



Original article

Predictors of microvascular complications in type 1 diabetic patients at onset: The role of metabolic memory

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ABSTRACT

Background: Several epidemiological studies showed a close association between metabolic control and microvascular complications in type 1 Diabetes Mellitus (T1DM). The aim of our longitudinal observational study was to evaluate the predictive role of the main clinical and biochemical parameters in determining microvascular complications.

Methods: 376 T1DM patients, hospitalized in our division from 1991 to 2005 (mean follow-up = 10.93 ± 4.26 years) were studied. Stepwise Cox regression analysis was used to identify the influence of residual β -cell function, β -cell autoimmunity, HbA1c levels and other clinical and laboratory parameters in the development of microalbuminuria and retinopathy.

Results: The probability of developing microalbuminuria was higher in males than in females (HR 1.82; 95% CI 1.01–3.28; $p = 0.044$), in patients with higher mean HbA1c values (HR 2.80; 95% CI 1.63–4.83; $p < 0.001$), longer duration of disease (HR 1.98; 95% CI 1.10–3.57; $p = 0.022$) and younger age of diabetes onset (HR 0.53; 95% CI 0.03–0.92; $p = 0.026$). An increased probability of developing retinopathy was found in patients with higher mean HbA1c levels during follow-up (HR 2.35; 95% CI 1.34–4.12, $p = 0.003$), as well as at onset (HR 1.85; 95% CI 1.06–3.24; $p = 0.030$).

Conclusions: Our study suggests that among the clinical, metabolic, immunological and biochemical factors evaluated at onset, only HbA1c is predictive for the microangiopathy development in T1DM.

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1. Introduction

Several epidemiological studies have shown a close association between metabolic control and microvascular complications in type 1 Diabetes Mellitus (T1DM) [1–4]. In particular, the Diabetes Control and Complications Trial (DCCT) has emphasized that optimization of blood glucose levels with intensive insulin treatment reduces the development and progression of microvascular complications [5,6]. Concerning micro- and macroalbuminuria, the cumulative prevalence in T1DM patients is 34% after about 18 years of diabetes, regardless of age at onset [7], while cumulative prevalence of microalbuminuria in patients with childhood onset is 12–25% after 5–10 years of disease [8–12]. As regards retinopathy, the 2000 ADA recommendations suggest that vision-threatening retinopathy does not occur during the first 3–5 years, although it is developed by almost all T1DM patients about 20 years

after onset. By contrast, the WESDR study (conducted on 137 patients) reported 49% positive findings for retinopathy when diabetes duration is ≤ 4 years [13]. These results were further confirmed by DCCT (1613 patients) which showed a percentage of retinopathy of at least 67.1% with diabetes duration < 5 years [5]. Concerning residual β -cell function, the Diabetes Prevention Trial-1 (DPT-1) indicated that a decrease in insulin secretion occurs from pre-diabetes to clinical onset [14,15]. Fasting C-peptide is usually maintained at diagnosis [16,17]. In addition, DCCT reported that a stimulated C-peptide level of ≥ 0.2 pmol/ml was correlated with improved metabolic control and a decreased rate of complications when compared to patients with C-peptide levels below this threshold [18]. Stimulated C-peptide levels > 0.2 pmol/ml were found in 33% of subjects < 18 years of age and in 48% of subjects > 18 years of age with a disease duration of < 5 years. As regards autoimmunity, several cross-sectional studies indicated absence of correlation between diabetes-associated autoantibodies and long-term development of microvascular complications [19,20]. The aim of this longitudinal observational study was to evaluate in newly-diagnosed T1DM patients, admitted to our Division of Endocrinology and Metabolic Diseases, the predictive role of residual β -cell function, β -cell specific autoantibodies, HbA1c levels and other clinical parameters regarding the development of microvascular complications.

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2. Methods

Among the 1118 Sicilian patients consecutively hospitalized in our Division from 1991 to 2005 for T1DM, we selected only those with diabetes at onset who were treatment-naïve (376 patients), with specific beta-cell autoimmunity (with or without ketosis or ketoacidosis at onset) or with ketosis or ketoacidosis at onset in the absence of autoimmunity, with weight loss >10% (from at least 6 months) and/or polyuria for more than 6 months before diagnosis. The selected patients [224 males (59.6%) and 152 females (40.4%); aged 20.23 ± 8.47 years (range 2–46)] were longitudinally studied from diagnosis, with a mean follow-up of 10.93 ± 4.26 (range 4–18) years. All patients included had been subsequently treated with conventional basal-bolus insulin therapy. During follow-up, patients underwent at least two medical examinations per year and clinical reports were recorded in our case files. Specifically, we extrapolated the following data: gender, age at onset, duration of disease, use of tobacco (current or former smoker/never smoker), body mass index (BMI) at onset and after 1 year, mean systolic and diastolic blood pressures, fundus oculi and intraocular pressure (tonometry), microalbuminuria, Glucagon Stimulated C-peptide (GSC-pep) test at onset and at year 1, insulin requirement (unit/kg body weight) at onset and at year 1, HbA1c (%)

at onset and at year 1, mean HbA1c, pancreatic autoimmunity [GAD antibodies (GADA), IA-2 antibodies (IA-2A) and insulin autoantibodies (IAA)]. IAA were determined at onset, while GADA and IA-2A at onset and after 1 year. Mean values of HbA1c, and systolic and diastolic blood pressures were calculated during the entire follow-up period (for patients without complications up to 2009) or until the onset of microalbuminuria and/or diabetic retinopathy (for patients with complications). The Institutional Review Board of the University of Palermo approved the study and patients gave informed consent for recording and scientific use of their data.

