Clinical significance of nonalbuminuric renal impairment in type 2 diabetes

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Objective **In type 2 diabetes, prevalence of nonalbuminuric renal impairment is increasing worldwide, though its clinical significance remains unclear. This large-cohort study aimed at evaluating the association of this phenotype with cardiovascular risk factors and other complications.**

Methods **Type 2 diabetic patients from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study ($n = 15\,773$), visiting consecutively 19 hospital-based Diabetes Clinics in years 2007–2008, were examined. Serum creatinine was assessed by the Jaffe method; albuminuria was measured by immunonephelometry or immunoturbidimetry.**

Results **Of patients with renal impairment, as identified by an estimated glomerular filtration rate (eGFR) less than 60 ml/min per 1.73 m^2, 56.6% were normoalbuminuric, 30.8% were microalbuminuric, and 12.6% were macroalbuminuric. Percentages were similar when GFR was estimated using the more accurate Chronic Kidney Disease Epidemiology Collaboration equation instead of the simplified Modification of Diet in Renal Disease formula, and were independent of age, thus indicating that the increasing prevalence of this phenotype does not reflects misclassification of elderly patients. Nonalbuminuric renal impairment was not associated with HbA_{1c} and correlated less strongly with retinopathy and hypertension than albuminuria, either alone or associated with reduced eGFR. It was associated with a higher prevalence of cardiovascular disease (CVD) than albuminuria alone, but lower than albuminuric renal impairment. Female sex correlated with nonalbuminuric renal impairment and male sex with the albuminuric forms.**

Conclusions **These data show that type 2 diabetic patients with nonalbuminuric renal impairment exhibit distinct clinical features, suggesting predominance of macroangiopathy as underlying renal pathology, and that this phenotype is associated with significant CVD burden.**
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Abbreviations: A/C, albumin/creatinine ratio; ACE-Is, angiotensin-converting enzyme inhibitors; AER, albumin excretion rate; ARBs, angiotensin II-receptor blockers; BP, blood pressure; CKD, chronic kidney disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CVD, cardiovascular disease; eGFR, estimated GFR; ESRD, end-stage renal disease; GFR, glomerular filtration rate; HbA_{1c}, glycated hemoglobin; IDMS, isotope dilution mass spectrometry; KDOQI, Kidney Disease Outcomes Quality Initiative; MDRD, Modification of Diet in Renal Disease; NEFRON, National Evaluation of the Frequency of Renal impairment cOexisting with NIDDM; NHANES III, Third National Health and Nutrition Examination Survey; NKF, National Kidney Foundation; OR, odds ratio; RIACE, Renal Insufficiency And Cardiovascular Events; UKPDS, U.K. Prospective Diabetes Study

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Introduction

The natural history of diabetic nephropathy has been derived mainly from studies in patients with type 1 diabetes. In these patients, microalbuminuria is the first sign of renal damage and may eventually progress to macroalbuminuria, which predicts subsequent decline of glomerular filtration rate (GFR) [1]. Therefore, assessment of urinary albumin excretion rate (AER) has traditionally played a central role in the screening,

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diagnosis, and management of diabetic nephropathy. However, in patients with long-standing type 1 diabetes, initial GFR loss may occur in the absence of albuminuria [2] and this is even more frequent in type 2 diabetes. Among 301 patients attending an outpatient clinic in Australia [3] and 1197 patients from the Third National Health and Nutrition Examination Survey (NHANES III) [4], of patients with GFR less than 60 ml/min per 1.73 m^2 body surface area, that is stage $\geq$3 chronic kidney disease (CKD) according to the classification of the National Kidney Foundation (NKF)'s Kidney Disease Outcomes Quality Initiative (KDOQI) [5], 39 and 36%, respectively, were normoalbuminuric. Recently, the National Evaluation of the Frequency of Renal impairment cOexisting with NIDDM (NEFRON) 11, an incident-driven survey of 3893 patients with type 2 diabetes in the primary care setting, has suggested that nonalbuminuric renal impairment has become the predominant form of stage $\geq$3 CKD [6]. This was confirmed in the cohorts form the UK Prospective Diabetes Study (UKPDS) [7] and the Action in Diabetes and Vascular disease: preterAx and diamicroN-MR Controlled Evaluation trial [8].

