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Article

Combined Association of Creatinine, Albuminuria, and Cystatin C with All-Cause Mortality and Cardiovascular and Kidney Outcomes

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Summary

Background Estimated GFR by serum creatinine ($eGFR_{creatinine}$) is a pivotal measure of kidney function in clinical practice but can be affected by several non-GFR determinants, resulting in misclassification. Combining multiple kidney markers to predict risk is an area of substantial interest.

Design, setting, participants, & measurements This study followed 9489 adults from visit 4 (1996–1998) of the Atherosclerosis Risk in Communities Study for a median of 11.2 years, and assessed joint association of $eGFR_{creatinine}$, $eGFR_{cystatin}$, and urinary albumin/creatinine ratio (ACR) with mortality, coronary heart disease, heart failure, AKI, and ESRD using Cox proportional hazards models. The predictive ability of ACR and $eGFR_{cystatin}$ beyond $eGFR_{creatinine}$ was also investigated.

Results Lower $eGFR_{creatinine}$ and $eGFR_{cystatin}$ as well as elevated ACR were independently associated with risk for all outcomes. $eGFR_{creatinine}$ <60 was not associated with risk of mortality, coronary heart disease, or heart failure if $eGFR_{cystatin}$ ≥60 with ACR <30 mg/g compared with those with all three markers above CKD cutoffs (*i.e.*, $eGFR_{cystatin}$ ≥60, $eGFR_{creatinine}$ ≥60, and ACR<30), whereas risk association with kidney outcomes remained: Hazard ratio (95% confidence interval), 0.96 (0.66, 1.39) for mortality, 0.85 (0.55, 1.31) for coronary heart disease, 0.99 (0.60, 1.63) for heart failure, 1.61 (0.92, 2.82) for AKI, and 3.53 (1.06, 11.68) for ESRD. Adding ACR to the fully adjusted model with $eGFR_{creatinine}$ or adding $eGFR_{cystatin}$ to both $eGFR_{creatinine}$ and ACR improved risk classification for all outcomes ($P \leq 0.01$).

Conclusions $eGFR_{cystatin}$ can be a useful confirmatory marker in those with $eGFR_{creatinine}$ <60 and whose ACR is <30 mg/g. This approach improves risk classification, and provides reassurance to a large group of individuals with $eGFR_{creatinine}$ <60.

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Introduction

CKD affects an estimated 19 million people in the United States, and is associated with mortality, cardiovascular disease, and kidney complications (1,2). Direct measurement of GFR using radioactive agents gives the most unbiased measure of GFR; however, this is not only expensive but is also inconvenient to do, and is thus practically impossible to utilize at the population level. Creatinine-based estimated GFR ($eGFR_{creatinine}$) is considered a key measure of kidney function in clinical practice. However, several non-GFR determinants of creatinine (e.g., muscle mass and diet) bias GFR estimates, resulting in misclassification. This is an issue particularly in elderly individuals or in those who are sick and therefore have muscle wasting where $eGFR_{creatinine}$ overestimates GFR.

Cystatin C, an alternate marker of kidney function, estimates GFR as accurately as serum creatinine (3). Data suggest that eGFR by cystatin C ($eGFR_{cystatin}$) has a stronger association with mortality and cardiovascular disease than does $eGFR_{creatinine}$ (4–11).

Because of these complex issues, there is an interest in investigating how multiple kidney markers combine to predict risk, in particular, using cystatin C as a supplement to traditional CKD markers such as $eGFR_{creatinine}$ and urinary albumin/creatinine ratio (ACR) (12). Data from the study Reasons for Geographic and Racial Differences in Stroke (REGARDS), utilizing a multimarker approach to predict future risk of mortality and ESRD showed that combining $eGFR_{creatinine}$, $eGFR_{cystatin}$, and albuminuria significantly improves risk prediction (13). However, more research is needed to investigate utility of multiple kidney markers in clinical practice; in particular, it is important to investigate who can benefit the most from this approach. It is also important to extend this approach to additional outcomes, especially cardiovascular outcomes such as coronary heart disease (CHD) and heart failure (HF), which constitute a

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major proportion of morbidity and mortality in the CKD population. Furthermore, current knowledge is limited regarding the association of multiple kidney markers with AKI risk, which is very high in patients with CKD (14).

