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High cardiovascular risk in patients with Type 2 diabetic nephropathy: the predictive role of albuminuria and glomerular filtration rate. The NID-2 Prospective Cohort Study

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Abstract

Background. In Type 2 diabetic patients, clinical diagnosis of diabetic nephropathy (DN) is generally based on the concomitant presence of abnormal albuminuria and severe retinopathy. In this high-risk population, cardiovascular (CV) outcome has never been evaluated.

Methods. A cohort of 742 Type 2 diabetic patients with DN from 17 national centres was selected by the presence of persistent albuminuria $\geq$30 mg/day and severe diabetic retinopathy and was followed prospectively. Time to CV event (CV death, non-fatal myocardial infarction, non-fatal stroke, revascularization, major amputation) was the primary composite end point and it was analysed by multivariable Cox's proportional hazards model. The interaction between albuminuria and glomerular filtration rate (GFR) was specifically investigated.

Results. Median follow-up was 4.6 years. Overall 242 events (26% of which fatal) were observed in 202 patients. The proportion of CV events increased from 19 to 40% as GFR declined from the highest ($\geq$90 mL/min/1.73m^2) to the lowest ($<$45 mL/min/1.73m^2) category and was equal to 25 and 33% in microalbuminuria and macroalbuminuria, respectively. In multivariable analysis, the interaction between albuminuria and GFR was statistically significant ($P = 0.012$). Albuminuria, indeed, had a remarkable prognostic effect in subjects with high GFR that virtually disappeared as GFR became $<$30 mL/min/1.73m^2. Age, smoking habit, previous occurrence of myocardial infarction or stroke and proliferative retinopathy were all found to have a statistically significant prognostic effect on CV outcome.

Conclusions. A clinically based diagnosis of DN in Type 2 diabetes allows the identification of subjects with high CV risk. Albuminuria has a relevant prognostic effect on CV morbidity and mortality; its effect is especially pronounced when GFR is normal or near normal.

Keywords: albuminuria; cardiovascular; diabetic nephropathy; GFR; proliferative retinopathy

Introduction

Diabetic nephropathy (DN) is defined by severe retinopathy and albuminuria ≥30 mg/day and occurs in a minority of Type 2 diabetic patients [1]. DN is different from renal disease occurring in diabetic patients and shows a more rapid decline of renal function than nephropathy due to other causes [2]. However, studies on cardiovascular (CV) outcome in Type 2 DN are still lacking.

Although both albuminuria and glomerular filtration rate (GFR) are believed to be risk factors for CV events, there are limited data as to whether these two factors are associated with adverse outcomes independent not only of other known CV risk factors but also of each other in patients with Type 2 diabetes. Recently, the ADVANCE study [3] showed that high albuminuria and low GFR are independent risk factors for CV events among patients with Type 2 diabetes. However, the two main clinical features defining DN, that is diabetic retinopathy and albuminuria, were detected in only 7.1 and 29.3% of patients, respectively [4]. More important, 62% of patients with a GFR <60 mL/min did not have concurrent albuminuria [3]. Overall, these data suggest that the ADVANCE study does not provide information on the outcome of 'true' DN.

The Nephropathy In Diabetes-Type 2 (NID-2) study [5] was originally designed to investigate the prevalence of CV risk factors, their management and the achievement of international guideline targets in a large population of Type 2 diabetic patients with a clinical diagnosis of DN (concomitance of albuminuria and severe diabetic retinopathy), followed up in the tertiary care setting. The cross-sectional phase of the NID-2 study pointed out that patients with DN are characterized by clusters of risk factors, not at target, compatible with a high CV risk profile [5]. Moreover, the NID-2 study showed that frank chronic kidney disease (CKD) (GFR < 60 mL/min) was present in 38 and 62% of micro- and macroalbuminuric DN patients, respectively.

The aim of this prospective study was to evaluate CV prognosis and determinants in the NID-2 cohort of 742 Type 2 diabetic patients, selected by the presence of abnormal albuminuria and severe diabetic retinopathy. In particular, we investigated how renal risk factors, albuminuria and GFR affected CV outcome in this sub-group of diabetic patients.