Whether the increasing prevalence of nonalbuminuric renal impairment in type 2 diabetes reflects heterogeneity of the pathology of renal disease [9,10] or a classification bias introduced by the NKF's KDOQI system is a matter of debate. This system has been criticized because it identifies patients with an eGFR less than 60 ml/min 1.73 m^2, without concomitant evidence of kidney damage, such as albuminuria, or reference to age [11]. Thus, though this CKD phenotype is very common, the significance of stage $\geq$3 CKD without albuminuria and, even more important, its prognosis in terms of both cardiovascular disease (CVD) risk and renal outcome have not been clarified yet. This represents an important limitation for patients' classification in both clinical and epidemiological settings.

In order to gain a better insight on the clinical meaning attributable to this specific, yet common condition, we have examined in a large cohort of patients with type 2 diabetes, the association of nonalbuminuric renal impairment with CVD risk factors and other complications, as compared to albuminuric CKD with either reduced (stage $\geq$3) or nonreduced (stages 1–2) eGFR.

Methods

Study population

We used the anamnestic, clinical and laboratory data obtained at the baseline visit for the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study (registered with ClinicalTrials.gov, NCT00715481; URL http://clinicaltrials.gov/ct2/show/NCT00715481), an observational, prospective cohort study on reduced eGFR as independent predictor of CVD morbidity and mortality (and renal outcome) in type 2 diabetes. The study protocol was approved by the locally appointed ethics committees. The RIACE population consists of 15 933 Caucasian patients with type 2 diabetes (American Diabetes Association criteria), visiting consecutively 19 hospital-based Diabetes Clinics of the National Health Service throughout Italy (see on-line appendix) in years 2007–2008. Exclusion criteria were dialysis or renal transplantation. The quality and completeness of data were controlled and 160 patients were excluded due to missing or implausible values; data from the remaining 15 773 patients were subsequently analyzed.

Albuminuria and estimated glomerular filtration rate

Albumin excretion rate was obtained from timed (24 h) urine collections or calculated from albumin/creatinine ratio (A/C) in early-morning, first-voided urine samples using a conversion formula developed in type 1 diabetes [12] and preliminary validated in a subgroup of patients from the RIACE cohort. Patients were instructed to discharge urine at start and to save urine for the next 24 h or to collect midstream urine for AER and A/C, respectively. Albuminuria was measured in fresh urine samples by immunonephelometry or immunoturbidimetry, in the absence of symptoms and signs of urinary tract infection or other interfering clinical conditions. As recommended [13], the analytical coefficient of variation of both methods was largely less than 15%, that is 2.1–5.2% for immunonephelometry vs. 3.4–8.1% for immunoturbidimetry, with a detection limit of 1.7 and 3.0 mg/l, respectively, according to previous studies [14]. As an external quality control of urinary albumin assays, 50 samples from each center were re-analyzed at the reference laboratory of the coordinating center using the immunonephelometry method to verify whether the coefficient of variations between the peripheral and the central values were below 15% at least in the relevant clinical range of 15–500 mg/l. Results showed that the coefficient of variations between the peripheral and the central values were lower than 15% in 94% of samples included in the 15–500 mg/l interval. As expected, coefficient of variations were more frequently higher for values outside this range, almost exclusively for very low albumin concentrations, with no impact on patient classification into albuminuria classes. One to three measurements for each patient were obtained; in case of multiple measurements, the geometric mean of two or three values was used for analysis. Patients were then assigned to one of the following classes of albuminuria (mg/24 h): normoalbuminuria (AER $<$30), microalbuminuria (AER 30–299), or macroalbuminuria (AER $\geq$300). In addition, normoalbuminuric patients were further classified as having normal (AER $<$10) or low albuminuria (AER 10–29), according to the recent definition of the NKF [15].

Serum creatinine was measured by the modified Jaffe method; the same assay was used for assessing urinary creatinine concentration. One to three measurements for

each patient were obtained and eGFR was calculated by the four-variable Modification of Diet in Renal Disease (MDRD) Study Eq. [16] using the mean serum creatinine value in case of multiple measures. As recommended [17], one of two versions of this equation was used, depending on whether or not serum creatinine methods had been calibrated to be traceable to an isotope dilution mass spectrometry (IDMS) reference method. Patients were then assigned to one of the following classes of eGFR (ml/min per 1.73 m^2): 1 ($\geq$90); 2 (60–89); 3 (30–59); 4 (15–29); and 5 (<15). GFR was also estimated using the recently developed Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, which was found to be more accurate and to provide lower estimates of CKD prevalence than the MDRD Study formula [18]. To this end, non-IDMS serum creatinine values were standardized using the following equation: $-0.166 + 1.10 \times$ [measured serum creatinine (mg/dl)]. To derive this equation, 201 frozen samples previously analyzed using a non-IDMS method were re-assayed by an IDMS reference method.

