Progression to Overt Nephropathy in Type 2 Diabetes: the Casale Montferrato Study

Progression to Overt Nephropathy in Type 2 Diabetes

The Casale Monferrato Study

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OBJECTIVE — The first sign of diabetic nephropathy is microalbuminuria, but its predictive role of progression to overt nephropathy in type 2 diabetes has not yet been clarified. The aims of this study were to assess during 7 years of follow-up the incidence rate of overt nephropathy and the predictive role of microalbuminuria and other baseline variables (blood pressure, lipids, fibrinogen, uric acid, smoking, and HbA_{1c} cumulative average during follow-up).

RESEARCH DESIGN AND METHODS — A prospective population-based study was performed in Casale Monferrato, Italy, including 1,253 type 2 diabetic patients recruited at baseline (1991–1992), 765 with normoalbuminuria (albumin excretion rate [AER] <20 μg/min) and 488 with microalbuminuria (AER 20–200 μg/min). All measurements were centralized. A nested case-control study within the cohort was performed, selecting four control subjects, frequency matched for age and attained individual time of follow-up with each case. Conditional regression analysis was performed to assess variables independently associated with risk of progression to overt nephropathy.

RESULTS — Of 1,253 total patients, 1,103 (88.0%) were included in the follow-up examination (median 5.33 years); their age and duration of disease at baseline were 68.4 ± 10.5 years and 10.4 ± 6.6 years, respectively. Cases of overt nephropathy were 202, giving an incidence rate of 37.0/1,000 person-years (95% CI 32.3–42.6). In conditional logistic regression analyses, microalbuminuria provided a 42% increased risk with respect to normoalbuminuria (95% CI 0.98–2.06), independently of duration of diabetes, hypertension, and systolic blood pressure. Other variables independently associated with progression to overt nephropathy were HbA_{1c} cumulative average ($P = 0.002$), apolipoprotein B ($P = 0.013$), fibrinogen ($P = 0.02$), and HDL cholesterol ($P = 0.03$).

CONCLUSIONS — Of type 2 diabetic patients, 3.7% progress every year to overt nephropathy. Microalbuminuria is associated with a 42% increased risk of progression to overt nephropathy. Other independent predictors are HbA_{1c}, HDL cholesterol, apolipoprotein B, and fibrinogen.

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Temporal trends of end-stage renal disease (ESRD) caused by diabetic nephropathy are increasing worldwide, so that diabetes represents the second leading cause of dialysis in most centers (1,2). The first sign of renal involvement is microalbuminuria, which affects 20–40% of patients with type 2 diabetes (3). When macroalbuminuria occurs, glomerular filtration rate declines, with an average reduction of 10–12 ml · min^{-1} · year^{-1} (3).

The predictive role of microalbuminuria in progression to overt nephropathy in type 2 diabetes has not yet been clarified, however (4–6). With respect to previous studies, more recent ones have suggested a lesser predictive role (5,6). A benefit of lipid-lowering treatment on risk of progression to overt nephropathy has also been hypothesized (7). Abnormalities in lipoprotein metabolism, such as elevations in triglycerides and apolipoprotein B (apoB) and reduced HDL cholesterol, have been shown in microalbuminuric type 2 diabetic patients (7,8). The clinical relevance of these alterations on progression to overt nephropathy, however, has not yet been established. Most of the studies are limited by the recruitment of either a low number of clinic-based rather than population-based patients or selected ethnic groups at higher risk for nephropathy than Caucasians (5,9–13). We have previously identified in Casale Monferrato, Italy, a large population-based cohort of type 2 diabetic patients, who were characterized as normo-, micro-, and macroalbuminuric (14,15). The aims of this study were to assess during a 7-year follow-up of this cohort the incidence rate of overt nephropathy and the predictive role of microalbuminuria and other baseline variables (blood pressure, lipids, fibrinogen, uric acid, and smoking), independent of HbA_{1c} cumulative average during follow-up.