The main objectives of this study were to investigate the joint association of $eGFR_{creatinine}$, $eGFR_{cystatin}$, and ACR with all-cause mortality, cardiovascular disease including CHD and HF, and kidney outcomes including AKI and ESRD in the general population. We also wanted to investigate if this multimarker approach would improve risk classification.

Materials and Methods

Study Population

The Atherosclerosis Risk in Communities (ARIC) study is a population-based cohort study in which 15,792 participants, aged 45–64 years, were recruited from 1987 to 1989. Participants were recruited from four US communities: Washington County, Maryland; Jackson, Mississippi; Forsyth County, North Carolina; and Minneapolis, Minnesota. They were followed prospectively by annual telephone calls (response rate >90%) and four standardized examinations each approximately 3 years apart until 1998. The fifth examination is currently in progress. For this study, we selected visit 4 as the baseline visit because cystatin C concentration and ACR were measured at visit 4 in the entire cohort. Of 11,656 participants who attended visit 4, we excluded those with race other than African American and white (n=31), those with missing data (n=1302), and those with prevalent cardiovascular disease at baseline (n=834). We had a final sample size of 9489 participants.

Data Collection

Trained interviewers collected data on demographic and behavioral characteristics at each visit (15). Body mass index (BMI) was calculated by weight (kilograms) divided by square of height (meters). Diabetes mellitus was defined based on a glucose concentration of ≥126 mg/dl (fasting), ≥200 mg/dl (nonfasting), self-reported physician diagnosis of diabetes, or use of hypoglycemic medications. Enzymatic methods were used to determine plasma cholesterol, triglyceride, and HDL cholesterol concentrations, and the Friedewald equation was used to calculate LDL cholesterol. We included hypertension as a categorical variable defined as systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or use of antihypertensive medications. An immunonephelometric assay by Siemens Healthcare Diagnostics measured high-sensitivity C-reactive protein.

Assessment of Exposure

Serum creatinine concentration was measured using a modified kinetic Jaffe method. Creatinine was calibrated to Cleveland Clinic laboratory by the addition of 0.18 mg/dl for interlaboratory differences, and then multiplied by 0.95 for standardization to the Roche enzymatic method (16,17). The reliability coefficient for 439 blinded replicates was 0.95. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation was used to estimate $eGFR_{creatinine}$ as follows:

$$eGFR_{creatinine} = 141 \times (\text{minimum of standardized serum creatinine [mg/dl]}/\kappa \text{ or } 1)^{\alpha} \times (\text{maximum of standardized serum creatinine [mg/dl]}/\kappa \text{ or } 1)^{-1.209} \times 0.993^{age} \times (1.018 \text{ if female}) \times (1.159 \text{ if black}),$$

where κ is 0.7 if female and 0.9 if male and α is −0.329 if female and −0.411 if male (18).

Plasma cystatin C concentration was measured by a Siemens' BN II nephelometer by a particle-enhanced immunonephelometric assay in the ARIC core laboratory at Baylor College of Medicine in 2008 from frozen stored samples collected at visit 4. The reliability coefficient for 421 blinded replicates was 0.65 (0.94 after removing 10 pairs of outliers). Cystatin C was calibrated to the International Federation for Clinical Chemists provided reference material (19), and $eGFR_{cystatin}$ was calculated using the latest CKD-EPI cystatin C equation as follows (20):

$$eGFR_{cystatin} = 133 \times \min(\text{serum cystatin}/0.8, 1)^{-0.499} \times \max(\text{serum cystatin}/0.8, 1)^{-1.328} \times 0.996^{age} \times 0.932 \text{ [if female]}.$$

Min indicates the minimum of serum cystatin/κ or 1, and max indicates the maximum of serum cystatin/κ or 1.

ACR was calculated from a random urine sample from urine albumin and urine creatinine concentrations. The Jaffe method measured urine creatinine, whereas urine albumin was measured using the nephelometric method on either the Dade Behring BN100 or Beckman Image Nephelometer. The reliability coefficient for a log-transformed ACR for 516 blinded replicates was 0.95.

