 6–71) (22 with Graves' disease, 3 with Graves' disease and vitiligo, 2 with Graves' disease and type A chronic atrophic gastritis, 25 with Hashimoto's thyroiditis or idiopathic myxoedema, 1 with Hashimoto's thyroiditis and type A chronic atrophic gastritis, 5 with vitiligo, 3 with type I autoimmune polyendocrine syndrome (APS), 3 with type II APS, 2 with idiopathic Addison's disease, 2 with alopecia areata, and 1 with type A chronic atrophic gastritis).

In all the 116 OSAD patients without ICA (i.e., < 2.5 JDF-U), the OGTT initially performed showed NGT. During periodic testing, 7 patients became ICA positive, and from then were included into *Class I* of ICA. Seroconversion was documented after a mean observation period of 25.2 months (range 3–44). Therefore, the persistently ICA-negative group included 109 OSAD patients (Fig. 1). Ninety-two were females and 17 males (F/M = 5.4), with a mean age of 40 years (range 9–70) (55 with Graves' disease, 25 with Hashimoto's thyroiditis or idiopathic myxoedema, 8 with Addison's disease or with adrenal autoantibodies but without overt hypoadrenalism, 12 with type II APS, 3 with type I APS, 4 with vitiligo, and 2 with alopecia areata).

Classes of ICA, fluctuating ICA, CF-ICA

Of the 69 OSAD patients with ICA of 2.5 JDF-U or more, 46 (66.7%) belonged to *Class I*, and 23 (33.3%) to *Class II*.

Of the 46 *Class I* patients, 20 belonged to *Subclass IA*, and 26 to *Subclass IB*. Of the 20 *Subclass IA* patients, 2 showed fluctuation of ICA, 3 (15%) were persistently positive for CF-ICA, 4 (20%) were occasionally CF-ICA positive, and 13 (65%) were found to be repeatedly negative. Of the 26 *Subclass IB* patients, none showed ICA fluctuation, 22 (84.6%) had persistent CF-ICA, 3 (11.5%) had occasional CF-ICA, and 1 (3.8%) was persistently negative (Table 1).

Of the 23 *Class II* patients, 14 revealed fluctuation of ICA, and none had CF-ICA.

None of the 109 *Class III* patients had CF-ICA.

A significant association was found between persistent high titres of ICA-IgG (*Subclass IB*) and persistence of CF-ICA ($p = 0.001 \times 10^{-2}$, chi-square test).

Table 1. Classes of ICA in OSAD patients: relationship with CF-ICA and progression to diabetes

Classes of ICA	Mean duration of follow-up (months)	*n*	CF-ICA			Progression to diabetes		
			Persistent *n* (%)	Transient *n* (%)	Negative *n* (%)	*n*	Predictive value %	Annual Incidence (CI)
A I	31.5	20	3 (15.0)	4 (20.0)	13 (65.0)	6	30.0	0.114 (0.052–0.248)
B	48.3	26	22 (84.6)[a]	3 (11.5)	1 (3.8)	8	30.8	0.076 (0.038–0.150)
Total	40.7	46	25 (54.3)	7 (15.2)	14 (30.4)	14	30.4[b]	0.090 (0.053–0.150)
II	49.3	23	0	0	23 (100)	0	–	–
III	52.1	109	0	0	109 (100)	3	2.7	0.006 (0.002–0.018)

[a] $p < 0.001$ vs Subclass IA; [b] $p = 0.008$ vs Class II and < 0.0001 vs Class III.
OSAD, organ-specific autoimmune disease; ICA, islet cell antibodies; CF, complement-fixing; CI, confidence interval; Class I, ICA persistently > 5 JDF-U; Subclass IA, ICA 10–40 JDF-U; Subclass IB, ICA ≥ 80 JDF-U; Class II, ICA 2.5–5 JDF-U or fluctuating; Class III, ICA persistently < 2.5 JDF-U

