Cross-Classification of Microalbuminuria and Reduced Glomerular Filtration Rate

Associations Between Cardiovascular Disease Risk Factors and Clinical Outcomes

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Background: Chronic kidney disease is defined by reduced estimated glomerular filtration rate (reduced eGFR) or by microalbuminuria (MA). Concordance between reduced eGFR and MA and associated cardiovascular disease (CVD) and all-cause mortality according to these definitions is uncertain.

Methods: Participants (n=2966 [52.6% were women], mean age, 59 years) were drawn from the Framingham Offspring Cohort. Participants were classified into 4 groups based on the presence or absence of reduced eGFR (eGFR < 59 mL/min/1.73 m² in women, < 64 mL/min/1.73 m² in men or MA (spot urinary albumin to creatinine ratio of at least 30 mg/g). Cox proportional hazard models were used to determine the combined risk of CVD events and all-cause mortality for each group.

Results: Of the participants, 9.9% (n=295) had reduced eGFR, and 12.2% (n=362) had MA. Among those with reduced eGFR and without MA and those without reduced eGFR and with MA, were at significantly increased risk of all-cause mortality (HR 2.2 [95% CI, 1.4-3.6] and HR 1.7 [95% CI, 1.1-2.6], respectively).

Conclusions: Reduced eGFR and MA are relatively common conditions with different risk factor profiles. The coexistence of reduced eGFR and MA was present in 2.8% of the study sample and conferred substantial increased risk for CVD and all-cause mortality, in part because of a heavy burden of CVD risk factors.

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CHRONIC KIDNEY DISEASE (CKD) affects an estimated 19 million adults in the United States. It is an independent risk factor for cardiovascular disease (CVD) and all-cause mortality and is associated with a high burden of CVD risk factors, including hypertension, diabetes mellitus, dyslipidemia, and smoking. Chronic kidney disease is defined as an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m², or the presence of microalbuminuria (MA) for at least 3 months. Microalbuminuria has also been shown to be independently associated with an increased risk of CVD events, and in a study using National Health and Nutritional Examination Survey (NHANES) III data, the use of MA and renal insufficiency based on an eGFR as separate criteria identified different groups within the study sample. Many large epidemiologic studies do not have contemporaneous collection of both serum creatinine and urinary albumin excretion measurements and are therefore limited in their definition of CKD according to eGFR criteria. As a result, it has not been established how the addition of MA to CKD staging affects the concomitant CVD risk factor burden and how this relates to CVD outcomes and all-cause mortality in the setting of CKD.

METHODS

STUDY SAMPLE

The Framingham Offspring Study was assembled in 1971 and included children of the original cohort as well as the spouses of the children. Enrollment in the offspring cohort con...
sisted of 5124 men and women. Members of the offspring cohort participated in clinic examinations approximately every 4 years, and the design and methods of these examinations are described elsewhere.\textsuperscript{13} Offspring cohort participants who underwent an examination during the sixth examination cycle (1993-1998) were included in the present investigation; that cycle was chosen because of the contemporaneous measurement of both serum creatinine and urinary albumin excretion. The study was approved by the institutional review boards of the Boston University Medical Center. All subjects provided written informed consent.

Of a total of 3532 attendees at the sixth examination cycle, 80 were excluded owing to missing serum creatinine values, and 486 were excluded owing to missing urinary albumin–creatinine ratio (UACR) values, resulting in a final study sample of 2966 participants.

MEASUREMENTS AND DEFINITIONS

The eGFR was calculated using the simplified Modification of Diet in Renal Disease (MDRD) Study Equation.\textsuperscript{10,14,15} The National Kidney Foundation Disease Outcome Quality Initiative working group defines CKD as a glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m\textsuperscript{2}. Our definition of CKD is based on this group’s definition, but we modified ours to be a GFR of less than 59 mL/min/1.73 m\textsuperscript{2} in women or less than 64 mL/min/1.73 m\textsuperscript{2} in men.\textsuperscript{16} This modification was implemented because the use of the 60 mL/min/1.73 m\textsuperscript{2} cutoff point identified 30% more women than men as having CKD. For the remainder of this article, we will use the term reduced eGFR to differentiate clinical CKD (which by definition includes individuals with MA) from our working definition of reduced GFR.