Patients were then classified as having no CKD or CKD stages 1–5, based on the presence or absence of micro or macroalbuminuria and the value of eGFR, according to the NKF's KDOQI [5]. Patients assigned to CKD stages (and GFR classes) 4 and 5 were pooled together.

Other parameters

The following information was collected by a structured interview: age at the time of the interview, family history of diabetes, hypertension, dyslipidemia, and premature (i.e. <55 years of age) CVD in a first degree relative, smoking status (never, former, current), known diabetes duration, current glucose, blood pressure (BP) and lipid-lowering therapy, with indication of the class of drug, comorbidities, and previous documented major acute CVD events, including myocardial infarction, stroke, ulcer or gangrene, amputation (minor or major), coronary, carotid, and lower limb revascularization (endovascular or surgical), and surgery for aortic aneurysm. CVD events were adjudicated based on hospital discharge records or specialist visits by an ad hoc committee in each participating center.

At physical examination, weight and height were assessed for body mass index (BMI) calculation; then BP was measured with a sphygmomanometer after the patient had been seated for at least 5 min.

Glycated hemoglobin (HbA_{1c}) was measured by high-performance liquid chromatography using Diabetes Control and Complications Trial (DCCT)-aligned methods, whereas triglycerides, high-density lipoprotein (HDL) and total cholesterol were assessed by standard methods and low-density lipoprotein (LDL) cholesterol was calculated using the Friedwald formula.

Retinopathy was assessed by an expert ophthalmologist by ophtalmoscopy or retinography (high-quality stereoscopic photographs) and classified into absent, non-advanced or advanced. Advanced retinopathy included maculopathy, preproliferative and proliferative retinopathy (or history of previous photocoagulation), and blindness, if less than 1/10 normal vision or 20/200 on the Snellen test.

Statistical analysis

Data are expressed as median [interquartile range (IQR)] or number of cases and percentages. The following statistical tests were applied: Kruskal–Wallis one-way ANOVA for continuous variables or Pearson chi-squared for categorical variables. Logistic regression analyses with stepwise variable selection were performed with no CKD (eGFR $\geq$60 ml/min per 1.73 m^2 without albuminuria) as reference category, nonalbuminuric or albuminuric stage $\geq$3 CKD (eGFR <60 ml/min per 1.73 m^2 with or without albuminuria), or stages 1–2 CKD (albuminuria with nonreduced eGFR) as dependent variable, as reference, and age, male sex, smoking status, known diabetes duration, HbA_{1c}, hypertension, triglycerides, HDL and LDL-cholesterol, lipid-lowering treatment, BMI, non-advanced and advanced retinopathy, and any previous CVD event as covariates. Hypertension was defined by systolic BP at least 140 mmHg and/or diastolic BP at least 90 and/or antihypertensive treatment. Conversely, triglycerides, HDL and LDL-cholesterol, and lipid-lowering treatment were considered separately in the regression models, to assess the contribution of each of them. To further investigate the relation of age with nonalbuminuric vs. albuminuric renal impairment, separate analysis was performed with each phenotype as dependent variable and age classes (<55, 55–64, 65–74, and $\geq$75 years) as covariates. Results of these analyses were expressed as odds ratios (ORs) with their 95% confidence interval (CI).

All statistical analyses were performed using SPSS 10.0 statistical software (SPSS Inc., Chicago, Illinois, USA).

Results

According to the MDRD Study formula, the prevalence rates of eGFR classes 1, 2, 3, and 4–5 was 29.6, 51.7, 17.1, and 1.6%, respectively. Prevalence of normo, micro, and macroalbuminuria was 73.1, 22.2, and 4.7%, respectively. Of the 11 538 patients with normoalbuminuria, 5515 (47.8%) had low albuminuria and 6023 (52.2%) had normal albuminuria. According to the NKF's KDOQI classification, 9865 patients (62.5%) had no CKD, whereas 1053 (6.7%), 1896 (12.0%), 2701 (17.1%), and 258 (1.7%) had stage 1, 2, 3, and 4–5 CKD, respectively.