Materials and methods

The cross-sectional phase of the NID-2 study evaluated diabetic patients at 17 national centres during a 6-month period from November 2002 to May 2003. Inclusion criteria were: Type 2 diabetes mellitus, age ≥40 years, therapy with diet and/or oral hypoglycaemic agents during the first year of the diagnosis of diabetes, persistent albuminuria ≥30 mg/day in at least two of three recordings in the last 6 months, a severe diabetic retinopathy as judged by means of fundus oculi and fluorangiography when necessary. Severe DR was defined as a proliferative diabetic retinopathy or a severe non-proliferative diabetic retinopathy, with vascular closure. This last criterion was chosen as the clinical hallmark of DN [1, 2, 6], thus excluding other possible causes for increased albuminuria since renal biopsies are not indicated for diagnostic purposes in microalbuminuric diabetic patients. Exclusion criteria were prior dialysis or renal transplant, diagnosis of diabetes at <30 years of age, insulin therapy during the first year of diagnosis of the disease (in order to exclude unknown autoimmune diabetes of adults), severe liver or heart failure and known neoplastic or psychiatric disease. All the participant physicians declared that they adhered to recommendations of the clinical practice guidelines issued by the American Diabetes Association [7]. Ethical committees approved the prospective phase of the study and all the patients signed informed consent.

Active follow-up, with control visits planned every 6 months, was completed on 30 November 2009. Baseline information included past medical history, with particular reference to major CV events (myocardial infarction and stroke), blood pressure (BP) measurement (calculated as a mean of three measurements taken in a sitting position after 10 min of rest), height and body weight as well as laboratory and therapeutic features. Laboratory tests were performed locally and included glycaemic, lipidic and renal function assessment. GFR was calculated by the four-variable Modification of Diet in Renal Disease equation and albuminuria was measured on 24-h urine collection; microalbuminuria and macroalbuminuria were defined by values of 30–300 and >300 mg/day, respectively.

The primary composite end point was time to CV events, defined as time from basal visit to CV death, non-fatal myocardial infarction, non-fatal stroke, revascularization or major amputation, whichever occurred first. When a CV event was suspected, hospital records were collected to make the diagnosis according to European Society of Cardiology and American College of Cardiology criteria [8, 9]. Death certificates and autopsy reports were used to establish the underlying cause of death and to adjudicate CV deaths, through the ninth revision of the International Classification of Diseases.

Statistical analysis

For descriptive purpose, patients were categorized into four groups according to GFR values (GFR ≥ 60 and <60 mL/min/1.73m^2) and the presence of micro- or macroalbuminuria. A regression model was used to assess the main effects of the two factors (GFR categories and micro- and macroalbuminuria) and their interaction effect. A multiple linear regression model was used for continuous dependent variables, while a logistic regression model was used for categorical ones. Median follow-up was estimated by inverse Kaplan–Meier curve. Multivariable Cox proportional hazards model, stratified by centre, was used to estimate hazard ratio (HR) and corresponding 95% confidence intervals (CIs). Covariates included predefined baseline risk factors [age, gender, body mass index (BMI), smoking, previous cardiovascular disease, HbA1c, cholesterol, systolic BP and proliferative retinopathy] and interaction between albuminuria and GFR. Interaction between either albuminuria or GFR and covariates included in the model was also tested. For each variable, restricted cubic splines were used to take into account the non-linear association with endpoint and were tested by means of likelihood ratio test. All statistical tests were two tailed and $P < 0.05$ was considered significant. Statistical analyses were performed using SPSS version 12.0 (SPSS Inc, Chicago, IL) and R version 2.9.2 (R Foundation for Statistical Computing, Vienna, Austria) software packages.