Table 2. CF-ICA and progression to diabetes in OSAD patients

Classes of ICA	CF-ICA status	Mean duration of follow-up (months)	*n*	Progression to diabetes		
				n	Predictive value %	Annual Incidence (CI)
I (A + B)	CF-ICA PP	48.9	25	10	40.0	0.098 (0.053–0.180)
	CF-ICA T/N	31.0	21	4	19.0	0.073 (0.028–0.189)
Total		40.7	46	14	30.4	0.090 (0.053–0.150)
II	CF-ICA PP	–	–	0	–	–
	CF-ICA T/N	49.3	23	0	–	–
III	–	52.1	109	3	2.7	0.006 (0.002–0.018)

CF-ICA, complement-fixing islet cell antibodies; PP, persistently positive; T, transiently positive; N, persistently negative

ICA pattern

Of the 42 subjects evaluated, 17 showed the *whole* and 25 the *selective* pattern of ICA. All sera with the *whole* pattern maintained the same reaction after absorption with rat brain homogenate, while all sera with the *selective* pattern lost this reactivity. The *whole* pattern was found in 82 % of patients with persistently high ICA titres (*Subclass IB*), and in 76 % of those with persistent CF-ICA. These associations, however, were not statistically significant ($p = 0.09$ and $p = 0.20$, respectively).

Classes of ICA and progression to diabetes (Table 1)

During follow-up 17 patients with OSAD developed diabetes. Of the 46 *Class I* patients, 14 (30.4 %) developed diabetes, with a mean latency period of 36.6 months (range 2–129) (annual incidence (AI) = 0.090; CR = 70 %, CI 56–84) (Table 1, Fig. 2a). In particular, diabetes occurred in 6 patients (30 %) of *Subclass IA* (AI = 0.114; CR = 76 %, CI 38–114), and in 8 (30.8 %) of *Subclass IB* (AI = 0.076; CR = 62 %, CI 48–76) (Table 1, Fig. 2b). No significant difference was shown in the cumulative risk for diabetes between *Subclass IA* and *IB*. In general, the ICA titres in patients developing the disease did not vary more than two dilutions with respect to the initial antibody detection. After the onset of diabetes, ten patients were further followed-up for a mean period of 3.5 years. Five patients became ICA-negative, three showed a decrease in ICA titres, while two maintained the same ICA trend. None of the 23 *Class II* patients developed diabetes. Of the 109 patients belonging to *Class III*, 3 (2.7 %) developed diabetes after a mean observation period of 11.7 months (range 7–15) (AI = 0.006; CR = 4 %, CI 0–8). The cumulative risk for diabetes in *Class I* patients was significantly increased if compared