Prevalence of albuminuria, retinopathy and CVD increased according to eGFR classes (Tables 1 and 2). Of the 2959 patients (18.8%) with renal impairment (i.e. eGFR <60 ml/min per 1.73 m^2), 1673 (56.6%) were normoalbuminuric, 861 (51.5%) with low albuminuria and

Table 1 Prevalence (number of cases and percentage in parentheses) of micro and macroalbuminuria according to classes of GFR as estimated by the MDRD Study and the CKD-EPI[a] formulas (ml/min per 1.73 m^2)

eGFR	Normoalbuminuria		Microalbuminuria	Macroalbuminuria
<30	69 (26.8%)		87 (33.7%)	102 (39.5%)
	81 (29.6%)		*110 (36.2%)*	*113 (37.2%)*
	Normal 23 (33.3%)	Low 46 (66.7%)		
	Normal 31 (38.3%)	Low 50 (61.7%)		
30–59	1604 (59.4%)		825 (30.5%)	272 (10.1%)
	1402 (58.2%)		*756 (31.4%)*	*253 (10.5%)*
	Normal 789 (49.2%)	Low 815 (50.8%)		
	Normal 664 (47.4%)	*Low 738 (52.6%)*		
60–89	6255 (76.7%)		1653 (20.3%)	244 (3.0%)
	5381 (75.5%)		*1515 (21.3%)*	*228 (3.2%)*
	Normal 3358 (53.7%)	Low 2897 (46.3%)		
	Normal 2861 (53.2%)	*Low 2520 (46.8%)*		
≥90	3610 (77.4%)		932 (20.0%)	120 (2.6%)
	4674 (78.8%)		*1116 (18.8%)*	*144 (2.4)*
	Normal 1853 (51.3%)	Low 1757 (48.7%)		
	Normal 2467 (52.8%)	*Low 2207 (47.2%)*		

[a] In italics.

812 (48.5%) with normal albuminuria, whereas 912 (30.8%) were microalbuminuric and 374 (12.6%) were macroalbuminuric. Normoalbuminuric renal impairment was less frequent in younger patients with reduced eGFR aged less than 55 years (35.0%) than in those aged 55–64 years (54.8%), 65–74 years (58.9%), and at least 75 years (56.7%). Of patients with reduced eGFR, 2028 (68.5%) had no retinopathy, 472 (16.0%) had nonadvanced retinopathy, and 459 (15.5%) had advanced retinopathy; moreover, 1280 patients (43.2%) had neither albuminuria nor retinopathy and only 538 (18.2%) had both albuminuria and retinopathy. Finally, out of patients with reduced eGFR, 1855 patients (62.7%) had no history of CVD, with 1145 (38.7%) presenting with neither albuminuria nor prior CVD, whereas major acute CVD events were documented in 1104 individuals (37.3%), with only 576 (19.5%) having both albuminuria and prior CVD. When eGFR was recalculated using the CKD-EPI equation, 2715 patients (17.2%) had values below 60 ml/min per 1.73 m^2, since 311 patients were reclassified from class 3 to class 2 and the opposite occurred for 67 patients, with the percentage of individuals with renal impairment and normoalbuminuria decreasing only slightly (53.9%) (Table 1). Also percentages of patients with retinopathy and CVD did not change significantly when patients were redistributed among eGFR classes using the CKD-EPI equation (Table 2).

Table 2 Prevalence (number of cases and percentage in parentheses) of nonadvanced and advanced retinopathy and previous CVD according to classes of GFR as estimated by the MDRD Study and the CKD-EPI[a] formulas (ml/min per 1.73 m^2)

eGFR	No retinopathy	Nonadvanced retinopathy	Advanced retinopathy
<30	138 (53.5%)	46 (17.8%)	74 (28.7%)
	163 (53.6%)	*59 (19.4%)*	*82 (27.0%)*
30–59	1890 (70.0%)	426 (15.8%)	385 (14.2%)
	1682 (69.8%)	*378 (15.7%)*	*351 (14.6%)*
60–89	6483 (79.5%)	961 (11.8%)	708 (8.7%)
	5578 (78.3%)	*916 (12.9%)*	*630 (8.8%)*
≥90	3768 (80.8%)	540 (11.6%)	354 (7.6%)
	4853 (81.8%)	*621 (10.5%)*	*460 (7.8%)*

eGFR	No CVD	Previous CVD
<30	139 (54.1%)	119 (45.9%)
	159 (52.3%)	*145 (47.7%)*
30–59	1716 (63.6%)	985 (36.4%)
	1496 (62.0%)	*915 (38.0%)*
60–89	6372 (78.2%)	1780 (21.8%)
	5436 (76.3%)	*1688 (23.7%)*
≥90	3891 (83.5%)	771 (16.5%)
	5027 (84.7%)	*907 (15.3%)*

[a] In italics.