2.1. Laboratory analysis

Routine chemistry/hematology blood analyses were performed on patients in external laboratories. HbA1c, C-peptide, pancreatic autoantibodies and microalbuminuria were assessed in our centralized laboratory. HbA1c was determined by means of High Pressure Liquid Chromatography (HPLC) with ion-exchange resin (HA8121, Hi-AutoA1c, Menarini, Italy). Serum C-peptide was measured by ELISA (DRG C-peptide ELISA, Germany). The conversion factor for the International System (ng/dl versus nmol/L) was 0.33. All subjects were evaluated for pancreatic autoimmunity at onset and at month

Table 1
Characteristics of T1DM patients grouped on the basis of age at onset tertiles.

	<15 years	15–23 years	>23 years	Total
	No 125	No 126	No 125	No 376
Age at onset (yrs)*	11.24 ± 3.42	19.13 ± 2.09	30.32 ± 5.46	20.23 ± 8.47
Duration of disease (yrs)*	12.02 ± 4.25	10.88 ± 4.22	9.90 ± 4.08	10.93 ± 4.26
Gender				
Male	81 (64.8)	67 (53.2)	76 (60.8)	224 (59.6)
Female	44 (35.2)	59 (46.8)	49 (39.2)	152 (40.4)
BMI at onset (kg/m ²)*	19.93 ± 2.87	21.60 ± 2.50	23.04 ± 2.52	21.52 ± 2.92
BMI at 1 year (kg/m ²)*	20.30 ± 2.59	21.98 ± 2.17	23.16 ± 2.83	21.81 ± 2.79
Positive autoantibodies at onset				
GADA	76 (60.8)	92 (73)	85 (68)	253 (67.3)
IA-2A^a	52 (41.6)	62 (49.2)	40 (32)	154 (40.9)
IAA^b	37/115 (32.2)	26/102 (25.5)	14/94 (14.9)	77/311 (24.8)
Positive autoantibodies 1 at 1 year				
GADA^c	64 (51.2)	85 (67.5)	74 (59.2)	223 (59.3)
IA-2A^d	50 (40)	59 (46.9)	37 (29.6)	146 (38.8)
Glucagon-stimulated C-peptide (nmol/L) at onset				
0'	0.22 ± 0.19	0.28 ± 0.24	0.24 ± 0.23	0.25 ± 0.22
1'	0.35 ± 0.27	0.45 ± 0.40	0.41 ± 0.49	0.41 ± 0.40
3'	0.38 ± 0.28	0.47 ± 0.40	0.45 ± 0.53	0.43 ± 0.42
6'	0.40 ± 0.33	0.50 ± 0.49	0.42 ± 0.42	0.44 ± 0.42
10'	0.34 ± 0.29	0.38 ± 0.27	0.44 ± 0.59	0.39 ± 0.41
15'	0.36 ± 0.26	0.48 ± 0.51	0.42 ± 0.46	0.42 ± 0.43
Glucagon-stimulated C-peptide (nmol/L) at 1 year				
0'	0.26 ± 0.14	0.25 ± 0.16	0.23 ± 0.15	0.25 ± 0.15
1'	0.44 ± 0.23	0.46 ± 0.33	0.43 ± 0.31	0.44 ± 0.29
3'	0.48 ± 0.25	0.46 ± 0.23	0.47 ± 0.28	0.47 ± 0.25
6'	0.59 ± 0.40	0.56 ± 0.40	0.59 ± 0.48	0.58 ± 0.43
10'	0.61 ± 0.44	0.55 ± 0.37	0.56 ± 0.38	0.57 ± 0.40
15'	0.53 ± 0.25	0.58 ± 0.47	0.52 ± 0.27	0.55 ± 0.34
AUC _{GSC-pep} 15 min at onset	5.48 ± 4.00	6.70 ± 5.28	6.44 ± 6.87	6.21 ± 5.52
AUC _{GSC-pep} 15 min at 1 year	8.21 ± 4.51	7.93 ± 4.98	7.92 ± 4.98	8.02 ± 4.82
Insulin requirement at onset (U/kg)*	0.60 ± 0.28	0.47 ± 0.21	0.34 ± 0.18	0.47 ± 0.25
Insulin requirement at 1 year (U/kg)*	0.51 ± 0.25	0.44 ± 0.25	0.32 ± 0.18	0.42 ± 0.24
HbA1c (%) at onset	9.36 ± 2.10	9.48 ± 2.26	9.27 ± 2.29	9.37 ± 2.21
HbA1c (%) at 1 year*	7.70 ± 1.68	7.40 ± 1.55	6.82 ± 1.58	7.31 ± 1.64
Mean HbA1c (%) before the onset of complications or of the entire disease	8.14 ± 1.31	7.69 ± 1.25	7.62 ± 1.17	7.82 ± 1.27
Presence of diabetic retinopathy	17 (13.6)	19 (15.1)	14 (11.2)	50 (13.3)
Presence of microalbuminuria	18 (14.4)	16 (12.7)	22 (17.6)	56 (14.9)
Diabetic retinopathy onset* (semester from diagnosis) ^e	22.74 ± 8.25	20.44 ± 7.96	18.67 ± 7.56	20.61 ± 8.08
Microalbuminuria onset* (semester from diagnosis) ^e	22.13 ± 8.84	20.41 ± 8.52	17.91 ± 7.91	20.15 ± 8.58

Data are presented as mean ± SD for quantitative variables and No (%) for categorical variables.

* Significant differences among the 3 groups (Univariate ANOVA; p < 0.001).