Results

From the original cohort of 847 patients, previously described [5], three centres following 72 patients refused to participate in the follow-up study and 33 patients were lost to follow-up. Therefore, 742 (95.7%) patients were included in the present study. Demographic and clinical characteristics of patients lost to follow-up did not differ from the study subjects. Baseline characteristics are reported in Table 1. One-third of the patients ($n = 247$) was aged >70 years, 287 (38.7%) were obese (BMI > 30 kg/m^2) and 323 (43.5%) were overweight (BMI 25–30 kg/m^2). Diagnosis of diabetic neuropathy was reported in 309/660 (46.8%) patients and autonomic neuropathy was diagnosed in 128/634 (20.2%) patients. BP target (<130/80 mmHg) was attained in 137 patients (18.5%) with a 2-fold greater prevalence of diastolic BP target (55.0%) than systolic target (28.0%). Oral hypoglycaemic agents alone and insulin alone were used by 57.8 and 19.3% of patients,

Table 1. Demographic and clinical characteristics in patients stratified for albuminuria and GFR (expressed as mL/min/1.73m^2)[a]

		Microalbuminuria		Macroalbuminuria		P-value[b]		
	Overall ($n = 742$)	GFR ≥ 60 ($n = 384$)	GFR < 60 ($n = 219$)	GFR ≥ 60 ($n = 59$)	GFR < 60 ($n = 80$)	GFR < 60 versus GFR ≥ 60	Micro versus Macro	Interaction[c]
Age (years)	65.8 ± 8.9	64.2 ± 9.0	68.2 ± 8.1	63.9 ± 9.3	67.5 ± 8.8	<0.0001	0.543	0.828
Male gender (%)	46.6	52.1	34.2	62.7	42.5	<0.0001	0.045	0.826
BMI (kg/m^2)	29.3 ± 4.9	29.2 ± 4.6	29.4 ± 5.2	30.0 ± 5.2	29.1 ± 5.4	0.437	0.613	0.253
Smokers (%)	23.3	27.9	14.6	28.8	21.3	0.007	0.270	0.369
CVD (%)	23.3	17.9	29.2	18.6	40	<0.0001	0.201	0.400
Proliferative DR (%)	70.1	75.5	64.4	69.5	60	0.019	0.229	0.775
HbA1c (%)	7.53 ± 1.24	7.58 ± 1.26	7.33 ± 1.13	7.90 ± 1.34	7.51 ± 1.30	0.007	0.036	0.537
Cholesterol (mg/dL)	197 ± 40	196 ± 37	195 ± 39	201 ± 44	201 ± 48	0.907	0.137	0.772
HDL (mg/dL)	48 ± 11	49 ± 11	47 ± 11	47 ± 14	48 ± 11	0.552	0.744	0.272
LDL (mg/dL)	119 ± 35	119 ± 33	117 ± 35	122 ± 41	121 ± 38	0.627	0.238	0.955
Triglycerides (mg/dL)	152 ± 79	145 ± 75	156 ± 74	167 ± 112	165 ± 75	0.584	0.041	0.390
Haemoglobin (g/dL)	13.2 ± 1.4	13.5 ± 1.2	12.9 ± 1.3	13.6 ± 1.6	12.3 ± 1.7	<0.0001	0.038	0.011
GFR (mL/min/1.73m^2)	66 ± 24	82 ± 16	45 ± 11	84 ± 15	39 ± 12			
UAlb (mg/day)	100 (54–222)	71 (42–113)	98 (58-163)	384 (348–535)	391 (350–500)			
Systolic BP (mmHg)	136 ± 13	135 ± 13	138 ± 13	138 ± 13	140 ± 14	0.044	0.010	0.654
Diastolic BP (mmHg)	78 ± 7	78 ± 7	78 ± 7	80 ± 7	78 ± 8	0.068	0.104	0.049
Anti-hypertensive drugs	1.8 ± 1.1	1.5 ± 1.0	2.1 ± 1.1	1.7 ± 1.1	2.3 ± 1.2	<0.0001	0.008	0.856
CEI and/or ARB (%)	73.5	68	81.3	72.9	78.8	0.021	0.862	0.381
OHA (%)	64.8	71.6	58.4	72.9	43.8	<0.0001	0.197	0.110
Insulin (%)	26.3	19.8	30.6	27.1	45	0.001	0.014	0.619
Statins (%)	32.9	27.9	39.7	30.5	40	0.019	0.731	0.774
Aspirin (%)	44.9	40.9	48.9	52.5	47.5	0.752	0.278	0.171