RESEARCH DESIGN AND METHODS

— The study base was 1,253 patients (765 normoalbuminuric and 488 microalbuminuric) with known type 2 diabetes, residing in 1988 in the town of Casale Monferrato, in the northwest of Italy (93,477 inhabitants), and invited at a baseline examination in 1991–1992 to assess the prevalence of cardiovascular risk factors (14–16). They were identified by using multiple sources of ascertainment: *1)* diabetes clinic, *2)* general

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Abbreviations: AER, albumin excretion rate; apo, apolipoprotein; CHD, coronary heart disease; ECG, electrocardiogram; ESRD, end-stage renal disease.

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

practitioners, 3) hospital discharges, 4) prescriptions, and 5) sale records of reagent strips and syringes (17). Patients exclusively cared for by their general practitioners were 23.8% of the cohort (18). A high estimated completeness of ascertainment was obtained (19). A comparative assessment of surveys conducted within Italy showed they were representative of Italian diabetic patients as regards age, sex, duration of diabetes, BMI, and pattern of antidiabetic treatment (20).

All patients were interviewed and examined by three investigators after a training period to standardize data collection and blood pressure measurement. Informed consent was obtained from all patients. Height and weight were measured in indoor clothing without shoes with a beam balance and a stadiometer, and BMI was calculated. Blood pressure was measured with mercury sphygmomanometers to the nearest 2 mmHg, in the right arm at the start of examination, in sitting position, three consecutive times after an initial 5-min rest. Reported values are the average of second and third readings (phase 1 for systolic and phase 5 for diastolic). Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or treatment with antihypertensive drugs. Venous blood samples were collected in the fasting state for determinations of plasma glucose, triglycerides, total cholesterol, uric acid (enzymatic-colorimetric methods), HDL cholesterol (enzymatic-colorimetric method, after precipitation with Mn^{2+}), apoA1 and apoB (turbidimetric method; BM/hitachi 717, BBR), fibrinogen (Clauss method), and HbA_{1c} (high-performance liquid chromatography; Daiichi; laboratory reference range 3.8–5.5%). Urinary albumin concentration was measured by a nephelometric method (Behring Nephelometer Analyzer; Behring Institute) from a timed overnight collection after having excluded urinary tract infections, congestive heart failure, and other known causes of renal diseases. Albumin excretion rate (AER) was calculated as the albumin concentration divided by timed urine volume. A single measurement of AER was performed at baseline.

Smoking habit was classified into one of three categories: never smoker, ex-smoker (stopped at least 1 month before the visit), and smoker. Duration of diabetes was defined as the time from diabetes diagnosis to the time of baseline examination (1991–1992). Standard supine 12-lead electrocardiograms (ECGs) were recorded and read according to the Minnesota code criteria in duplicate (21). Coronary heart disease (CHD) was defined by ECG abnormalities, including probable (major Q and QS items, codes 1.1 and 1.2) and possible (minor Q and QS items, S-T/T items, codes 1.3, 4.1–4.4, 5.1–5.3) indications of CHD together. RR and QT intervals were measured in five consecutive cycles on lead V5 of the resting ECG using a graduated lens.

During the follow-up period (1991–1997), patients were examined three to four times a year at the diabetes clinic or by general practitioners, according to physician scheduled plans, with centralized laboratory measurements. In 1996–1997, all living patients were reexamined to assess progression to overt nephropathy (AER >200 μg/min in at least two consecutive overnight urine collections). For patients who died or migrated from the area, the last measurements of AER were obtained from computerized files of the central laboratory. Cumulative individual averages of HbA_{1c} during follow-up were calculated. Differences in clinical characteristics of patients were assessed using the *t* test for continuous variables and χ^2 test for ordinal variables; results are shown as mean (SD) and geometric mean (interquartile range) for normally and nonnormally distributed variables, respectively. Incidence rates were calculated by dividing the number of new cases occurring during the study period by the number of person-years of observations. The time period was calculated from the baseline study to the follow-up examination or to the last examination if patient had died or had moved from the area.