^a Percentages differ significantly (χ^2 test, p = 0.021).

^b Percentages differ significantly (χ^2 test, p = 0.015).

^c Percentages differ significantly (χ^2 test, p = 0.032).

^d Percentages differ significantly (χ^2 test, p = 0.019).

^e Six-month period.

12. GADA and IA-2A measurements were performed with radio-immunologic test (RIA) (Adaltis Kit). Values were considered positive for GADA >4.39 relative units (RU) and for IA-2A >0.81 RU. For IAA measurement we used a custom-made RIA test. Cut-off values were based on the 99th percentile of more than 40 Sicilian normal subjects, aged 12–30 years. Measurements were validated by our participation in the Diabetes Autoantibody Standardization Program (DASP), which showed sensitivity of 87% and specificity of 96% for GADA assay, sensitivity of 83% and specificity of 100% for IA-2A assay and sensitivity of 79% and specificity of 100% for IAA.

Microalbuminuria was assessed by immunoturbidimetric assay (Array Protein System 360, Beckman, USA) on a 24-hour urine collection.

2.2. GSC-pep test

Pancreatic β -cell function was assessed in all patients by GSC-pep test at diagnosis (after correction of ketosis or ketoacidosis and stabilization of blood glucose levels) and at year 1. Basal and stimulated C-peptide were performed in the morning after an overnight fasting period of 8–10 h. Patients took no short-acting insulin for at least 6 h and bedtime long-acting insulin at least 12 h before the test. The test was performed only if fasting glucose was 72 to 200 mg/dl (4 to 11.1 mmol/L). 1 mg of glucagon (0.03 mg/kg of weight in children, maximum 1 mg) was intravenously injected within 10 s [21]. Samples were drawn at 1, 3, 6, 10 and 15 min after the end of glucagon administration.

2.3. Assessment of microalbuminuria

Microalbuminuria was defined for albumin levels of 30–300 mg in a 24-h urine collection [22]. Assessment was performed routinely every 6–12 months, along with a microbiological urinalysis to exclude infections. The onset was established as the semester in which microalbuminuria was first found positive in two out of three consecutive tests.

2.4. Assessment of diabetic retinopathy

Diabetic retinopathy was classified according to the Diabetic Retinopathy Working Party screening recommendations [23]. The onset was established as the semester in which at least one single red lesion (dot or larger) or bright lesion (hard exudate, cotton wool spot) was observed in at least one eye. Screening was performed once a year and standard retinal photographs were taken.

2.5. Statistical analysis

All data were analyzed using statistical software SPSS Version 17 for Windows. Baseline characteristics were calculated as mean \pm standard deviation (SD) for continuous variables and as rates and proportions for categorical data. The C-peptide area under the curve (AUC) during GSC-pep ($AUC_{GSC-pep\ 15\ min}$) was computed from all timed collections (including the basal one) using the trapezoidal method. Normality of distribution for quantitative data was assessed by the Kolmogorov–Smirnov test. The one-way ANOVA was used for differences in means within more than two groups and by χ^2 -test and Fisher's exact test (when appropriate) for categorical variables. The ANOVA trend analysis and χ^2 -test for trend were used to assess means and proportions of the population characteristics across grouped type 1 diabetic patients on the basis of β -cell function. The Kaplan–Meier survival analysis was used to estimate the probability of developing microalbuminuria and diabetic retinopathy and the log-rank test was performed to compare survival curves. Two forward stepwise Cox proportional-hazard regression models, with p values for entry of covariates into the regression set at 0.10 were generated to investigate the contribution of each covariate in the development of complications (microalbuminuria, and diabetic retinopathy). Mean

HbA1c, systolic and diastolic blood pressures were defined as the mean values calculated from the onset of diabetes to the onset of complications (in patients with complications) or mean values of the entire disease (in patients without complications). Variables were removed from the models for p value >0.10. In the models, the variable for time was the duration of diabetes expressed in semesters and the variables for status were onset of microalbuminuria and diabetic retinopathy, treated as dichotomous categorical values. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Clinical characteristics

Fifty-six subjects (14.9%) out of the total cohort of 376 patients developed microalbuminuria and fifty patients (13.3%) developed diabetic retinopathy. After subdividing the total population into three groups according to disease duration, we found the following prevalence distribution: group 4–8 years [microalbuminuria 11.8% (16/136), retinopathy 3.7% (5/136)]; group 9–13 years [microalbuminuria 5% (6/121), retinopathy 3.6% (8/121)]; group 14–18 years [microalbuminuria 28.6% (34/119), retinopathy 31.1% (37/119)]. Mean age at onset of diabetes was 20.23 ± 8.47 years (range 2–46) and showed Gaussian distribution. When dividing patients into three groups in relation to tertiles of age at diagnosis (<15 years, 15–23 years and >23 years) we did not find any significant difference between BMI at onset and 1 year later, though a trend of progressive increase was seen