Assessment of Outcomes

Participants were followed until December 31, 2008 for all-cause mortality, CHD, HF hospitalization, AKI hospitalization, and ESRD. For all cardiovascular disease-related hospitalizations and mortality, continuous comprehensive surveillance was conducted and all potential CHD events were adjudicated according to published criteria (15). An incident CHD event was defined as a hospitalized definite or probable myocardial infarction, fatal CHD (CHD death) or a coronary revascularization procedure. Diagnosis of incident HF hospitalization was based on International Classification of Diseases (ICD) diagnostic codes (ICD9: 428 and ICD10: I50).

AKI was based on validated AKI events from hospital discharge diagnosis using ICD codes (ICD9: 584.5–584.9, ICD10: N17.0–N17.9) (21). We also included participants who died with AKI as the cause of death on their death certificate.

Incident ESRD was based on ICD9 or ICD10 diagnostic codes that were specified for kidney transplant, dialysis, or procedural codes indicating dialysis. ESRD cases also included those patients with an earlier diagnosis of CKD who had an underlying cause of death being ARF on their death certificate. If the transplant or dialysis code had the same event date as the code for ARF and the individual did not have any prior CKD, they were not included. Participants with an ICD code for traumatic anuria were also not included.

Statistical Analyses

We divided the cohort into eight exposure categories based on the current clinical CKD cutoffs (22) as follows: (*1*) no CKD by any marker ($eGFR_{creatinine}$ ≥60 and $eGFR_{cystatin}$ ≥60 and ACR <30 [reference]); CKD by either

(2) $eGFR_{creatinine}$ (<60 ml/min per 1.73 m^2) or (3) $eGFR_{cystatin}$ (<60 ml/min per 1.73 m^2) or (4) ACR (≥30 mg/g) only; CKD by both (5) $eGFR_{creatinine}$ and $eGFR_{cystatin}$ or (6) $eGFR_{creatinine}$ and ACR; or (7) $eGFR_{cystatin}$ and ACR and (8) CKD by all three markers. Although most of those with $eGFR_{creatinine}$ <60 who are reclassified up to >60 by $eGFR_{cystatin}$ are in the 30–59 $eGFR_{creatinine}$ range and *vice versa*, we also conducted sensitivity analysis after excluding those with eGFR <30 ml/min per 1.73 m^2 to rule out that our findings were derived by extreme cases. We also presented estimates for two additional categories: CKD stage 1–2 (ACR ≥30 with normal $eGFR_{creatinine}$ [≥60], ignoring $eGFR_{cystatin}$) and CKD stage 3+ ($eGFR_{creatinine}$ <60, ignoring ACR and $eGFR_{cystatin}$). This was done to compare risk in these two categories with the multiple marker subcategories. We used the *t* test and ANOVA for continuous variables, whereas the chi-squared test was used for categorical variables. Cox proportional hazards models were used to estimate hazard ratios (HRs) for outcomes of interest adjusting for age, race, and sex, as well as total cholesterol, diabetes, current smoking, hypertension, high-sensitivity C-reactive protein, and BMI.

For risk prediction, we calculated the Harrell *C* statistic from three separate models. Model I was the fully adjusted model + $eGFR_{creatinine}$, model II was model I + ACR, and model III was model II + $eGFR_{cystatin}$. We compared model II versus model I and model III versus model II in terms of *C* statistics and continuous net reclassification improvement (cNRI). These were done to see if the addition of ACR to $eGFR_{creatinine}$ (model I), and $eGFR_{cystatin}$ to both $eGFR_{creatinine}$ and ACR (model II) improved risk prediction. The hierarchy of three kidney markers is based on recommendations in clinical guidelines and general availability in clinical practice (23,24). We calculated cNRI as follows: [(Difference between number of individuals moving to the appropriate risk category and those moving to the inappropriate risk category among cases)/total number of cases + (Difference between number of individuals moving to the appropriate risk category and those moving to the inappropriate risk category among noncases)/total number of noncases].

P values <0.05 were considered statistically significant. All statistical analyses were done using Stata 12 software (25).