At baseline, the Casale Monferrato Study recruited a prevalence cohort of patients, i.e., a heterogeneous cohort as regards duration of disease and stage of complications. The severity affecting baseline factors is associated with selective mortality of the cohort, and the expected effect on our study was lower attained individual time of follow-up (the lag between baseline and reference examination) of more severe patients (22).To take into account this effect, which would have biased our results, we performed a nested case-control study within the cohort of Casale Monferrato, selecting four control subjects (subjects who did not progress to overt nephropathy during follow-up), frequency matched for age and attained time of follow-up with each case (subjects who progressed to overt nephropathy during follow-up). By doing so, we compared predictors of progression to overt nephropathy in subsets of case and control subjects having similar age and followed for an equal period of time. Conditional regression analysis was then performed to assess variables independently associated with risk of progression to overt nephropathy. Continuous variables were categorized in quartiles of their distribution. The –2 log likelihood ratio test was used to test the significance of variables. The possible interactions between variables were assessed. All analyses were performed using Stata (Stata Release 7.0; Stata Corporation).

RESULTS — Of 1,253 patients with normo- or microalbuminuria not caused by other diseases at baseline examination, 1,103 were recruited during follow-up, 677 of 765 (88.5%) normoalbuminuric and 426 of 488 (87.3%) microalbuminuric. Patients lost to follow-up were those who died without centralized assessments of AER (n = 53 normoalbuminuric, n = 57 microalbuminuric), migrated from the area (n = 18), or refused to be retested (n = 22).

At the baseline examination, microalbuminuric subjects were treated more often with insulin and had higher values of HbA_{1c}, triglycerides, fibrinogen, creatinine, and systolic and diastolic blood pressure than normoalbuminuric patients (Table 1). The distribution of AER values in the cohort was skewed, with mean 28.8 μg/min, median 15.7 μg/min, and interquartile range 9.38–28.12.

During the follow-up period (median 5.33 years, range 0.05–7.86), 202 cases of overt nephropathy were identified of 5,452.7 person-years of observations, giving an incidence rate of 37.0/1,000 person-years (95% CI 32.3–42.6); of those, 84 were normoalbuminuric and 118 microalbuminuric at baseline, giving incidence rates per 1,000 of 25.8 (95% CI 20.9–32.0) and 53.6 (95% CI 44.7–64.2), respectively. Relative risk (RR) of progression to overt nephropathy was twofold higher in micro- than in normoalbuminuric patients (2.07, 95% CI

Table 1—*Baseline characteristics of normo- and microalbuminuric patients of the Casale Monferrato Study recruited for follow-up*

	Normoalbuminuric	Microalbuminuric	*P*
n	677	426	
Age (years)	67.2 ± 10.0	67.9 ± 10.6	0.22
Men	273 (40.3)	204 (47.9)	0.01
Duration of diabetes (years)	9.8 ± 6.3	11.1 ± 7.0	0.001
BMI (kg/m^2)	27.4 ± 4.8	27.8 ± 4.8	0.20
Antidiabetic treatment			
Diet	98 (14.5)	30 (7.1)	<0.0001
Oral drugs	496 (73.5)	299 (70.7)	
Insulin	81 (12.0)	94 (22.2)	
CHD	123 (21.5)	84 (23.7)	0.43
Smoker	89 (13.4)	60 (14.4)	0.02
Ex-smoker	119 (18.0)	103 (24.7)	
Nonsmoker	454 (68.6)	254 (60.9)	
Hypertension	554 (81.8)	363 (85.2)	0.08
Systolic blood pressure (mmHg)	153.1 ± 21.1	156.1 ± 22.5	0.03
Diastolic blood pressure (mmHg)	87.0 ± 10.0	88.3 ± 10.7	0.03
Treatment with ACE inhibitors	140 (20.7)	94 (22.1)	0.58
HbA_{1c} (%)	7.8 ± 2.3	8.4 ± 2.3	<0.0001
Total cholesterol (mmol/l)	5.81 ± 1.20	5.80 ± 1.25	0.85
HDL cholesterol (mmol/l)	1.46 ± 0.43	1.42 ± 0.52	0.26
Triglycerides (mmol/l)	1.44 (1.02–1.91)	1.55 (1.07–2.15)	0.02
ApoA1 (mg/dl)	137.1 ± 35.5	131.8 ± 29.8	0.01
ApoB (mg/dl)	101.6 ± 35.0	102.2 ± 38.6	0.79
Fibrinogen (g/l)	3.55 ± 0.87	3.69 ± 0.97	0.01
Uric acid (μmol/l)	313 ± 92	323 ± 92	0.09
Creatinine (μmol/l)	88 (74–97)	90 (80–104)	0.007