[a]Values are mean ± SD, median (IQR) or percent. CVD, history of myocardial infarction or stroke; GFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; DR, diabetic retinopathy; UAlb, urinary albumin excretion; CEI, converting enzyme inhibitors; ARB, angiotensin II receptor blockers; OHA, oral hypoglycaemic agents.
[b]P-values were calculated from multiple linear regression model and logistic regression model for continuous and categorical-dependent variables, respectively.
[c]GFR < 60 versus GFR ≥ 60, macroalbuminuria versus microalbuminuria and their interaction were used as covariates in each model.

respectively, and in combination by 7.0% of patients. Diet was the only treatment for 15.9% of the study subjects.

In our cohort, albuminuria was 100 mg/day [interquartile range (IQR) 54–222]. Microalbuminuria (30–300 mg/day) was found in 603 (81.3%) patients, (median value 80 mg/day, IQR 48–127) and macroalbuminuria (>300 mg/day) in 18.7% (median value 385 mg/day, IQR 350–516); among macroalbuminuric patients, 7.2% had an albuminuria >1000 mg/day. Mean GFR was 66 ± 24 mL/min/1.73m^2 with a prevalence of CKD from Stages 1 to 5 being 16.2, 43.5, 34.4, 5.1 and 0.8%, respectively.

Demographic and clinical characteristics of patients are reported in Table 1. No difference among sub-groups was detected for BMI and dyslipidaemia. Patients with GFR <60 mL/min/1.73m^2 ($N = 299$, 40.3%) were older, predominantly females and with more frequent history of CV disease and proliferative retinopathy (Table 1). They also had a slightly higher systolic BP and consequently, these patients received more anti-hypertensive drugs. As expected, in patients with lower GFR, prescription of insulin was higher and that of oral hypoglycaemic agents was lower (Table 1). In comparison with microalbuminuric patients, those with macroalbuminuria were characterized by a larger prevalence of male gender, greater renal impairment (57.8 ± 26.2 versus 68.2 ± 22.8 mL/min/1.73m^2, $P < 0.0001$) and higher systolic BP, with a consequent higher number of anti-hypertensive drugs. HbA1c and haemoglobin values were influenced by both GFR and albuminuria.

Nephrology consultation was requested in 137 patients (18.5%). This occurred more frequently in the sub-group with low GFR and in macroalbuminuric patients. Specifically, nephrology consultation rate was 9.5, 24.1, 19.2 and 45.2% in patients with microalbuminuria and GFR ≥60 mL/min/1.73m^2, microalbuminuria and GFR <60 mL/min/1.73m^2, macroalbuminuria and GFR ≥60 mL/min/1.73m^2 and macroalbuminuria and GFR <60 mL/min/1.73m^2, respectively.

Median follow-up was equal to 4.6 years (IQR 3.2–6.3). Overall, 242 CV events occurred in 202 (27.2%) patients (CV death $n = 64$, non-fatal myocardial infarction $n = 80$, non-fatal stroke $n = 51$, revascularization $n = 43$, amputation $n = 4$). Eight patients died of non-CV cause and seven patients progressed to end-stage renal disease. Incidence rate of CV events occurring during the study are reported in Table 2. At multivariable Cox regression analysis, taking microalbuminuria and normal GFR as the reference, HR was 1.43 (95% CI 1.00–2.04) for microalbuminuria and GFR <60 mL/min/1.73m^2, 1.83 (95% CI 1.04–3.02) for macroalbuminuria and GFR ≥60 mL/min/1.73m^2 and 1.72 (95% CI 1.08–2.76) for macroalbuminuria and GFR <60 mL/min/1.73m^2. The final multivariable Cox regression analysis was built replacing the four groups with GFR and albuminuria as continuous variables (Table 3 and Figure 1). Age, history of CV disease, smoking habit, proliferative retinopathy, independently increased the risk of CV events. As testified by restricted cubic spline analysis,

Table 2. Incidence rate of CV events occurring during the study overall and in sub-groups