Patients with nonalbuminuric renal impairment were more frequently female and nonsmoker, had shorter diabetes duration, lower levels of HbA_{1c}, triglycerides, and serum creatinine (and higher eGFR), and lower rate of retinopathy, CVD and antihypertensive treatment, including angiotensin-converting enzyme inhibitors (ACE-Is)/angiotensin II-receptor blockers (ARBs), than those with albuminuric renal impairment (Table 3). Also when compared with individuals with stages 1–2 CKD, patients with nonalbuminuric stage ≥3 CKD were more frequently female and nonsmoker, and had lower levels of HbA_{1c}, and rate of retinopathy but higher prevalence of any CVD and each major acute CVD event except ulcer/gangrene or amputation (Table 3).

Logistic regression analysis with stepwise variable selection (Table 4) showed that independent correlates of the three CKD phenotypes (nonalbuminuric and albuminuric stage ≥3 CKD, stages 1–2 CKD) vs. no CKD were age, diabetes duration, triglycerides, and BMI, with similar extent of association, except for age, which correlated less strongly with stages 1–2 CKD than with stage ≥3 CKD, with or without albuminuria. Further analysis of the relation of age with nonalbuminuric vs. albuminuric stage ≥3 CKD showed that the likelihood of having the albuminuric phenotype increased significantly (OR = 1.88; 95% CI 1.15–3.05) for age class 55–64 years as compared with younger individuals, whereas it remained

Table 3 Clinical characteristics of patients with nonalbuminuric and albuminuric renal impairment (eGFR <60 ml/min per 1.73 m^2, stage ≥3 CKD), albuminuria with nonreduced eGFR (eGFR ≥60 ml/min per 1.73 m^2, stages 1–2 CKD) and no CKD (eGFR ≥60 ml/min per 1.73 m^2 without albuminuria)