Data are means ± SD, *n* (%), or medians (interquartile range).

1.57–2.74; $P < 0.0001$), partially because of the effect of duration of diabetes (adjusted RR 1.75, 95% CI 0.95–3.23; $P = 0.07$). Patients who died during follow-up were 85 of 677 (12.6%) normoalbuminuric and 76 of 426 (17.8%) microalbuminuric patients ($P = 0.016$).

At the baseline examination, patients who developed overt nephropathy were older (71.8 ± 10.7 vs. 66.8 ± 10.1 years; $P < 0.001$) and had a longer duration of diabetes (11.8 ± 7.8 vs. 10.0 ± 6.3 years) than nonprogressors. In addition, they had higher values of cumulative average HbA_{1c} (7.7 ± 2.0 vs. 7.3 ± 1.6%; $P = 0.001$) and fibrinogen (3.79 ± 0.89 vs. 3.55 ± 0.90 g/dl; $P = 0.001$) and lower values of total cholesterol (5.63 ± 1.36 vs. 5.83 ± 1.18 mmol/l; $P = 0.046$) and HDL cholesterol (1.35 ± 0.42 vs. 1.46 ± 0.46 mmol/l; $P = 0.001$) but similar values of other metabolic variables.

No differences between progressors to overt nephropathy and nonprogressors were found at baseline in either systolic blood pressure (155.2 ± 27.0 vs. 153.5 ± 22.1 mmHg; $P = 0.35$) or diastolic blood pressure (86.5 ± 13.6 vs. 87.4 ± 10.7; $P = 0.30$). Even at the follow-up examination, blood pressure values were similar (150.9 ± 20.3 and 83.3 ± 9.7 mmHg in progressors; 148.5 ± 18.5 and 83.2 ± 8.9 mmHg in nonprogressors); both systolic and diastolic blood pressure values, however, were lower than at baseline.

In patients treated for hypertension, frequencies of treatments were slightly different at baseline ($n = 555$ patients) than at follow-up ($n = 610$ patients): 42.2 vs. 61.2% were treated with ACE inhibitors, either alone or in combination with other drugs; 6.3 vs. 12.0% were treated with Ca^{2+} antagonists, 21.1 vs. 10.0% with diuretics, 4.1 vs. 3.1% with β-blockers, 20.0 vs. 5.7% with vasodilators or alfa adrenergic inhibitors, and 6.3 vs. 8.0% with two or more drugs in combination. Frequencies were similar in subgroups defined by AER categories at baseline and in patients cared for by general practitioners, apart from treatment with ACE inhibitors, which was more frequent at baseline (25.1 vs. 10.0%; $P = 0.002$) and at follow-up (42.5 vs. 28.9%; $P = 0.02$) in microalbuminuric patients cared for by diabetes clinics. Frequencies of treatment for hyperlipidemia were similar at baseline and at follow-up examinations (5.4 vs. 5.9%) in the whole cohort as well as in subgroups defined by AER categories.