		Microalbuminuria		Macroalbuminuria	
	Overall ($n = 742$)	GFR ≥ 60 ($n = 384$)	GFR < 60 ($n = 219$)	GFR ≥ 60 ($n = 59$)	GFR < 60 ($n = 80$)
CV death					
Number of events	64	22	26	4	12
Event rate (100 patient-years)	1.98	1.28	2.69	1.71	3.84
Non-fatal CV event					
Number of events	150	71	46	12	21
Event rate (100 patient-years)	5.13	4.51	5.26	6.00	7.61
Fatal/non-fatal events					
Number of events	202	88	68	15	31
Event rate (100 patient-years)	6.90	5.59	7.77	7.50	11.23

association of continuous variables with risk outcome was always linear (data not shown). Interaction between GFR and albuminuria was statistically significant ($P = 0.012$), suggesting that either effect varied as the other one was changing. Conversely, no interaction was detected between either GFR or albuminuria and other covariates included in the model. The combined HRs from Cox regression model and for specific values of albuminuria and GFR are reported in Figure 1; reference values are 90 mL/min/1.73m^2 for GFR and 30 mg/day for albuminuria. Albuminuria had a remarkable prognostic effect in subjects with high GFR that virtually disappeared as GFR became very low. At the same time, a predictive role for GFR can be detected only in microalbuminuric patients.

Table 3. HR and 95% CI for fatal and non-fatal CV events by Cox regression analysis[a]

	β-Coefficient	HR	95% CI	P
Age (years)	0.0291	1.03	1.01–1.05	0.003
Male gender	0.1469	1.16	0.85–1.58	0.352
BMI (kg/m^2)	−0.0005	1.00	0.97–1.03	0.976
Smokers (yes versus no)	0.4276	1.53	1.08–2.17	0.016
History of MI or stroke (yes versus no)	0.8455	2.33	1.70–3.19	<0.0001
Proliferative DR (yes versus no)	0.4599	1.58	1.10–2.28	0.013
HbA1c (%)	0.0780	1.08	0.96–1.22	0.212
Total cholesterol (mg/dL)	−0.0010	1.00	1.00–1.00	0.612
Systolic BP (mmHg)	0.0067	1.01	1.00–1.02	0.274
GFR (5 mL/min/1.73m^2)[b]	−0.0688			0.002
Albuminuria (g/day)[b]	−1.0830			0.165
Interaction eGFR × albuminuria[b]	0.1799			0.012

[a]DR, diabetic retinopathy.
[b]For HR, see Figure 1.

Discussion

This study originally evaluated the impact of risk factors on CV outcome in a prospective cohort of 742 Type 2 diabetic patients with persistent albuminuria and severe retinopathy. In particular, we investigated the prognostic role of the two main renal risk factors (albuminuria and GFR).

It is well established that diabetic subjects with associated nephropathy present an increased CV risk and that most of them do not develop end-stage renal disease because of prior fatal CV events [10]. All the previous investigations, however, examined patients with 'unspecific' microalbuminuria or macroalbuminuria [3, 10, 11]. This is a critical point of difference with our study because the absence of retinopathy constitutes the strongest clinical evidence to consider albuminuria as a non-diabetic lesion by renal histological analysis, with very high sensitivity (87%) and specificity (93%) [12]. On the other hand, in clinical practice, kidney biopsy, which is the ideal diagnostic tool to discern between true and false DN, is hardly feasible for ethical reasons in patients without significant proteinuria. Therefore, the NID-2 study investigated a specific sub-group of diabetic patients never studied before, but easily identifiable.

The first result to be underlined is the high CV risk of the population studied. Overall, 27.2% of the patients, during the follow-up (4.6 years on median), experienced at least one CV event, fatal in about one-third of cases. The incidence rates are definitely higher than those observed in studies with similar duration of follow-up conducted in 'generic' Type 2 diabetic populations [13]. Notably, CV event rate is even greater than that observed in the diabetic microalbuminuric group of HOPE Study and MICROHOPE sub-study [14].