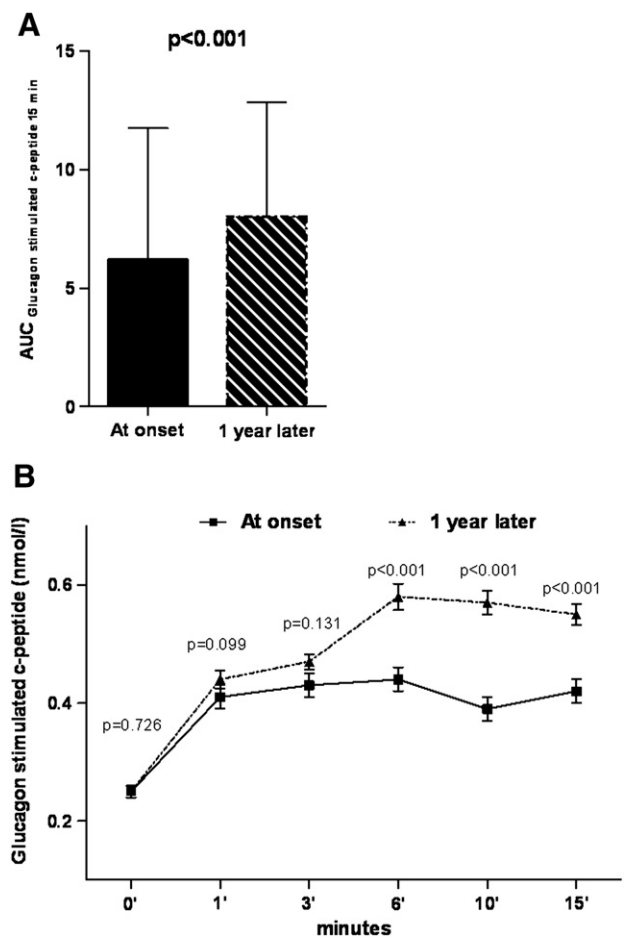


Fig. 1. Beta cell function assessed through GSC-pep test showing significantly higher values at 1 year in comparison to diabetes onset. A. Error bars represent standard deviation (SD) of the means. B. Error bars represent standard error (SE) of the means. Significant differences (paired t-test).

as the tertile increased (Table 1). With regard to GADA, no difference was found at onset between the three groups, while after 1 year the group 15–23 years showed a higher prevalence of positivity (67.5%). The higher prevalence of IA-2A was found in the 15–23 year group, both at onset and after 1 year. IAA at onset were higher in the first tertile. The percentage of negative patients for all the antibodies both at onset and after 1 year was 19.68% (74/376). No difference was found between the three groups concerning C-peptide secretion (GSC-pep, $AUC_{GSC-pep\ 15\ min}$ both at onset and 1 year later). Based on age at diagnosis, the first tertile (younger patients) showed significantly higher levels of insulin requirement and HbA1c both at onset and after 1 year. With regard to microalbuminuria and retinopathy prevalence we found no differences between the three groups (Table 1).

3.2. β -cell function during the first year of disease

A difference in $AUC_{GSC-pep\ 15\ min}$ was found in all 376 patients, with significantly higher values at 1 year in comparison to onset (8.02 ± 4.82 versus 6.21 ± 5.52 ; $p < 0.001$): this difference was not related to GSC-pep values at 0', 1' and 3', but to those at 6', 10' and 15' (Fig. 1). In addition, patients were subdivided on the basis of insulin secretion changes 1 year after diabetes onset calculated as the ratio between $AUC_{GSC-pep\ 15\ min}$ (after 1 year) and $AUC_{GSC-pep\ 15\ min}$ (at onset) ($AUC_{GSC-pep}$ ratio). To identify those subjects who showed deteriorated, unchanged or improved pancreatic β -cell function 1 year after diagnosis, we divided all patients into two groups (those with $AUC_{GSC-pep}$ ratio \leq or > 1). The patients with $AUC_{GSC-pep}$ ratio between 0.81 (equivalent to the 75th percentile of the first group) and 1.32 (equivalent to the 25th percentile of the second group) were defined as

having an unchanged function. We therefore identified three groups (Table 2):

- patients with $AUC_{GSC-pep}$ ratio ≤ 0.81 [Deteriorated β -Cell Function after 1 year (D β CF)];
- patients with $AUC_{GSC-pep}$ ratio > 0.81 and ≤ 1.32 [Unchanged β -Cell Function after 1 year (U β CF)];
- patients with $AUC_{GSC-pep}$ ratio > 1.32 [Improved β -Cell Function after 1 year (I β CF)].

The I β CF group showed no differences in the mean HbA1c levels in comparison to D β CF and U β CF. A significant increasing trend of prevalence of GADA negative patients (D β CF = 8.2%; U β CF = 28.6%; I β CF = 34.8%; $p < 0.001$) and absence of both antibodies (D β CF = 3.3%; U β CF = 17.6%; I β CF = 25%; $p < 0.001$) from onset to a one-year cut-off point was observed among the three groups, while GADA positivity had an inverse trend (D β CF = 73.8%; U β CF = 57.1%; I β CF = 50%; $p = 0.001$). No significant difference was found for IA-2A positivity, BMI and insulin requirement (Table 2).

3.3. Contribution of different clinical and laboratory parameters to the development of microalbuminuria and diabetic retinopathy in multivariate analysis

The variables that showed a p value < 0.10 at log-rank test (Fig. 2) were included in the multivariate analysis. For the development of microalbuminuria the variables not included were GAD and IA2 at 1 year, $AUC_{GSC-pep\ 15\ min}$ at 1 year, C-peptide trend ($AUC_{GSC-pep}$ ratio) during the first year, HbA1c at onset and use of tobacco (current or former smoker). In the final model, the probability of developing

Table 2

Characteristics of T1DM patients grouped on the basis of β -cell function.