In nested case-control analyses, 202 case subjects and 785 control subjects, frequency matched for age and attained time of follow-up, were compared. In univariate analyses we showed increased, although not statistically significant, odds ratios (ORs) for progression in patients treated with insulin (OR 1.43, 95% CI 0.82–2.48) and smokers (OR 1.31, 95% CI 0.81–2.13). No effects of hypertensive status, systolic or diastolic blood pressure, CHD, or treatment with ACE inhibitors were evident. The OR for macroalbuminuria was 1.55 (95% CI 1.11–2.16). The upper quartile of HbA_{1c} cumulative individual average (values >8.3%) had OR 1.66 (95% CI 1.08–2.55) versus the lowest quartile (values <6.2%). As regards fibrinogen, increased ORs for progression were found in the upper quartiles (OR 1.94, 95% CI 1.23–3.07, for values between 3.49 and 4.12 g/l; OR 1.56, 95% CI 0.97–2.52, for values >4.12; reference values <3.00 g/l).

As regards lipids, a decreasing trend of ORs for increasing values of HDL was evident (OR 0.50, 95% CI 0.32–0.76, for values between 1.35 and 1.60 mmol/l; OR 0.57, 95% CI 0.30–0.72, for values >1.60 mmol/l; reference values <1.14 mmol/l). Higher values of triglycerides were associated with risk of progression (OR 1.27, 95% CI 0.80–2.00, for values between 1.44 and 2.03 mmol/l; OR 1.60, 95% CI 1.01–2.52, for values >2.03 mmol/l; reference values <1.07 mmol/l).

In multivariate conditional logistic regression analyses (Table 2), after having taken into account the effects of confounders (duration of diabetes) and known risk factors for overt nephropathy (hypertension, systolic blood pressure, and cumulative individual average of HbA_{1c}), microalbuminuria provided a 42% increased risk of progression with respect to normoalbuminuria (95% CI 0.98–2.06; $P = 0.06$). Other variables independently associated with progression to overt nephropathy were HbA_{1c} cumulative average ($P = 0.002$), apoB ($P = 0.013$), fibrinogen ($P = 0.02$), and HDL cholesterol ($P = 0.03$). No effect of tri-

Table 2—*Results of conditional logistic regression analyses of variables associated with progression to overt nephropathy in the Casale Monferrato Study*

	OR (95% CI)
Duration (years)	
<5.5	1.00
5.5–8.5	1.10 (0.67–1.80)
8.6–14.9	1.44 (0.89–2.35)
>14.9	1.39 (0.85–2.28)
Hypertension	
No	1.00
Yes	0.67 (0.35–1.28)
Systolic blood pressure (mmHg)	
<140	1.00
141–152	0.87 (0.47–1.60)
153–170	1.53 (0.87–2.68)
>170	1.21 (0.67–2.18)
AER	
Normoalbuminuria	1.00
Microalbuminuria	1.42 (0.98–2.06)
HbA_{1c} cumulative average (%)	
<6.16	1.00
6.16–7.37	0.52 (0.31–0.88)
7.38–8.32	0.67 (0.40–1.12)
>8.32	1.26 (0.78–2.03)
Fibrinogen (g/l)	
<3.00	1.00
3.00–3.48	1.07 (0.63–1.83)
3.49–4.12	1.93 (1.18–3.16)
>4.12	1.54 (0.92–2.58)
HDL cholesterol (mmol/l)	
<1.14	1.00
1.14–1.34	0.56 (0.34–0.93)
1.35–1.60	0.55 (0.34–0.87)
>1.60	0.54 (0.33–0.89)
ApoB (mg/dl)	
<74	1.00
74–94	0.82 (0.48–1.40)
95–122	1.73 (1.05–2.87)
>122	1.34 (0.81–2.24)

glycerides was evident after including HDL cholesterol in the model.