Results

Baseline Characteristics

Overall, the mean age was 63 years, and 22% of participants were African American and 58% were female. Our results showed that 16.2% of participants (*n*=1539) had CKD by any marker (either $eGFR_{creatinine}$ <60 or $eGFR_{cystatin}$ <60 or ACR ≥30). In addition, 5.5% (*n*=524) and 8.6% (*n*=820) had eGFR <60 ml/min per 1.73 m^2 by creatinine and cystatin C, respectively, whereas only 2.9% (*n*=281) had eGFR <60 ml/min per 1.73 m^2 by both $eGFR_{creatinine}$ and $eGFR_{cystatin}$. Of the participants, 6.9% (*n*=659) had high albuminuria. Of those with $eGFR_{creatinine}$ <60, 42% had normal $eGFR_{cystatin}$ (≥60 ml/min per 1.73 m^2) and normal ACR (<30 mg/g). Diabetes and hypertension prevalence were 15% and 45%, respectively. Table 1 shows baseline characteristics of the participants. Participants without CKD were younger compared with those with one or more abnormal markers. Diabetes and hypertension prevalence was lower in those without CKD than those with CKD by one or more markers with the exception of those with abnormal $eGFR_{creatinine}$ only where diabetes prevalence was similar to those without CKD. If ACR was elevated, diabetes prevalence was higher.

Mortality and Cardiovascular Outcomes

Table 2 shows the number of events, total person-time in years, and crude incidence rates per 1000 person-years. Median follow-up time was 11.2 years. Incidence rates for mortality, CHD, and HF were higher for CKD stages 1–2 and CKD stage 3+ compared with no CKD. In contrast, incidence rates for mortality, CHD, or HF were not higher in those with abnormal $eGFR_{creatinine}$ only compared with those with no CKD. However, if either $eGFR_{cystatin}$ or ACR were in the CKD range, incidence rates were higher compared with no CKD. When the combination of two markers was abnormal, incidence rates were usually further higher. Incidence rates were the highest in individuals with all three markers in the CKD range. Similar risk gradients were observed even after adjusting for potential confounders (Table 3). Associations observed were weaker for CHD compared with those for mortality or HF.

Kidney Outcomes

CKD stages 1–2 and CKD stage 3+ were both associated with higher incidence of AKI and ESRD compared with no CKD. In contrast to mortality and cardiovascular outcomes, low $eGFR_{creatinine}$ only (normal $eGFR_{cystatin}$ and ACR) as well as abnormality of $eGFR_{cystatin}$ only or ACR only were associated with increased rates of kidney outcomes compared with no CKD; however, rates were lower than those observed for CKD stages 1–2 or CKD stage 3+ (Table 2). For AKI, incidence rates were higher when either ACR or $eGFR_{cystatin}$ was abnormal compared with those with CKD by $eGFR_{creatinine}$ only. For ESRD, risk according to $eGFR_{creatinine}$ <60 was similar to $eGFR_{cystatin}$ in the CKD range (incidence rates per 1000 person-years of 1.2 for $eGFR_{creatinine}$ only versus 1.1 for $eGFR_{cystatin}$ only, and 8.2 in $eGFR_{creatinine}$ + ACR versus 9.1 for $eGFR_{cystatin}$ + ACR). When all three markers were in the CKD range, incidence rates were the highest (15-fold higher for AKI and >150-fold higher for ESRD compared with no CKD). We observed consistent results in our adjusted analyses (Table 3).

For all outcomes, our results remained similar when we used the Modified Diet in Renal Disease study equation instead of the CKD-EPI study equation (data not shown).

eGFR Category 30–59 ml/min per 1.73 m^2

In Figure 1, we present associations after excluding individuals with eGFR <30 to evaluate risk implications of eGFR category 30–59 ml/min per 1.73 m^2. Our findings were similar to our primary analysis (Table 3). With reference of no CKD by any marker, risk of nonkidney outcomes was not elevated in $eGFR_{creatinine}$ 30–59 when $eGFR_{cystatin}$ and ACR were both normal ($eGFR_{cystatin}$ ≥60 and ACR<30) (Figure 1A). Risk was increased when either ACR or $eGFR_{cystatin}$ was in the CKD range (except for CHD in which ACR was not associated with increased risk). For kidney outcomes, $eGFR_{creatinine}$ 30–59 was associated with