	D β CF patients No 61	U β CF patients No 91	I β CF patients No 224	p value for trend
Age at onset (years)	21.75 \pm 8.08	19.71 \pm 8.74	20.03 \pm 8.91	0.281 ^a
Gender				0.279 ^b
Male	35 (57.4)	49 (53.8)	140 (62.5)	
Female	26 (42.6)	42 (46.2)	84 (37.5)	
BMI at onset (kg/m ²)	21.84 \pm 3.28	21.19 \pm 2.54	21.57 \pm 2.96	0.829 ^a
Delta BMI (kg/m ²)	0.31 \pm 1.24	0.45 \pm 1.10	0.21 \pm 1.58	0.372 ^a
$AUC_{GSC-pep\ 15\ min}$ at onset*	13.55 \pm 9.13	7.05 \pm 2.83	3.87 \pm 2.24	<0.001 ^a
$AUC_{GSC-pep\ 15\ min}$ at 1 year*	5.57 \pm 3.95	7.64 \pm 2.61	8.84 \pm 5.45	<0.001 ^a
Positivity of IAA at onset	17/61 (27.8)	20/91 (21.9)	40/224 (17.8)	0.078 ^b
Evolution of GAD autoimmunity (1 year)				
GADA – onset/GADA – 1 year*	5 (8.2)	26 (28.6)	78 (34.8)	<0.001 ^b
GADA + onset/GADA + 1 year*	45 (73.8)	52 (57.1)	112 (50)	0.001 ^b
GADA – onset/GADA + 1 year	4 (6.6)	3 (3.3)	7 (3.1)	0.268 ^b
GADA + onset/GADA – 1 year	7 (11.5)	10 (11)	27 (12.1)	0.844 ^b
Evolution of IA2 autoimmunity (1 year)				
IA2 – onset/IA2 – 1 year	29 (47.5)	48 (52.7)	124 (55.4)	0.281 ^b
IA2 + onset/IA2 + 1 year	24 (39.3)	30 (33)	71 (31.7)	0.297 ^b
IA2 – onset/IA2 + 1 year	1 (1.6)	10 (11)	10 (4.5)	0.975 ^b
IA2 + onset/IA2 – 1 year	7 (11.5)	3 (3.3)	19 (8.5)	0.883 ^b
Number of antibodies (first year of disease)				
No antibodies*	2 (3.3)	16 (17.6)	56 (25)	<0.001 ^b
Only GADA	27 (44.3)	32 (35.2)	68 (30.4)	0.042 ^b
Only IA2	3 (4.9)	10 (11)	22 (9.8)	0.368 ^b
GADA + IA2*	29 (47.5)	33 (36.3)	78 (34.8)	0.098 ^b
IR at onset (U/kg)	0.48 \pm 0.23	0.44 \pm 0.23	0.47 \pm 0.27	0.803 ^a
Delta IR (U/kg)	–0.03 \pm 0.23	–0.06 \pm 0.24	–0.02 \pm 0.23	0.525 ^a
HbA1c at onset	9.45 \pm 2.44	9.63 \pm 2.24	9.24 \pm 2.14	0.311 ^a
Delta HbA1c (%)	–2.10 \pm 2.93	–2.39 \pm 2.80	–1.91 \pm 2.45	0.350 ^a
Mean HbA1c (%) before the onset of complications or of the entire disease	7.92 \pm 1.25	7.78 \pm 1.38	7.80 \pm 1.23	0.633 ^a

D β CF: Deteriorated β -Cell Function after 1 year; U β CF: Unchanged β -Cell Function after 1 year; I β CF: Improved β -Cell Function after 1 year.

Data are presented as mean \pm SD for quantitative variables and No (%) for categorical variables.

^a ANOVA trend analysis.

^b Chi-squared test for trend.

* Significant values when $p < 0.05$.