Variables	Stage ≥3 CKD nonalbuminuric	Stage ≥3 CKD albuminuric	Stages 1–2 CKD	No CKD	*P**
N (%)	1673 (10.6)	1286 (8.2)	2949 (18.7)	9865 (62.5)	
Age, years	73 (67–79)	73 (66–79)	66 (59–73)	58 (65–71)	<0.0001
Male sex, *n* (%)	576 (34.4)	773 (60.1)	2085 (70.7)	5526 (56.0)	<0.0001
Smoking, *n* (%)					<0.0001
No	1104 (66.0)	685 (53.3)	1444 (49.0)	5695 (57.7)	
Ex	424 (25.3)	436 (33.9)	922 (31.3)	2652 (26.9)	
Yes	145 (8.7)	165 (12.8)	583 (19.7)	1518 (15.4)	
Diabetes duration, years	14 (6–23)	18 (9–26)	12 (6–21)	9 (4–18)	<0.0001
HbA_{1c}, %	7.4 (6.6–8.4)	7.6 (6.8–8.8)	7.6 (6.7–8.7)	7.2 (6.5–8.1)	<0.0001
BMI, kg/m^2					
Men	27.9 (25.5–31.0)	28.2 (25.6–31.1)	28.7 (25.9–31.6)	27.5 (25.1–30.4)	<0.0001
Women	29.0 (25.8–33.0)	29.8 (26.2–33.9)	29.9 (26.4–34.2)	28.8 (25.4–32.8)	<0.0001
Triglycerides (mg/dl)	129 (96–182)	142 (104–201)	126 (90–179)	112 (82–156)	<0.0001
Total-chol (mg/dl)	183 (161–209)	182 (156–210)	181 (156–208)	183 (160–207)	0.039
HDL-chol (mg/dl)					
Men	43.0 (36.0–51.0)	42.0 (35.0–51.0)	44.4 (38.0–53.0)	46.0 (39.0–55.0)	<0.0001
Women	50.0 (42.0–59.5)	48.0 (40.1–59.0)	51.0 (43.0–60.0)	52.9 (45.0–62.0)	<0.0001
LDL-chol (mg/dl)	105 (86–129)	102 (81–125)	104 (83–127)	107 (87–128)	<0.0001
SBP (mmHg)	140 (125–150)	140 (130–150)	140 (130–150)	135 (125–150)	<0.0001
DBP (mmHg)	80 (70–80)	80 (70–80)	80 (71–85)	80 (70–85)	<0.0001
Serum creatinine (mg/dl)	1.21 (1.03–1.40)	1.40 (1.25–1.72)	0.89 (0.75–1.00)	0.84 (0.71–0.95)	<0.0001
Albuminuria (mg/24 h)	10.2 (5.3–17.0)	124.9 (56.7–383.9)	69.5 (42.6–145.5)	9.3 (5.1–15.2)	<0.0001
eGFR MDRD (ml/min per 1.73 m^2)	52.1 (44.9–56.8)	46.6 (36.3–53.6)	82.5 (71.9–97.7)	83.7 (73.3–96.9)	<0.0001
eGFR CKD-EPI (ml/min per 1.73 m^2)	52.3 (44.3–57.9)	46.1 (35.0–54.1)	87.4 (75.2–97.4)	89.1 (78.0–97.8)	<0.0001
Retinopathy [*n* (%)]					<0.0001
No	1280 (76.5)	747 (58.1)	2067 (70.1)	8182 (82.9)	
Nonadvanced	219 (13.1)	252 (19.6)	448 (15.2)	1052 (10.7)	
Advanced	174 (10.4)	287 (22.3)	434 (14.7)	631 (6.4)	
Any CVD event [*n* (%)]	528 (31.6)	576 (44.8)	794 (26.9)	1756 (17.8)	<0.0001
AMI [*n* (%)]	271 (16.2)	267 (20.8)	346 (11.7)	874 (8.9)	<0.0001
Stroke [*n* (%)]	75 (4.5)	91 (7.1)	123 (4.2)	226 (2.3)	<0.0001
Ulcer/gangrene or amputation [*n* (%)]	72 (4.3)	127 (9.9)	150 (5.1)	183 (1.9)	<0.0001
Coronary revascularization [*n* (%)]	246 (14.7)	228 (17.7)	315 (10.7)	794 (8.0)	<0.0001
Carotid revascularization [*n* (%)]	132 (7.9)	170 (13.2)	193 (6.5)	372 (3.8)	<0.0001
Lower limb revascularization, *n* (%)	68 (4.1)	104 (8.1)	102 (3.5)	182 (1.8)	<0.0001
Lipid-lowering treatment [*n* (%)]	909 (54.3)	704 (54.7)	1363 (46.2)	4310 (43.7)	<0.0001
Antihypertensive treatment [*n* (%)]	1424 (85.1)	1167 (90.7)	2318 (78.6)	6246 (63.3)	<0.0001
ACE-I/ARB treatment [*n* (%)]	1159 (69.3)	992 (77.1)	1998 (67.8)	5016 (50.8)	<0.0001

ACE-I, ACE inhibitors; Alb, micro + macroalbuminuria; AMI, acute myocardial infarction; ARB, angiotensin II receptor blockers; chol, cholesterol; CVD, cardiovascular disease. Classes of eGFR were identified using the MDRD Study formula. *Kruskal–Wallis one-way ANOVA or Pearson chi-square.

Table 4 Independent correlates of nonalbuminuric and albuminuric renal impairment (eGFR <60 ml/min per 1.73 m^2, stage ≥3 CKD) and albuminuria with nonreduced eGFR (eGFR ≥60 ml/min per 1.73 m^2, stages 1–2 CKD) vs. no CKD (eGFR ≥60 ml/min per 1.73 m^2 without albuminuria)

	Stage ≥3 CKD nonalbuminuric		Stage ≥3 CKD albuminuric		Stages 1–2 CKD	
	OR	95% CI	OR	95% CI	OR	95% CI
Age (year)	1.101	1.093–1.109	1.092	1.083–1.101	1.019	1.014–1.024
Male sex	0.444	0.392–0.503	1.365	1.182–1.576	2.089	1.891–2.308
Smoking						
Ex	–	–	–	–	1.075	0.969–1.193
Current	–	–	–	–	1.409	1.247–1.592
Diabetes duration	1.006	1.000–1.012	1.019	1.012–1.026	1.026	1.020–1.032
HbA_{1c}	–	–	1.061	1.014–1.110	1.179	1.144–1.214
BMI (unit)	1.027	1.015–1.039	1.034	1.020–1.049	1.035	1.026–1.034
Triglycerides (×10 mg/dl)	1.046	1.036–1.056	1.070	1.059–1.081	1.027	1.020–1.034
HDL-cholesterol (×5 mg/dl)	0.942	0.919–.965	0.936	0.910–.963	–	–
Lipid-lowering treatment	1.216	1.082–1.367	–	–	–	–
Hypertension	1.612	1.317–1.974	2.534	1.922–3.340	1.804	1.576–2.065
Retinopathy						
Nonadvanced	1.086	0.910–1.297	1.944	1.624–2.327	1.400	1.229–1.594
Advanced	1.447	1.184–1.769	3.927	3.261–4.730	2.252	1.953–2.597
Previous CVD	1.661	1.454–1.898	2.274	1.982–2.610	1.211	1.090–1.345