The modified Jaffe method was used to measure serum creatinine levels. Variations in the measurement of serum creatinine can occur in different laboratories, so serum creatinine measurements were calibrated in a 2-step process. In the first step, a correction factor of 0.23 mg/dL (to convert serum creatinine to micromoles per liter, multiply by 88.4) was applied to the NHANES III serum creatinine values.\textsuperscript{17} Next, our serum creatinine values were calibrated by age (20-39, 40-59, 60-69, and \(\geq 70\) years) and sex according to the corresponding NHANES III age- and sex-specific means among white NHANES III participants; this alignment is described elsewhere.\textsuperscript{16}

At the time of the examination, 3-mL spot urine samples were obtained and kept at \(-20^\circ\)C until quantification. Urinary creatinine concentration was measured using a modified Jaffe method; the intra-assay coefficient of variation varied from 1.7% to 3.8%. Urinary albumin concentration was assessed using immunoturbimetry (Tina-quant albumin assay; Roche Diagnostics, Indianapolis, Indiana). Differences in urine concentrations were accounted for by taking the UACR values (urinary albumin [in milligrams] to creatinine [in grams] ratio). The UACR has been validated and is a reliable measure of urinary albumin excretion. The UACR is also correlated with albumin excretion rates determined using a 24-hour urine collection.\textsuperscript{18,19} We defined MA as a UACR of at least 30 mg/g.\textsuperscript{10}

CVD AND RISK FACTOR ASSESSMENT

At each examination, participants underwent blood testing and were assessed for CVD. Diabetes mellitus was defined as a fasting blood glucose level of 126 mg/dL or higher (to convert glucose to millimoles per liter, multiply by 0.0555) at a Framingham Heart Study examination or if a participant was receiving insulin and/or oral hypoglycemic treatment for diabetes mellitus. Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or receiving medication for treatment of hypertension. The mean of the 2 readings taken by an examining physician was used for the systolic and diastolic blood pressure measurements. A low-density lipoprotein cholesterol value was defined as a value lower than 40 mg/dL in men and lower than 50 mg/dL in women, and a high triglyceride level was defined as a value of 150 mg/dL or higher (to convert high-density lipoprotein cholesterol and triglyceride level to millimoles per liter, multiply by 0.0259 and 0.0113, respectively). Current smoking status was defined as smoking at least 1 cigarette per day during the year prior to the examination. Central obesity was defined as a waist circumference greater than 88 cm in women and 102 cm in men. Metabolic syndrome was defined using modified Adult Treatment Panel III criteria.\textsuperscript{20} A 3-physician panel adjudicated all CVD end points. Coronary heart disease (CHD) included recognized myocardial infarction, coronary insufficiency, and angina pectoris; CVD included CHD, unrecognized myocardial infarction, stroke, transient ischemic attack, and intermittent claudication. Diagnostic criteria for prevalent CVD and CHD events are described elsewhere.\textsuperscript{21}

STATISTICAL ANALYSIS

Participants were categorized into 4 groups based on the presence or absence of reduced eGFR and/or MA. The prevalence of CVD risk factors were compared among these 4 groups; age- and sex-adjusted \(P\) values were obtained from analysis of variance models for continuous variables; logistic regression models were used for dichotomous variables.\textsuperscript{22} The UACR was log-transformed prior to its inclusion in the analysis of variance model owing to its skewed distribution. The comparisons between (1) those with reduced eGFR and with MA compared with those with reduced eGFR and without MA and (2) those with reduced eGFR and without MA compared with those without reduced eGFR and with MA were chosen a priori based on where we felt key differences might lie. Age- and sex-adjusted incidence rates of the combined end point of CVD and all-cause mortality were calculated, with follow-up of all events through December 31, 2004. Cox proportional hazards models were used to calculate the hazards ratios (HRs) of CVD and all-cause mortality within each subgroup. Models were first age- and sex-adjusted and then additionally adjusted for systolic blood pressure, hypertension treatment, body mass index (calculated as weight in kilograms divided by height in meters squared), current smoking, diabetes mellitus, total–high-density lipoprotein cholesterol ratio, and prevalent CVD. Statistical analyses were performed using SAS statistical software (version 8; SAS Inc, Cary, North Carolina).\textsuperscript{23} A 2-tailed \(P<.05\) was defined as statistically significant.

PREVALENCE OF REDUCED eGFR AND MA

Participants with reduced eGFR comprised 9.9% of the study sample; 12.2% of the overall study sample had MA. Among those with reduced eGFR, 28% also had MA (95% confidence interval, 23.1-33.7). Overall, participants with reduced eGFR and with MA comprised 2.8% of the study sample, whereas those with reduced eGFR and without MA comprised 7.1% of the study sample, and those without reduced eGFR and with MA comprised 9.4% of the study sample.
Participants with reduced eGFR had a higher risk factor burden compared to those without reduced eGFR. Specifically, participants with reduced eGFR and with MA were more likely to have hypertension, diabetes mellitus, and CVD, as well as a lower mean eGFR. Overall, there were 396 events over a mean duration of follow-up of 7.6 years. Age- and sex-adjusted incidence rates for all 4 subgroups are presented in Table 2. When compared with participants without reduced eGFR and without MA, those with reduced eGFR and with MA had an increased risk of CVD and all-cause mortality in age-, sex-, and multivariable-adjusted models (Table 3 and Figure). Most of these observations seemed to be driven by the all-cause mortality end point (Table 3). In multivariable analyses, those with reduced eGFR and with MA had a 2-fold increased risk of death (P = .01), those with reduced eGFR and without MA had a 70% increased risk of death (P = .01), and those without reduced eGFR and with MA had a nonsignificant 20% increased risk of death (P = .34).