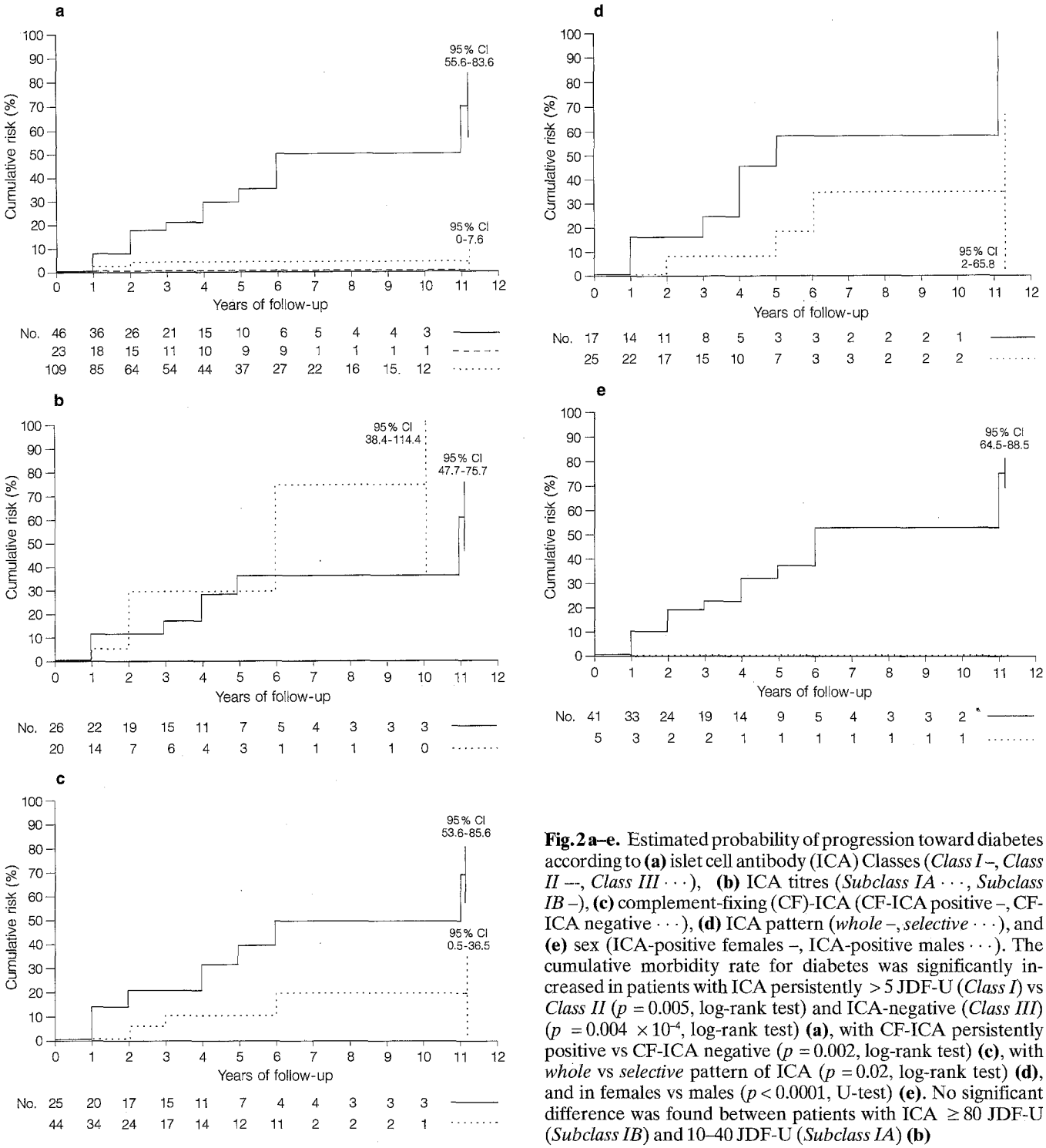

Fig. 2 a–e. Estimated probability of progression toward diabetes according to **(a)** islet cell antibody (ICA) Classes (*Class I* –, *Class II* --, *Class III* · · ·), **(b)** ICA titres (*Subclass IA* · · ·, *Subclass IB* –), **(c)** complement-fixing (CF)-ICA (CF-ICA positive –, CF-ICA negative · · ·), **(d)** ICA pattern (*whole* –, *selective* · · ·), and **(e)** sex (ICA-positive females –, ICA-positive males · · ·). The cumulative morbidity rate for diabetes was significantly increased in patients with ICA persistently > 5 JDF-U (*Class I*) vs *Class II* ($p = 0.005$, log-rank test) and ICA-negative (*Class III*) ($p = 0.004 \times 10^{-4}$, log-rank test) **(a)**, with CF-ICA persistently positive vs CF-ICA negative ($p = 0.002$, log-rank test) **(c)**, with *whole* vs *selective* pattern of ICA ($p = 0.02$, log-rank test) **(d)**, and in females vs males ($p < 0.0001$, U-test) **(e)**. No significant difference was found between patients with ICA ≥ 80 JDF-U (*Subclass IB*) and 10–40 JDF-U (*Subclass IA*) **(b)**

both to *Class II* ($p = 0.005$) and *Class III* ($p = 0.004 \times 10^{-4}$, log-rank test) (Table 1, Fig. 2 a).