Logistic regression analysis with stepwise variable selection. Variable excluded: LDL-cholesterol. Classes of eGFR were identified using the MDRD Study formula.

quite stable for age classes 65–74 years (2.42; 1.53–3.80) and at least 75 years (2.05; 1.30–3.26). HDL-cholesterol was inversely associated to stage ≥3 CKD, but not to stages 1–2 CKD, which was related to smoking status. In addition, ORs for hypertension and advanced retinopathy were higher in patients with CKD with than in those without albuminuria. Conversely, OR for major acute CVD events in patients with nonalbuminuric renal impairment was higher (1.66) than that of individuals with albuminuria and nonreduced eGFR (1.21), though lower than that of patients with albuminuric renal impairment (2.27). Finally, HbA_{1c} and nonadvanced retinopathy correlated with albuminuric CKD (independent of stage), whereas female sex, and lipid-lowering treatment correlated with nonalbuminuric renal impairment.

Discussion

The study shows that nonalbuminuric renal impairment is the predominant form of stage ≥3 CKD in a large cohort of Italian patients with type 2 diabetes. This is at variance with previous studies in smaller cohorts from Australia [3] and US [4], but consistent with more recent studies [6–8]. Therefore, our findings support the concept of a shift in the phenotype of renal impairment among type 2 diabetic patients with a worldwide growing prevalence of the nonalbuminuric form.

Since definition of CKD stage in our study and previous surveys was based on GFR estimation by the MDRD Study formula, prevalence rates may, however, be affected by the estimation procedure or simply by the age of the diabetic population. For this reason, we have recalculated GFR using the more accurate CKD-EPI equation. The corresponding figures were almost identical to those obtained using the MDRD Study formula. Moreover, the extent of the association with age was similar in the two phenotypes of stage ≥3 CKD, with nonalbuminuric renal impairment being predominant in older individuals as well as in patients aged 55–64 years, who showed a risk of presenting the nonalbuminuric phenotype similar to that associated with older ages. Taken together, these findings indicate that the increasing prevalence of the nonalbuminuric phenotype is not a misclassification artifact and support the existence of both nonalbuminuric and albuminuric pathways to renal impairment in type 2 diabetes [19].

In interpreting the increasing prevalence of nonalbuminuric renal impairment one should consider the impact of modern therapeutic intervention. For instance, during the past two decades, the number of diabetic patients with hypertension and/or nephropathy treated with ACE-Is/ARBs has been dramatically increasing. In our own cohort, 58.1% of the patients were on such a treatment and an even higher percentage has been reported in the recent NEFRON 11 [6]. These figures are quite different from the 13% reported in the NHANES III in years 1988–1994 [4]. Although this is a plausible hypothesis, in our study, the prevalence of the nonalbuminuric phenotype was even higher in stage ≥3 CKD patients not on ACE-I/ARB treatment than in all individuals with reduced eGFR (63.6 vs. 56.6%). In fact, the use of these agents was more common in patients with than in those without albuminuria, yet this may be an indication effect.

In addition, the increasing prevalence of nonalbuminuric renal impairment may reflect changes in the underlying pathology of renal disease in type 2 diabetes, with macroangiopathy prevailing over microangiopathy. Such a shift may also be due to changes in treatment, particularly to tighter control of glucose, lipid, and BP levels that is being achieved in diabetic patients on the grounds of results of trials on intensive treatment. Our finding that HbA_{1c} was an independent correlate of albuminuric CKD, independent of eGFR, but not of nonalbuminuric CKD, provides strong support to this view and is consistent with the UKPDS results showing that HbA_{1c} is an independent risk factor for albuminuria, but not for GFR impairment [7]. Moreover, nonalbuminuric renal impairment was less strongly associated with the other microvascular complication of diabetes, that is retinopathy, than the albuminuric forms of CKD. The concept that microangiopathy is not the prevailing underlying pathology in these patients is in keeping with the finding that nonalbuminuric renal impairment, though very frequent in type 2 diabetic patients with CKD, is less common than in the general population, due to the contribution of diabetes (and diabetic microangiopathy) to the development of the albuminuric form [6]. However, in the Atherosclerosis Risk in Communities Study, the association between HbA_{1c} and incident CKD was present even in the absence of either albuminuria or retinopathy.