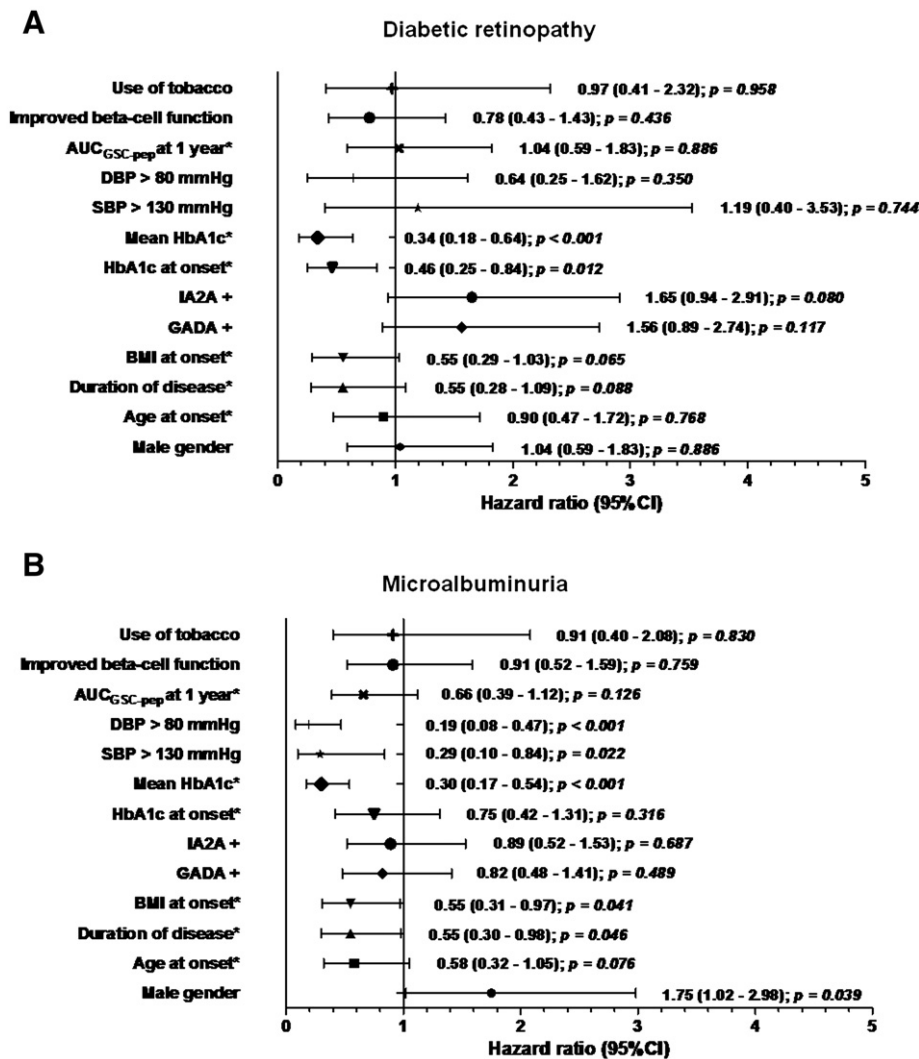


Fig. 2. Univariate analysis of cumulative risk for the development of microvascular complications (Kaplan–Meier survival curves). The variables that showed a p value < 0.10 at log-rank test were included in the multivariate analysis. The variables included for the development of microalbuminuria (A) were diastolic blood pressure (DBP), systolic blood pressure (SBP), mean HbA1c, BMI at onset, duration of disease, male gender, and age at onset. The variables included for the development of diabetic retinopathy (B) were mean HbA1c, HbA1c at onset, BMI at onset, IA2 positivity, and duration of disease. * Dichotomous variables (0 = I and II tertiles; 1 = III tertile).

microalbuminuria was higher in males compared to females (HR 1.82; 95% CI 1.01–3.28; $p=0.044$), in patients with higher mean HbA1c values (HR 2.80; 95% CI 1.63–4.83; $p<0.001$), longer duration of disease (HR 1.98; 95% CI 1.10–3.57; $p=0.022$), younger age of diabetes onset (HR 0.53; 95% CI 0.03–0.92; $p=0.026$) and lower diastolic blood pressure levels (HR 0.48; 95% CI 0.25–0.89; $p=0.021$) (Table 3).

With regard to the development of diabetic retinopathy, the variables not included in the multivariate analysis were age at onset, male gender, GAD at 1 year, AUC_{GSC-pep} 15 min at 1 year, C-peptide trend (AUC_{GSC-pep} ratio) during the first year, mean systolic and diastolic blood pressures and use of tobacco (current or former smoker). The final stepwise proportional hazard model showed an increased probability of developing retinopathy in patients with higher mean (HR 2.35; 95% CI 1.34–4.12; $p=0.003$) and at onset (HR 1.85; 95% CI 1.06–3.24; $p=0.030$) HbA1c levels (Table 3; Fig. 3).

4. Discussion

Our study investigated the occurrence of early microvascular complications in a group of 376 patients with type 1 diabetes. We found that 14.9% of patients developed microalbuminuria and 13.3% developed diabetic retinopathy during a mean follow-up period of

11 years (range 4–18 years). These results are in accordance with DCCT, which reported 10% for retinopathy and 15% for nephropathy over a 10-year period [5], and with previous studies [11,24–28]. With regard to the evaluation of pancreatic reserve, DCCT individuated the cut-off level of 0.2 mmol/L after a mixed meal tolerance test (MMTT) to identify patients with residual β -cell function. In our study we used GSC-peptide as our cohort is based on outpatients' clinical records tracing back to 1991. Since the GSC-pep test does not have any definite cut-off reference point, we evaluated residual β -cell function during the first year as the ratio between AUC_{GSC-pep} at 1 year and at onset, thus arbitrarily identifying D β CF, U β CF and I β CF patients. We observed significant improvement in the pancreatic β -cell function at 1 year in terms of AUC_{GSC-pep} in the entire cohort. It is to be noted that this improvement was mainly due to increased C-peptide values only in the late phases of the test (6', 10' and 15'). This suggests that probably there is no real recovery of β -cell mass, but a phenomenon of reggranulation of β -cells which were temporarily "exhausted" at the time of diagnosis of diabetes [29]. In addition, we cannot rule out the possibility of a non-physiological effect of glucagon on the residual β -cell mass, perhaps not occurring with the more physiological MMTT [30]. This hypothesis is further supported by the observation that the 227 I β CF patients (60.37% of the whole group) did not show any difference with respect to D β CF and U β CF regarding insulin

Table 3

Association of different variables with development of microalbuminuria and diabetic retinopathy using a forward stepwise Cox proportional hazards regression model.