CF-ICA and progression to diabetes (Table 2)

According to CF-ICA status within *Class I*, the onset of diabetes was found in 10 of 25 (40%) patients with persistent CF-ICA (AI = 0.098; CR = 70%, CI 54–86), compared to 4 of 21 (19%) with transient or negative CF-ICA (AI = 0.073; CR = 50%, CI 6–94). The risk for diabetes in patients with persistent CF-ICA was significantly higher than that of patients without or with occasional CF-ICA belonging to *Class I* and *II* (i.e. with ICA ≥ 2.5 JDF-U or fluctuating) (AI = 0.026; CR = 18.5%, CI 0.5–36.5) ($\chi^2 = 5.086$, $p = 0.02$, log-rank test) (Fig. 2 c). In *Subclass IA*, diabetes occurred in 3 of 3 (100%) patients with persistent CF-ICA (AI = 0.363,

CI 0.122–1.019; CR = 100 %), and in 3 of 17 (18 %) with transient or negative CF-ICA (AI = 0.067, CI 0.023–0.197; CR = 60 %, CI 31–89) ($\chi^2 = 3.12$, p = NS, log-rank test; $p = 0.006$, U-test). Within *Subclass IB*, diabetes developed in 7 of 22 (32 %) patients with persistent CF-ICA (AI = 0.075, CI 0.036–0.156; CR = 61 %, CI 47–75), compared to 1 of 4 (25 %) with transient or negative CF-ICA (AI = 0.081, CI 0.014–0.459; CR = 40 %, CI 9–71) (p = NS, log-rank and U-test).

ICA pattern and progression to diabetes

Of the 42 patients evaluated, 12 developed diabetes and 30 had NGT.

Of the 12 patients who developed diabetes, 8 (67 %) showed the *whole*, and 4 (33 %) the *selective* ICA pattern. Of the 30 patients who did not develop diabetes, 9 (30 %) had the *whole* and 21 (70 %) the *selective* pattern. Thus, diabetes occurred in 8 of 17 (47 %) patients with the *whole* pattern (AI = 0.132, CI 0.067–0.261; CR = 100 %), compared to 4 of 25 (16 %) patients with the *selective* pattern (AI = 0.042, CI 0.016–0.107; CR = 34 %, CI 2–66) ($\chi^2 = 4.710$, $p = 0.02$, log-rank test) (Fig. 2 d).

Six patients developed Type 1 diabetes with acute onset, while six had a slower onset. The *whole* pattern was found in five of the six (83 %) patients in whom diabetes presented acutely, with respect to three of the six (50 %) in whom diabetes presented as non-insulin requiring.

Sex and progression to diabetes

Of the 46 *Class I* patients, 41 were females and 5 males. All 14 patients who developed diabetes were females ($p < 0.0001$, U-test) (Fig. 2 e). Of 109 *Class III* patients, 92 were females and 17 males. The 3 patients who developed diabetes were females.

Family history of Type 1 diabetes in OSAD patients and in the patients with Type 1 diabetes

A family history for Type 1 diabetes was found in six (3 %), and for Type 2 (non-insulin-dependent) diabetes in nine (5 %) of our OSAD patients.

Of the 180 patients with newly-diagnosed Type 1 diabetes, 17 (9 %) were found to have one or more first degree relatives with Type 1 and 10 (5.5 %) with Type 2 diabetes. One diabetic patient had a family history of both Type 1 and Type 2 diabetes (0.5 %).

HLA-DR in OSAD patients

Of 59 OSAD patients typed for HLA, 31 (52.5 %) had DR3 and/or DR4 (17 DR3, 6 DR4, and 8 DR3/DR4). Specifically, 18 of 35 patients (51.4 %) with ICA had DR3 and/or DR4 (9 DR3, 3 DR4, and 6 DR3/DR4), while 17 had other DR haplotypes. Thirteen of 24 patients (54.2 %) without ICA had DR3 and/or DR4 (8 DR3, 3 DR4, and 2 DR3/DR4), while 11 had other haplotypes. No differences were observed in the prevalence of DR3 and/or DR4 between OSAD patients with or without ICA, whereas the prevalence of both DR3 and DR4 in OSAD patients was significantly increased with respect to the background population (DR3 = 19 %, and DR4 = 6 % in 134 normal control subjects) ($p = 0.0007$ and $p = 0.0005$, respectively).