Hypertension correlated less strongly with nonalbuminuric than with albuminuric renal impairment. This is in keeping with the view that increased BP levels represent a major risk factor for macroangioathy, but also for renal disease: albuminuria is regarded as a reliable marker of target organ damage in hypertension. This is also consistent with the inverse relationship between eGFR and intrarenal resistive index as well as indexes of systemic atherosclerosis, such as carotid intima–media thickness and arterial stiffness, reported in type 2 diabetes [20], though this association was shown to occur independent of albuminuria [21].

Our study provides clear evidence that nonalbuminuric renal impairment is significantly associated with acute major CVD events: type 2 diabetic patients with nonalbuminuric renal impairment had higher prevalence of CVD and stronger independent correlation with prior CVD events than those with albuminuria and nonreduced eGFR. This is consistent with the view that eGFR less than 60 ml/min per 1.73 m^2 is a powerful predictor of CVD morbidity and mortality in the general population and in diabetic patients, independent of

traditional CVD risk factors and albuminuria [8,22]. The even higher CVD prevalence/association observed in patients with albuminuric renal impairment is consistent with the multiplicative risk associated with the combination of albuminuria and reduced eGFR [23]. Interestingly, though patients with the albuminuric renal impairment were predominantly women (65.6%), as previously reported [6,7], prevalence rate of CVD was higher in male than in female patients with this phenotype (44.3 vs. 24.7%), as in the entire cohort (27.6 vs. 17.4%). This is consistent with the belief that the MDRD Study formula, which was derived from CKD patients, may overcorrect for female sex, since sex differences were lower when GFR was measured in individuals ranging over the entire GFR interval [24], and also with the recent finding that individuals reclassified upward with the CKD-EPI equation were predominantly women with a favorable cardiovascular risk profile [25]. It should be noted, however, that this is a cross-sectional analysis and longitudinal studies are required to confirm the association of the nonalbuminuric phenotype with CVD and also to clarify whether and to which extent reduced eGFR also predicts further loss of renal function, independent of albuminuria.

Other limitations should be considered in interpreting these data. Different methods were used to measure albuminuria and serum creatinine. Nonetheless, the prevalence of nonalbuminuric renal impairment was not affected by the method employed (56.1% with AER vs. 56.8% with A/C, 57.6% with immunonephelometry vs. 55.9% with immunoturbidimetry and 56.2% for IDMS-traceable vs. 57.1% for non-IDMS-traceable methods). Confirmation of albuminuria may be required to ensure reliable definition of the renal impairment phenotype. In our cohort, up to 74.2% of type 2 diabetic patients had only one assessment. However, in the remaining patients with two to three measurements, the concordance rate between the first value and the geometric mean of multiple measurements was above 90% [26]. Finally, retinopathy was evaluated by different ophthalmologists. Although this may be a potential source of variability, the inclusion of history of previous photocoagulation in the definition of diabetic retinopathy should have reduced the bias in detecting advanced conditions.

In conclusion, this large-cohort study shows that nonalbuminuric renal impairment is the predominant clinical phenotype of stage $\geq$3 CKD in type 2 diabetes, irrespective of patients' age and the equation use for GFR estimation. Moreover, patients with nonalbuminuric renal impairment exhibit distinct clinical features as compared with patients with albuminuria, either alone or combined with reduced eGFR (prevailing female sex and nonsmoker status, no or lower association with HbA_{1c}, retinopathy and hypertension) as well as a prevalence of major acute CVD events which is intermediate between that associated with these two forms of albuminuric CKD and is almost twice higher in men than in women. These data indicate that the increasing prevalence of this phenotype does not reflect misclassification of elderly patients (though it might be overestimated in women), but rather changes in the underlying pathology of renal disease in type 2 diabetes, possibly due to changes in treatment; and nonalbuminuric renal impairment is associated with clinically significant CVD burden.

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Conflicts of interest

There are no conflicts of interest.

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