### CVD AND ALL-CAUSE MORTALITY

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### SECONDARY ANALYSIS

In a secondary analysis, we used a definition of eGFR less than 60 mL/min/1.73 m² for both women and men;
findings were not materially different from the primary analyses. For the combined end point of CVD and all-cause mortality, the multivariable-adjusted HR was 1.9 (P = .002), 1.4 (P = .06), and 1.1 (P = .39) for those with reduced eGFR and with MA, with reduced eGFR and without MA, or without reduced eGFR and with MA, respectively. For all-cause mortality, the multivariable-adjusted HR was 2.2 (P = .003), 1.7 (P = .01), and 1.3 (P = .25), for those with reduced eGFR and with MA, with reduced eGFR and without MA, or without reduced eGFR and with MA, respectively. When an age-squared term was incorporated into the analyses, results were essentially unchanged (data not shown). When individuals with a UACR greater than 300 mg/g were excluded (n = 41), the results were somewhat attenuated, although the findings for all-cause mortality remained strong for those with reduced eGFR and with MA, as well for those with reduced eGFR and without MA (data not shown).

**COMMENT**

In the Framingham Heart Study, both reduced eGFR and MA were modestly common, but only about one-quarter of patients with reduced eGFR also had MA. Therefore, the cross-classification of reduced eGFR with vs without MA identified different groups of participants in our study sample. Those with reduced eGFR and with MA, although accounting for only 2.8% of our sample, had a high burden of CVD risk factors and a high risk of all-cause mortality. Lastly, those either with reduced eGFR or MA but not both were fairly common and had different risk factor profiles. Although we observed similar outcomes for the combined CVD and all-cause mortality end point, those with reduced eGFR alone were at increased risk for all-cause mortality, whereas those with MA alone were not. These findings underscore the differences in risk factor profiles and risk identified by these 4 subgroups in our study sample.

**IN THE CONTEXT OF THE CURRENT LITERATURE**

To the best of our knowledge, our findings are among the first data to compare CVD risk factor profiles and outcomes in these 4 groups of individuals with reduced eGFR and/or MA. These subgroups are enriched for CVD risk factors and increased CVD risk, which may be due to the known associations among reduced eGFR, MA, CVD risk factors, and CVD risk. Established risk factors for CVD, including hypertension, diabetes mellitus, dyslipidemia, obesity, and smoking, have also been shown to be associated with the development of CKD. Chronic kidney disease is associated with a high burden of traditional CVD risk factors among individuals with mild renal insufficiency as well as among those with end-stage renal disease. Microalbuminuria is also independently associated with several CVD risk factors, including hypertension, diabetes mellitus, smoking, and previous myocardial infarction. Chronic kidney disease has been identified as an independent risk factor for CVD, all-cause mortality, and noncardiac mortality. Microalbuminuria has been identified as a risk factor for CVD, and even among individuals free of hypertension or diabetes mellitus, very low levels of urinary albumin are associated with CVD. Results from the MICRO-HOPE study demonstrated that urinary albumin excretion is an independent risk factor for CVD, even after adjustment for serum creatinine. Furthermore, the HR of death from CVD, myocardial infarction, or stroke was 2-fold higher among individuals with both MA and renal insufficiency vs those without either condition.
The high risk factor burden among those with reduced eGFR and with MA provides insight as to why the outcomes in this subgroup were significantly worse when compared with those without reduced eGFR and without MA. However, the elevated risk in this group persisted despite adjustment for CVD risk factors, suggesting that additional mechanisms may be responsible for these observations. Proteinuria is considered a marker of endothelial dysfunction and compounded with reduced eGFR may significantly increase the risk of events. Furthermore, individuals with CKD have elevated markers of endothelial dysfunction, including plasminogen activator inhibitor-1, tissue plasminogen inhibitor, and forearm blood flow, suggesting that widespread vascular injury may be a hallmark of CKD and proteinuria.

It is less clear why those with reduced eGFR and without MA are at increased risk for all-cause mortality, but those with MA and without reduced eGFR are not. In this setting, low eGFR may be an overall marker of frailty and poor physical function; frailty has been shown to be increased among individuals with CKD. The additional observation that associations among those with MA but without reduced eGFR were attenuated on adjustment for CVD risk factors, whereas associations for those with both conditions were not, suggests that novel risk factors not accounted for in this study may be responsible for the observed increased mortality among those with both conditions. Further research is necessary to uncover the etiology of the differences in all-cause mortality in these 2 groups.