Age, history of CV disease, smoking habit, proliferative retinopathy, albuminuria and GFR independently increased the risk of CV events. Among the non-renal risk factors, the significant prognostic role of type of diabetic retinopathy is of great interest, confirming recent observations [15, 16]. Indeed, in an 18-year follow-up study [15], proliferative retinopathy predicted all-cause, CV and coronary death in Type 2 diabetic subjects. These associations were independent of current smoking, hypertension, total cholesterol, high-density lipoprotein cholesterol, glycaemic control, duration of diabetes and proteinuria. In a sub-analysis of the VADT study [16], a relevant relationship was observed between retinopathy and coronary atherosclerosis quantified by means of computed tomography-detectable coronary artery calcium. Individuals with proliferative retinopathy were ~6-fold more likely to have high coronary artery

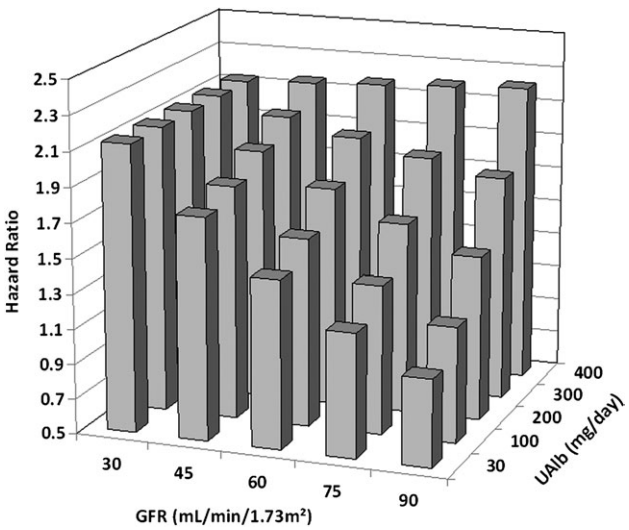

Fig. 1. Combined effects of albuminuria and GFR levels at baseline on the risk for fatal and non-fatal CV events. The estimates were calculated at specific values of GFR and albuminuria and adjusted for the covariates included in the Cox model (Table 2). Reference category GFR 90 mL/min/1.73m^2 and albuminuria 30 mg/day.

calcium than those with no proliferative retinopathy, even after adjustment for the other CV risk factors. Our and the previous data, therefore, suggest the presence of common background pathways for diabetic micro- and macrovascular disease. In particular, abnormal albuminuria could be a linkage between these diabetic vascular complications, being a marker of an early inflammatory state in the atherosclerotic disease process [17]. In this regard, a recent study has evidenced that the risk of developing retinopathy is greater in the diabetic patients with GFR <60 and macroalbuminuria with respect to diabetics with GFR <60 without macroalbuminuria [18].

The main measures of renal damage markedly influenced the CV outcome of these patients. In particular, proportion of CV events increased from 19 to 40% as GFR declined from the highest (≥90 mL/min/1.73m^2) to the lowest (<45 mL/min/1.73m^2) category and was equal to 25 and 33% in micro- and macroalbuminuria, respectively. In particular, the most intriguing findings resulted from multivariable analysis, showing an interaction between albuminuria and GFR (Figure 1). Albuminuria, indeed, showed a remarkable prognostic effect in subjects with high GFR that virtually disappeared in the lower strata of GFR. These results clash with the conclusions of another study. In a sub-analysis of the ADVANCE study [3], 10 640 Type 2 diabetic patients with levels of albuminuria and serum creatinine available at baseline were studied, and it was observed that both increased urinary albumin excretion and reduced GFR are independently and continuously associated with the risk for both CV and kidney outcomes in patients with Type 2 diabetes, but there was no evidence of any interaction between these risk factors.

On this matter, it is interesting to underline the great difference between NID-2 and ADVANCE studies. The two studies were similar for age, HbA1c, BMI of diabetic patients at baseline and for duration of follow-up, but the majority of patients of ADVANCE was normoalbuminuric (69%) and only 7% had retinopathy; therefore, it was a generic diabetic population, with and without nephropathy. Although patients with a previous CV event at baseline were 32% in ADVANCE versus 23% in NID study, and BP at baseline was higher in ADVANCE versus NID (145/81 versus 136/78 mmHg) patients, thus suggesting a major CV risk in ADVANCE group, the incidence rates of total major CV events and of death from CV causes were, respectively, 3 and 2.5-fold higher in NID group than in overall ADVANCE diabetic population. Therefore, the different role for GFR and albumin excretion rate (AER) on CV outcome observed in the two studies could be the result of two different diabetic populations.