Variables included in the equation	p value	Hazard ratio	95% CI for hazard ratio	
<i>Development of microalbuminuria</i>				
STEP 1				
III tertile of mean HbA1c	<0.001	2.78	1.64	4.72
STEP 2				
DBP <80 mm Hg	0.007	0.42	0.22	0.79
III tertile of mean HbA1c	0.001	2.52	1.47	4.32
STEP 3				
III tertile of disease duration	0.038	1.84	1.03	3.29
DBP <80 mm Hg	0.006	0.42	0.22	0.78
III tertile of mean HbA1c	0.001	2.52	1.47	4.32
STEP 4				
III tertile of age at onset	0.041	0.56	0.32	0.97
III tertile of disease duration	0.026	1.93	1.08	3.47
III tertile of mean HbA1c	<0.001	2.67	1.55	4.58
DBP <80 mm Hg	0.011	0.49	0.24	0.83
STEP 5				
Male gender	0.044	1.82	1.01	3.28
III tertile of age at onset	0.026	0.53	0.03	0.92
III tertile of disease duration	0.022	1.98	1.10	3.57
III tertile of mean HbA1c	<0.001	2.80	1.63	4.83
DBP <80 mm Hg	0.021	0.48	0.25	0.89
Variables not included in the model: GAD and IA2 autoimmunity at 1 year, AUCGSC-pep 15 min at 1 year, C-peptide trend during the first year, HbA1c at onset and use of tobacco (current or former smoker) (each p>0.10).				
<i>Development of diabetic retinopathy</i>				
STEP 1				
III tertile of mean HbA1c	0.001	2.49	1.42	4.35
STEP 2				
III tertile of HbA1c at onset	0.030	1.85	1.06	3.24
III tertile of mean HbA1c	0.003	2.35	1.34	4.12
Variables not included in the model: age at onset, gender (males), GAD at 1 year, AUCGSC-pep 15 min 1 year later, C-peptide trend during the first year, mean systolic and diastolic blood pressures and use of tobacco (current or former smoker) (each p>0.10).				

requirement, BMI and HbA1c levels, and results which could reasonably be expected if real β -cell recovery occurred. Certainly, it could be argued that the AUC_{GSC-pep} ratio does not provide absolute quantitative measurement of the residual β -cell function, but is a mere qualitative evaluation regarding the possible improvement or deterioration trend of the β -cell mass during the whole of the first year. Nevertheless, it is quite intriguing that D β CF patients (18.61% of the whole group) show the lowest rate (2.9%) of antibody negativity and the highest rate of double autoimmunity (GAD + IA-2) (51.4%). This observation confirms that the positivity for multiple diabetes-associated autoantibodies, rather than for the individual antibody, reflects a more severe disease process [31,32].

Concerning the evaluation of determinant factors for the development of microvascular complications, our results confirmed that the mean value of HbA1c from the diagnosis of diabetes to the onset of complication is predictive of both microalbuminuria and diabetic retinopathy. It is noteworthy that higher HbA1c at diagnosis only predicts retinopathy. As regards gender, the probability of developing microalbuminuria was higher in males compared to females (especially in patients with a higher onset age), while no correlations were found with retinopathy. This is in accordance with previous studies which showed higher prevalence of microalbuminuria in men compared with women [33], in particular in subjects with poor control [34]. Whether this is caused by a direct effect of sex hormones on the kidney or on body fat distribution leading to differences in albumin excretion rate remains to be determined [35]. BMI, pancreatic β -cell autoimmunity, basal and GSC-pep did not correlate with microvascular complications, while the duration of diabetes seemed to influence microalbuminuria. Lower diastolic blood pressure levels were associated with microalbuminuria without a cause-effect relationship; this apparently surprising datum can be explained by the confounding statistical effect due to the antihypertensive therapy administered to patients with microalbuminuria as soon as the latter was evidenced, as suggested by the ADA guidelines [22].

With regard to the influence of HbA1c levels, it is well known that the risk of developing microvascular complications is significantly reduced in the intensive versus the conventionally treated group during the first 4 years, despite the similar HbA1c levels over this period (so-called “metabolic memory”) [36]. This long-term influence of early metabolic control on clinical outcomes is supported by ample experimental evidence [37–40]. Surprisingly, our study revealed that the value of HbA1c at diagnosis only affects diabetic retinopathy and not microalbuminuria. This suggests that only retinal endothelium, and not glomerular endothelium, records the “first” glycotoxic insult. Hence, the reported abnormality in the eNOS/NO system of diabetic angiopathy could be an expression of the different behavior in different endothelial districts [41]. The primary role played by HbA1c at the onset of diabetes has recently been corroborated by an International Expert Committee, which suggested that standardized HbA1c values are useful for the diagnosis of diabetes [42]. Similarly, a recent study proposed a 6.6 to 7.0% HbA1c cut-off point for the diagnosis of diabetes, confirming it as the best value for the identification of individuals with mild and moderate retinopathy [43]. In our study, the onset of microvascular complications, both retinal and renal, did not seem to be affected by the pancreatic β -cell reserve expressed by AUC_{GSC-pep} after 1 year. This observation apparently disagrees with several studies reporting that the preservation of β -cell function results in easier and better glycaemic control and fewer end-organ complications, especially retinopathy [44–47]. In particular, the DCCT experience widely demonstrated the clinical benefits of preservation of β -cell function. DCCT described the relative risk of development and progression of retinopathy and nephropathy by comparing baseline responders (mixed-meal-stimulated C-peptide >0.2 mmol/L) versus non-responders (mixed-meal-stimulated C-peptide <0.2 mmol/L) separately within the intensive and conventional treatment groups [48]. However, the beneficial effect of β -cell preservation was only observed in the intensive therapy group, as conventionally treated responders had similar risks for the progression of retinopathy and for the