Table 1. Baseline characteristics of the study population

	No CKD	$eGFR_{creatinine}$ Only	ACR Only	$eGFR_{cystatin}$ Only	$eGFR_{creatinine}$ and $eGFR_{cystatin}$	$eGFR_{creatinine}$ and ACR	$eGFR_{cystatin}$ and ACR	All Abnormal
Participants (*n*)	7950	219	476	476	185	24	63	96
Age (yr)	62.1 (5.5)	65.4 (5.4)	63 (5.8)	65.3 (5.6)	67.5 (4.7)	65.6 (5.5)	66.1 (5.3)	66.8 (5.2)
White	78.5	82.7	59.5	84.2	84.3	54.2	76.2	66.7
Female	57.5	61.6	58.2	64.1	63.2	54.2	47.6	54.2
Total cholesterol (mg/dl)	201.8 (35.8)	211.6 (38.2)	200.7 (38.2)	198.5 (37.5)	205.2 (38.3)	226.3 (47.8)	202.6 (56)	205.9 (38.3)
LDL cholesterol (mg/dl)	123.4 (32.9)	130.2 (36.7)	120.8 (34.5)	120.9 (33)	127.7 (35.4)	145.6 (38.1)	120.4 (38.9)	123.2 (36.4)
CRP (mg/L)	4 (5.9)	3.7 (4.1)	5.6 (7.8)	6.2 (9.8)	7.1 (11.1)	6.8 (9.3)	9.6 (14.2)	6.8 (7.3)
BMI (kg/m^2)	28.4 (5.3)	28.4 (4.5)	29.5 (6.4)	30.3 (6.6)	30.2 (5.6)	29.6 (4.4)	29.8 (6.5)	29.3 (5.9)
Hypertension	40.8	50.2	72.3	52.9	74.1	70.8	81.0	89.6
Diabetes	12.9	12.3	38.5	14.7	20.0	50.0	43.6	39.6
Current smokers	14.4	4.1	21.9	20.0	15.8	4.4	31.8	21.9
ACR (mg/g)	3.3 [1.6–6.1]	2.6 [1.5–5.3]	64.0 [43.5–144.1]	4.0 [2.1–7.2]	3.8 [1.6–8.2]	93.8 [48.8–246.2]	152.1 [62.9–447.0]	220.8 [74.7–818.2]
Creatinine (mg/dl)	0.7 (0.2)	1.1 (0.2)	0.7 (0.2)	0.8 (0.2)	1.2 (0.2)	1.1 (0.2)	0.8 (0.2)	1.9 (2.3)
Cystatin C (mg/L)	0.8 (0.1)	0.9 (0.1)	0.8 (0.1)	1.4 (0.5)	1.3 (0.3)	0.9 (0.1)	1.2 (0.3)	1.8 (1.2)
$eGFR_{creatinine}$ (ml/min per 1.73 m^2)	87.3 (12.4)	55.7 (3.8)	89 (14.4)	77.4 (11.7)	50.3 (7.3)	55.4 (3.3)	75.9 (13.8)	42.9 (14.9)
$eGFR_{cystatin}$ (ml/min per 1.73 m^2)	88.5 (13.3)	73.8 (11.1)	86.4 (14.4)	48.0 (13.0)	47.6 (9.3)	70.2 (9.4)	50.9 (9.2)	38.6 (13.4)

All values expressed as mean (SD), percentages, or median [interquartile range]. eGFR, estimated GFR; CRP, C-reactive protein; BMI, body mass index; ACR, albumin/creatinine ratio.