HLA-DR and progression to diabetes

Fifteen of the 17 patients who developed diabetes were typed for HLA-DR, 10 (67 %) having DR3 and/or DR4 (5 DR3, 3 DR4, and 2 DR3/DR4), and 5 showing other DR haplotypes. Of 44 patients investigated for HLA-DR who did not progress to diabetes, 21 (48 %) had DR3 and/or DR4 (12 DR3, 3 DR4, and 6 DR3/DR4), while the remaining patients had other haplotypes. Despite the fact that two thirds of the patients who later developed diabetes had DR3 and/or DR4, no statistical difference was found when they were compared to OSAD patients who had not developed the disease.

HLA-DR and ICA pattern

Twenty-three patients with persistent ICA were evaluated for both ICA pattern and HLA-DR haplotype. Of 11 patients with the *whole* pattern, 7 (64 %) had DR3 and/or DR4, and 4 other haplotypes (none was DR2). Of 12 patients with the *selective* pattern, 4 (33 %) had DR3 and/or DR4, while 8 had other haplotypes, 3 of which (25 %) were DR2. Thus, DR3 and/or DR4 showed an increased frequency in sera with the *whole* pattern, although this was not statistically significant ($p = 0.30$).

Clinical features of patients who developed diabetes (Table 3)

Of the 69 ICA-positive patients, 14 progressed to diabetes. Upon entry to the study, 10 had NGT, 3 IGT, and 1 Prev-AGT, and all were females. Thus, the prevalence of diabetes in females was 34 % compared to 0 % in males. Three belonged to the group of the seven subjects who had seroconverted for ICA, so that the prevalence of diabetes in such individuals was 43 %. Diabetes occurred after a mean observation period of

Table 3. Immunologic, genetic and metabolic features in ICA-positive (patients 1–14) and ICA-negative (patients 15–17) OSAD patients who developed diabetes during follow-up

Patient	Sex	Pre-existing autoimmune diseases	Family history Type 1 diabetes	Family history Type 2 diabetes	Class of ICA	CF-ICA	ICA pattern	HLA-DR	OGTT at entry	Latency before Type 2 diabetes (months)[a]	Latency before Type 1 diabetes (months)[a]	Age of onset (years)
1	F	HT	+	–	IA	PP	n.t.	n.t.	NGT	–	24	36
2	F	GD	+	–	IB	PP	whole	1/5	IGT	–	6	64
3	F	GD	–	–	IA	PP	whole	3/5	IGT	–	3	30
4	F	HT-MG	–	–	IB	PP	whole	3/7	NGT	–	47	31
5	F	Vitiligo	–	–	IB	PP	whole	3/4	NGT	–	129	27
6	F	GD	–	–	IB	PP	n.t.	4/6	IGT	–	2	9
7	F	HT	–	–	IB[b]	PP	whole	3/4	NGT	–	46	38
8	F	GD	–	–	IA	N	selective	4/7	Prev-AGT	–	20	45
9	F	GD	–	–	IA	PP	whole	n.t.	NGT	60[c]	–	74
10	F	GD	–	–	IB	PP	selective	6/7	NGT	56	88	35
11	F	HT	–	+	IA[b]	T	selective	3/9	NGT	21	41	38
12	F	HT	–	–	IB	T	whole	3/x	NGT	28	42	28
13	F	GD	–	+	IA[b]	N	selective	2/x	NGT	63	–	58
14	F	APS II	–	–	IB	PP	whole	2/3	NGT	8	22	46
15	F	GD	–	–	III	N	–	2/4	Prev-AGT	–	7	33
16	F	Vitiligo	–	–	III[d]	N	n.t.	5/7	NGT	–	13	41
17	F	HT	–	–	III	N	–	5/8	NGT	15	63	53

[a] Period of observation before the onset of diabetes.
[b] Seroconverted for ICA before the onset Type 1 diabetes.
[c] Deceased.
[d] Seroconverted for ICA at the onset of Type 1 diabetes.
HLA, Human leucocyte antigens; OGTT, oral glucose tolerance test; HT, Hashimoto's thyroiditis; GD, Graves' disease; MG, myasthenia gravis; APS II, type II autoimmune polyglandular syndrome; AD, Addison's disease; PP, persistently positive; T, transiently positive; N, persistently negative; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; Prev-AGT, previous abnormal glucose tolerance; n.t., not tested