CONCLUSIONS — This study was addressed to investigate the association between microalbuminuria and the incidence of overt nephropathy in a 7-year follow-up of a type 2 diabetic cohort of Casale Monferrato. We provide evidence that patients with microalbuminuria have a 42% increased risk of progression to overt nephropathy versus those with normoalbuminuria, even after adjustment for confounders (age, duration of diabetes, and attained time of follow-up) and risk factors (blood pressure and cumulative HbA_{1c} average). We found that 3.7% of diabetic patients progress every year to overt nephropathy, 2.6% among normoalbuminuric and 5.4% among microalbuminuric patients. These estimates are consistent with those obtained in previous studies (4,12). Although numerous investigators have reported the association between microalbuminuria and the risk of diabetic nephropathy, few studies have been carried out in large population-based cohorts of Caucasians with type 2 diabetes (4,12). In addition, most studies were limited to patients younger than 65 years. The Casale Monferrato cohort is representative of the Italian diabetic population, being characterized by both better glycemic control than those generally recruited in Northern European countries and later age at diagnosis; 50% of patients recruited in this study were 68 years and older at baseline. Our finding that microalbuminuria is associated with an increased risk of overt nephropathy even in the elderly is relevant, highlighting that microalbuminuria is never a benign condition and that it should be carefully prevented at any age, in view of the effective intervention measures (3).

When we analyzed in a multivariate model the contribution of each risk factor adjusting for all the others, HDL cholesterol, apoB, and fibrinogen remained as independent risk factors, whereas the association with triglycerides disappeared. These results, therefore, provide evidence that HDL, apoB, and fibrinogen are per se predictors of progression to overt nephropathy, independently of the presence of microalbuminuria or hypertension. These findings are consistent with previous studies (7,23) and could at least partially explain the excess of cardiovascular morbidity and mortality of macroalbuminuric patients (24). Whether these findings reflect lipid abnormalities characterizing the earlier phases of diabetic nephropathy, or their direct involvement in its progression, is unknown.

We did not find any association between hypertensive status and progression to overt nephropathy. This finding, however, should be considered with caution considering that the prevalence of hypertension in this cohort was very high (80%), thus reducing the power to detect a significant effect of this variable. Moreover, blood pressure values at the follow-up examination were lower than at baseline, and a more aggressive approach to treatment of hypertension might have blunted its predictive effect on progression to overt nephropathy.

An association between smoking and loss of renal function has been shown in type 1 diabetes (25) and nondiabetic subjects (26), whereas no convincing data are available for type 2 diabetes (12). Consistently, we did not find a predictive role of smoking on risk of progression to overt nephropathy.

The prospective study design, the large population sample, and the high degree of longitudinal surveillance for diabetic nephropathy are strengths of the present study. At variance with previous studies, we dedicated great efforts to reduce selection bias by recording AER and

HbA_{1c} data of both living and dead patients, which were retrospectively extracted from the computerized files of the central laboratory. By using this method, we were able to provide a high degree of completeness of data at follow-up (88%). In addition, considering that attained individual time of follow-up (that is lag between baseline and reference examination) is associated with prognostic factors, which are likely to be stratified differently among normo- and microalbuminuric patients, a case-control analysis within the cohort was performed, matching case subjects (progressors) and control subjects (nonprogressors) for age and attained time of follow-up. Conditional logistic regression analysis of the case-control study design gave very different results from the unconditional logistic regression of the cohort study design. In the latter, the role of microalbuminuria was overemphasized, with a threefold increased risk with respect to normoalbuminuria (data not shown). This point is critical to state the strength of predictors and to compare results of different studies on this issue (22).

The main limitation of the study is the use of a single AER measurement at baseline, because it might have caused a misclassification of patients with regard to micro- and macroalbuminuria. However, this finding would have caused a bias toward the null value, that is, the underestimation of the strength of the relationship between microalbuminuria and the incidence of overt nephropathy. In ~30% of patients with type 2 diabetes and microalbuminuria but no retinopathy, the glomerular structure is normal or nondiabetic kidney diseases are present (27). Variability in the progression of renal disease has also been suggested, with more rapid loss of function in patients with typical diabetic glomerulopathy (28). Unfortunately, no data on retinopathy were available at baseline in the Casale Monferrato cohort to test this hypothesis.

In conclusion, our population-based study extends previous observations in several ways. First, we provide evidence that 3.7% of type 2 diabetic patients progress every year to overt nephropathy; second, we confirm the predictive role of microalbuminuria, which is associated with a 42% increased risk of progression; third, we show that HDL cholesterol, HbA_{1c}, apoB, and fibrinogen are independently associated with risk of progression.

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