The overlap among individuals with reduced eGFR and with MA has been previously reported in the literature, with varying results. In NHANES III, 50.7% of individuals with a GFR of 30 to 60 mL/min/1.73 m² also had MA, with MA defined as a UACR greater than 26.5 mg/g. This overlap between reduced eGFR and MA is higher than the 28% observed in our study sample. Potential explanations for these differences may include lower cutoff points for defining MA than those used in our study. The NHANES III data also represent a sample of multiethnic individuals, whereas the Framingham sample is composed primarily of white individuals. It is unclear how our results would compare with those from only the white participants from NHANES III. Results from the Kidney...
In our sample, those with MA and without reduced eGFR were characterized by a higher prevalence of diabetes mellitus. Indeed, in this subgroup, those with diabetes mellitus had a higher eGFR than those without (95 vs 89 mL/min/1.73 m²). Although diabetes mellitus status plays an important role in differentiating this subgroup from those with reduced eGFR and without MA, smoking is clearly another important contributor, because those with MA and without DM were characterized primarily by increased prevalence of smoking (23% among those with MA and without reduced eGFR vs 8.5% among those with reduced eGFR and without MA), suggesting that smoking may be an important correlate of elevated MA in the absence of reduced eGFR. Smoking has been shown to be related to urinary albumin excretion, and it is a risk factor for incident CKD; therefore, the effects of cigarette smoking on urinary albumin excretion may be in the causal pathway for CKD progression. It is also possible that the presence of both low GFR and MA reduces the potential misclassification in identifying those individuals who truly have CKD. This could also explain some of the increased risk seen in those with reduced eGFR and with MA.

STRENGTHS AND LIMITATIONS OF OUR STUDY

Strengths of the Framingham Heart Study sample include routine ascertainment of CVD risk factors. Furthermore, our sample was not selected for CKD. The creatinine measurements used to estimate GFR were indirectly calibrated to the Cleveland Clinic Laboratory, which helps correct for differences in creatinine measurements based on different laboratory standards. Some limitations exist. Our sample is composed primarily of white individuals from a limited geographical area. However, the risk factors and associated CVD outcomes seen in the Framingham Heart Study have been validated in other more diverse samples. In our study, the simplified MDRD Study Equation was used to estimate GFR instead of a direct measurement of GFR, and the MDRD Study Equation has been shown to underestimate GFR in individuals without CKD. However, this affects the dichotomous classification of CKD is uncertain, because this study was performed using continuous GFR values. The MDRD Study Equation works better with lower eGFRs and becomes more robust in classifying individuals with vs those without CKD. According to the National Kidney Foundation guidelines, CKD is classified as a reduction in GFR over a 3-month period. Our eGFR and MA estimations are based on a single serum creatinine measurement, which may lead to misclassification of CKD status and an underestimation of the magnitude of our findings. The UACR measurements in this study were based on a spot urine collection as opposed to 24-hour urine collection; however, spot urine collections estimate 24-hour collections. Our sample contains few individuals with macroalbuminuria; therefore, our findings are unlikely to be generalizable to this group. Lastly, we had few CVD events to adequately evaluate the cross-classification of reduced eGFR and MA for CVD alone.

IMPLICATIONS FOR FUTURE RESEARCH

Based on these results, participants with reduced eGFR and with MA have a high risk factor burden for CVD and are at an increased risk of CVD and all-cause mortality, and a substantially increased risk of death. Those with reduced eGFR, even in the absence of MA, are also at increased risk for death. This finding suggests that this group in particular should be targeted for CVD risk factor reduction, although further research is warranted to determine whether risk factor reduction in this setting of primarily stage 3 CKD and MA would help improve CVD outcomes. In particular, the role of smoking cessation in the prevention of CKD progression should be explored further.

Clinically, these results suggest that screening for both decreased eGFR and MA might help to identify different groups of at-risk individuals. Currently, screening guidelines for CKD include those with known risk factors, such as hypertension, diabetes mellitus, and non-steroidal anti-inflammatory drug use. Universal screening guidelines for MA are not in place; however, consensus statements have suggested that individuals with diabetes mellitus, hypertension, a family history of CKD, or CVD should be routinely tested, although data on optimal screening interval, aside from data on diabetes mellitus, do not exist.

In conclusion, the presence of reduced eGFR and MA identified 4 different groups of individuals with different CVD risk factor profiles. The highest risk factor burden was seen among individuals with reduced eGFR and with MA, and equivalent risk was observed among those with either reduced eGFR or MA. The etiology of the increased risk of mortality requires further exploration.

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