36.6 months (range 2–129) at a mean age of 40.3 years (range 9–74). Two patients had a family history of Type 1 diabetes, and two of Type 2 diabetes. In eight patients the disease presented acutely as insulin-dependent. The onset was non-acute in six individuals: four required insulin after 14–32 months of therapy with oral hypoglycaemic agents (OHA) and/or diet (patients no. 10, 11, 12, 14), one is still well-controlled by OHA after 15 months from diagnosis (patient no. 13), while one patient died of myocardial infarction after 20 months from the onset of the disease (patient no. 9). In the three seroconverted patients who developed diabetes (patients no. 7, 11, 13), the disease developed after a mean period of 43.3 months (range 21–63) from seroconversion.

Of the 109 OSAD patients without ICA, three progressed to diabetes after a mean observation period of 11.7 months (range 7–15) (patients no. 15, 16, 17), all were females. In one, the disease appeared as non-insulin-dependent (patient no. 17), and in two as insulin-dependent (patients no. 15, 16). Patient no. 17 required insulin therapy after 48 months. None had a family history of Type 1 or Type 2 diabetes. Patient no. 16 was found to be ICA positive at the onset of diabetes, having shown a negative ICA test 1 year before.

Of the remaining 32 patients belonging to *Class I*, all maintained NGT during follow-up. One 17-year-old male with type I autoimmune polyendocrine syndrome, with persistently high titres of ICA, CF-ICA, *selective* ICA-pattern, and DR5/DR7 HLA-haplotype, died after 121 months of follow-up from cerebral haemorrhage during a hypertensive crisis. He was also suffering from renal insufficiency. Histological investigation of his pancreas did not show any sign of lymphocytic insulitis [27].

Discussion

Over the past 10 years many prospective studies have been carried out on selected populations at risk for diabetes in order to characterise and, possibly, quantify immunological and genetic factors conferring susceptibility. Investigation of patients with autoimmune endocrinopathies is important because of the polyglandular involvement in their disease. However, only few studies are available thus far [20, 21]. The present investigation, which follows the recent "polyendocrine study" carried out in southern England [21], reports more extensive data about a prospective Italian study which began in 1976.

During screening, 3.6% of patients with OSAD had ICA of 2.5 JDF-U or more, and about two-thirds of them showed persistent ICA levels of more than 5 JDF-U. A group of patients showed fluctuations in ICA status, but they belonged almost exclusively to those with the lowest ICA titres. Thus, fluctuation seems more related to methodological variability than to actual modifications in ICA amounts. High ICA titres were strictly correlated with the ability to fix complement, confirming that

CF-ICA are likely to be the expression of high levels of ICA-IgG. After 11 years of follow-up, the predictive value for diabetes in OSAD patients having ICA persistently greater than 5 JDF-U was 30%, with an estimated actuarial risk of 70%. In the polyendocrine study, Bosi and colleagues [21] found a positive predictive value of 20%, with a cumulative risk of 41% after 10 years. Our elevated actuarial estimate at 11 years may be partly due to the smaller number of patients available for complete evaluation over the last years of follow-up, and to the bias introduced by the assumption that the future experiences of subjects lost to follow-up are similar to those remaining under observation. However, both the studies suggest that OSAD patients are generally less prone to develop diabetes if compared to FDR of Type 1 diabetic probands, where the predictive value ranges from 25 to 75% [14, 15, 28], or to identical twins, where the predictive value rises to 77% [12]. Such differences for the development of diabetes in FDR have been correlated to HLA identity or haploidentity with the diabetic proband [13].

The lower progression toward diabetes in our OSAD patients compared to FDR may be explained by the fact that a family history of Type 1 diabetes was ascertained in only 3%. This concept is supported by the observation that ICA-positive OSAD patients with a family history of Type 1 diabetes develop the disease at a similar rate to ICA-positive FDR [21]. These findings also suggest that, in one individual with ICA, a family history of Type 1 diabetes confers more risk for the future onset of the disease than the presence of organ-specific autoimmunity. If this is the case, the coincidence of OSAD and family history of Type 1 diabetes in one person would not provide more risk to develop diabetes than in one FDR without OSAD.