The explanation for this interaction is not readily apparent. We may speculate that in patients with normal or only mildly reduced GFR, the prognostic role on CV outcome of albuminuria is higher because under these conditions, albuminuria is a marker of endothelial dysfunction, therefore heralding as such the increased CV risk [19]. On the other hand, indirect evidence suggests that in the presence of more impaired renal function, albuminuria is more related to either glomerulosclerosis or advanced tubular damage rather than endothelial dysfunction [20–23].

It is supposable that our results could offer a different, less exacting, interpretation of the significant positive interaction of GRF and AER. In fact, for the low prevalence (<6%) of CKD at Stages 4 and 5, both measures could simply provide significant prognostic information about the risk of CV events, and their effects could be multiplicative. However, our intriguing interpretation of the findings seems to be supported by the current knowledge of CV risk in Type 2 diabetes.

The main limitation of this study is that the observational nature precludes a proper cause-effect analysis; however, results are hypothesis generating to design multifactorial intervention trials aimed at verifying the possible improvement of CV outcome in patients with true DN. In particular, our findings suggest an intensive treatment in the early phases of DN, when the increased albuminuria is coupled with normal (or slightly decreased) GFR. On the basis of our results, it is in fact intriguing to hypothesize that at this stage of nephropathy, an intensive approach to abnormal albuminuria may reduce CV risk. A potential area of improvement, to be verified by *ad hoc* randomized trials, may be represented by the dual blockade of renin-angiotensin system (RAS) in diabetics selected by abnormal albuminuria [24]. This intervention was rare in the NID-2 patients and in particular in the early phases of DN. In this regard, a specific role of a dual RAS blockade on cardiorenal risk in diabetic patients in the early phases of renal damage and without established CV diseases should be tested.

As above stated, another limitation of the study is the low prevalence (<6%) of GFR <30 mL/min.

Finally, the strict criteria used to select the population are also a limitation of the study because its results cannot be generalized to all diabetic patients. Nevertheless, the very high CV risk observed in the NID-2 cohort strengthens the prognostic usefulness of these selection criteria, independently of histological confirmation of DN. In fact, even if the co-existence of albuminuria and retinopathy in Type 2 diabetes was recently reported to be not always histologically

matched in DN [25], the possibility to clinically identify a sub-group of patients at very high CV risk represents the highlight of this study.

In conclusion, patients with Type 2 DN show a CV risk independently related to age, history of CV disease, smoking habit, proliferative retinopathy, albuminuria and GFR. Albuminuria has a relevant prognostic effect on CV morbidity and mortality; its effect is especially pronounced when GFR is normal or near normal. These findings strongly suggest an intensive treatment since the early phases of DN, especially for albuminuria and smoking cessation, but it has to be confirmed by an interventional study.

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F.C.S. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Contribution statement. F.C.S. and R.M.: conception and design, interpretation of data, drafting the article and final approval of the manuscript; P.C., O.C., L.D.N., G.C., S.S. and C.G.: analysis and interpretation of data, final approval of the version to be published; T.S., R.N., R.M., R.T.: revising the manuscript critically for important intellectual content, final approval of the version to be published.

Conflict of interest statement. None declared.

Appendix

NID-2 Study Group: F.C. Sasso, O. Carbonara, R. Torella, R. D'Urso, A. Lampitella Jr, N. Lascar, R. Nasti, A. Pagano, E. Pisa, L. Zirpoli, F. Zibella, M. Corigliano, G. Conte, L. De Nicola, R. Minutolo, P. Trucillo, V. Armentano, P. Calatola, G. Corigliano, E. Del Vecchio, N. De Rosa, G. Di Giovanni, A. De Matteo, O. Egione, A. Gatti, S. Gentile, L. Gesuè, L. Improta, A. Lampitella, A. Lanzilli, S. Masi, P. Mattei, V. Mastrilli, P. Memoli, E. Rossi, S. Sorrentino, R. Troise, A.A. Turco, S. Turco.

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