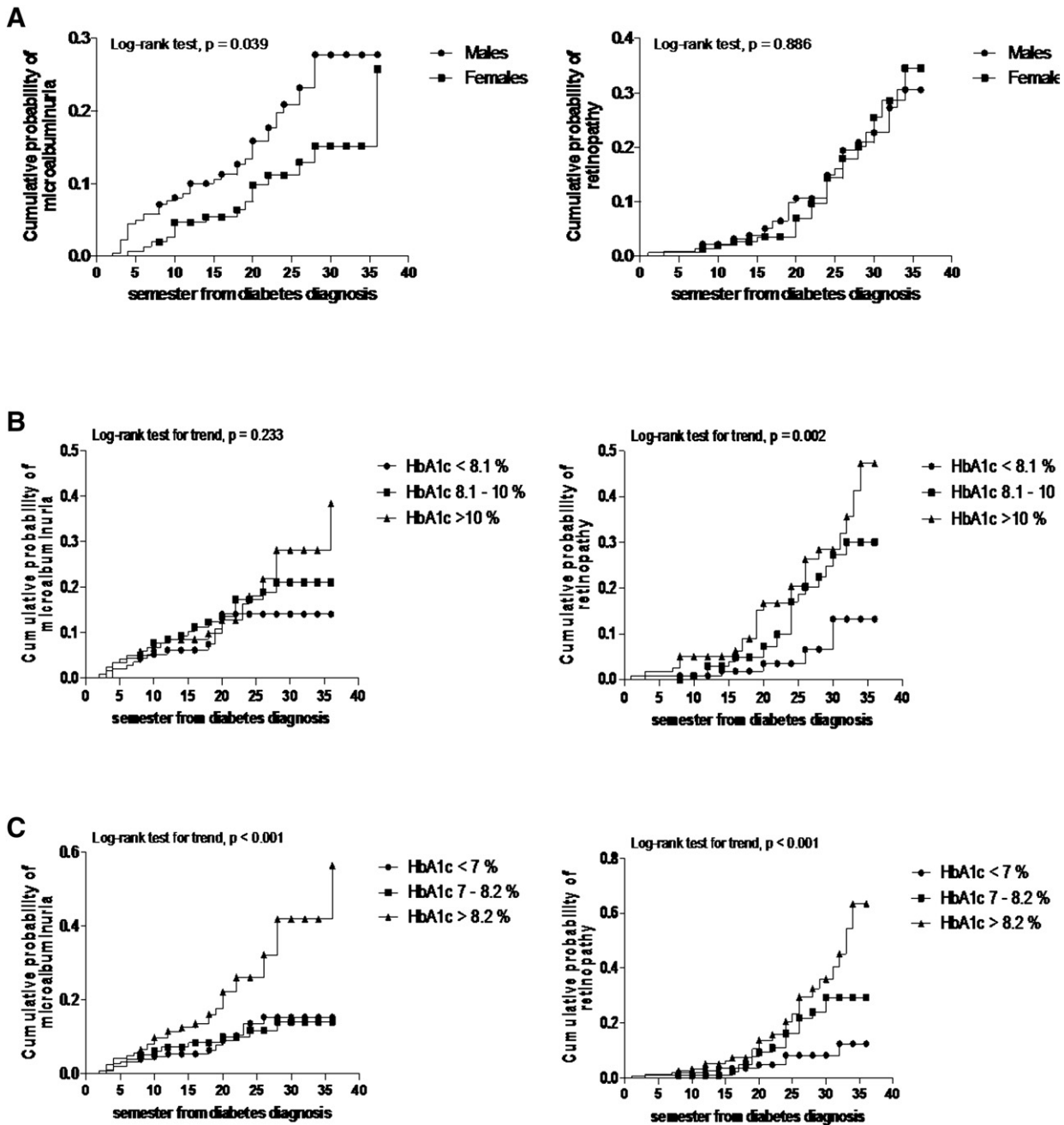


Fig. 3. Cumulative risk (Kaplan–Meier survival curves) for the development of microvascular complications (microalbuminuria, and diabetic retinopathy) for gender (A), for HbA1c at onset (based in tertiles of HbA1c) (B), for mean HbA1c before the onset of complications or of the entire disease (based in tertiles of HbA1c) (C).

development of microalbuminuria. This is therefore in accordance with our results, as our cohort of patients had higher mean HbA1c (7.82%) in comparison to the DCCT patients in the intensive therapy group, and similar mean HbA1c to the conventionally treated group. Furthermore, the multivariate models used in many analyses of the DCCT data used a correction for a lower number of covariates in comparison to our study. This might explain the lack of an independent influence of residual β -cell function on the development of complications found in our study. In our opinion, the independent protective effect of residual β -cell function on the development of microvascular complications in type 1 diabetes still requires confirmation in larger clinical studies.

Other additional factors could influence the risk to develop microvascular complications, such as familial insulin resistance (Type 2 diabetes in relatives of Type 1 diabetic patients) and shared genetic

basis for complications, perhaps independent of T1DM susceptibility, as previously suggested [49–51].

In conclusion, our study not only confirms what was already known about the role of HbA1c, but emphasizes the fact that HbA1c is the only predictive factor for the development of microvascular complications in T1DM. This is in accordance with the recent guidelines of several international diabetes associations (ADA, IDF, EASD, Italian Society of Diabetology, etc.) which point out that an intensive therapeutic approach is mandatory right from the disease onset, in order to reach optimum levels of HbA1c. In addition, our study suggests that metabolic memory exerts a different influence on diabetic retinopathy and microalbuminuria. By contrast, clinical, immunological and other biochemical factors are only useful for diagnosis and do not predict the onset of complications.

5. Learning points

- A close association between metabolic control and microvascular complications in T1DM is well known.
- The DCCT experience widely demonstrated the clinical benefits of preservation of β -cell function in preventing microvascular complications, but only in intensively treated T1DM.
- Our study confirms the already known role of HbA1c, emphasizing that in conventionally treated T1DM HbA1c is the only predictive factor for the development of microvascular complications.
- In addition, our study suggests that the metabolic memory only exerts a role in diabetic retinopathy development.
- Other clinical, immunological and biochemical factors are only useful for diagnosis and do not predict the onset of complications.

Conflict of interest statement

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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