Considering three different studies carried out on the general population (schoolchildren), the cumulative predictive value of ICA has been estimated to be about 10% [17–19]. Thus, ICA confer a declining risk for diabetes in identical twins, FDR, OSAD patients, and schoolchildren, respectively.

We did not find a significant difference in the risk for diabetes between middle and high ICA titre in our patients. These data agree with those recently reported by Bosi et al. [21], but they differ substantially from that observed in FDR, where ICA levels are proportionally related to the risk of progression to diabetes [15, 28].

A positive test for CF-ICA was an additional predictor of Type 1 diabetes if the ICA titres were generally 2.5 JDF-U or more, but not if they were persistently more than 80 JDF-U. Thus, the determination of CF-ICA is advantageous only when ICA are less than 80 JDF-U.

At the end of follow-up, diabetes had occurred in 11 patients with persistent ICA, in three who seroconverted for ICA during the observation period, and in three without ICA. However, one of the ICA-negative individuals was found to be positive at the onset of the disease. This patient might have been previously identified as positive if shorter antibody detection intervals had been performed.

It has recently been demonstrated that conventional ICA are heterogeneous, with at least two subsets which exhibit different patterns by standard indirect immunofluorescence [22]. One, known as *whole* or *non-restricted,* homogeneously stains all endocrine cells of pancreatic islets, while the other, known as *selective* or *restricted*, stains only beta cells. The target antigen of the *whole* ICA pattern has not yet been identified. By contrast, the *selective* pattern is due to an anti-glutamic acid decarboxylase (GAD) antibody, which is completely abolished by preincubation with tissues rich in GAD [22]. Preliminary observations indicate that the *whole* pattern is more strongly related to the onset of diabetes both in FDR [23] and in OSAD patients [22]. In the present study we demonstrated that the *whole* ICA pattern significantly increases the probability of developing diabetes with respect to the *selective* pattern. Thus, the detection of the *whole* pattern appears to be the immunological marker which confers the highest risk of progression to diabetes in OSAD patients. In addition, the *whole* pattern was demonstrated in most patients who developed Type 1 diabetes acutely (83%), while it was found in 50% of those in whom the progression to Type 1 diabetes was slow, i.e. preceded by a transient non-insulin-requiring stage. These data suggest that the identification of the *whole* or the *selective* pattern in ICA-positive patients provides further important information not only about the magnitude of the risk but also on the clinical presentation of the diabetic state.

In this cohort of OSAD patients, the prevalence of DR3 and/or DR4 was significantly increased with respect to the general population, and this could account for the lack of correlation found between these haplotypes and susceptibility to diabetes. Interestingly enough, patients with the *whole* ICA pattern have an increased prevalence of DR3 and/or DR4 when compared to those with the *selective* pattern, but these differences were not statistically significant, probably due to the small sample of patients available. In this regard, it has been recently reported that in FDR the presence of the *restricted* ICA pattern (which confers a protective effect) is related to DR2 haplotype [23]. On the basis of these data, it might be speculated that the production of specific ICA subsets are under genetic control also in OSAD patients.

The present study reveals many differences in the natural history of Type 1 diabetes between OSAD patients and FDR with ICA. All our OSAD patients who developed diabetes were females, the mean age at onset was 40 years, and the presentation of Type 1 diabetes was often slow, preceded by a variable period of non-insulin dependence. By contrast, in FDR the disease afflicts both males and females, and has an acute onset between ages 0–20 years.

In conclusion, we found that in OSAD patients the *whole* pattern of ICA is a stronger indicator for future development of overt diabetes than any serological marker which has been investigated, thus confirming similar findings reported in other susceptible populations. The definition of more reliable predictors for Type 1 diabetes represents an essential prerequisite in the selection of those individuals at a very high risk for progressive beta-cell failure and, hopefully, will have a considerable influence on the management of the prediabetic period.

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