Table 2. Number of events, total person-time in years, and crude incidence rates per 1000 person-years

	All-Cause Mortality		Coronary Heart Disease		Heart Failure		AKI		ESRD	
	Events (Time)	Incidence Rate	Events (Time)	Incidence Rate	Events (Time)	Incidence Rate	Events (Time)	Incidence Rate	Events (Time)	Incidence Rate
No CKD	918 (87,611)	10.5	802 (82,136)	9.8	499 (84,873)	5.9	252 (84,873)	3.0	31 (87,611)	0.4
CKD stages 1–2	146 (5476)	27.1	84 (5202)	16.5	102 (4928)	20.3	65 (5202)	12.4	22 (5202)	4.2
CKD stage 3+	151 (5202)	28.5	106 (4928)	21.6	118 (4928)	23.6	79 (5202)	15.4	55 (5202)	10.8
Multiple marker subcategories										
No CKD	918 (87,611)	10.5	802 (82,136)	9.8	499 (84,873)	5.9	252 (84,873)	3.0	31 (87,611)	0.4
CKD by $eGFR_{creatinine}$ only	29 (2409)	12.0	21 (2300)	9.1	16 (2355)	6.8	13 (2382)	5.5	3 (2409)	1.2
CKD by ACR only	117 (4928)	23.7	74 (4654)	15.9	79 (4654)	17.0	52 (4654)	11.2	17 (4654)	3.7
CKD by $eGFR_{cystatin}$ only	118 (4654)	25.4	62 (4381)	14.2	78 (4381)	17.8	44 (4654)	9.5	5 (4654)	1.1
CKD by $eGFR_{creatinine}$ and $eGFR_{cystatin}$	60 (1834)	32.7	42 (1670)	25.1	39 (1752)	22.3	32 (1780)	18.0	10 (1807)	5.5
CKD by $eGFR_{creatinine}$ and ACR	6 (257)	23.3	5 (246)	20.3	11 (222)	49.6	3 (246)	12.2	2 (244)	8.2
CKD by $eGFR_{cystatin}$ and ACR	29 (575)	50.4	10 (548)	18.3	23 (493)	46.7	13 (548)	23.7	5 (548)	9.1
CKD by all three markers	56 (794)	70.5	38 (684)	55.5	52 (657)	79.1	31 (712)	43.5	40 (657)	60.9

Time is person years. Incidence rates are per 1000 person-years. eGFR, estimated GFR; ACR, albumin/creatinine ratio.

Table 3. Joint association of $eGFR_{creatinine}$, ACR, and $eGFR_{cystatin}$ with outcomes

	All-Cause Mortality	Coronary Heart Disease	Heart Failure	AKI	ESRD
No CKD	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
CKD stages 1–2	1.73 (1.44, 2.08)	1.07 (0.85, 1.36)	1.95 (1.56, 2.45)	2.53 (1.88, 3.38)	5.02 (2.81, 9.00)
CKD stage 3+	1.80 (1.50, 2.15)	1.62 (1.31, 2.00)	2.47 (2.01, 3.05)	3.63 (2.79, 4.74)	24.20 (14.86, 39.40)
Multiple marker subcategories					
No CKD	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
CKD by $eGFR_{creatinine}$ only	0.96 (0.66, 1.39)	0.85 (0.55, 1.31)	0.99 (0.60, 1.63)	1.61 (0.92, 2.82)	3.53 (1.06, 11.68)
CKD by ACR only	1.66 (1.36, 2.04)	1.13 (0.88, 1.45)	1.85 (1.44, 2.37)	2.43 (1.77, 3.33)	4.74 (2.52, 8.93)
CKD by $eGFR_{cystatin}$ only	1.67 (1.37, 2.03)	1.21 (0.93, 1.58)	1.90 (1.49, 2.44)	2.44 (1.75, 3.41)	3.15 (1.20, 8.30)
CKD by $eGFR_{creatinine}$ and $eGFR_{cystatin}$	1.86 (1.42, 2.44)	1.85 (1.35, 2.55)	2.00 (1.44, 2.80)	3.90 (2.65, 5.74)	14.57 (6.75, 31.46)
CKD by $eGFR_{creatinine}$ and ACR	1.26 (0.52, 3.05)	1.03 (0.38, 2.76)	4.31 (2.28, 8.13)	2.19 (0.70, 6.9)	8.91 (2.06, 38.49)
CKD by $eGFR_{cystatin}$ and ACR	2.47 (1.70, 3.61)	0.93 (0.49, 1.74)	3.25 (2.10, 5.03)	3.96 (2.18, 7.18)	14.55 (5.38, 39.32)
CKD by all three markers	3.69 (2.79, 4.87)	3.01 (2.15, 4.20)	6.92 (5.14, 9.31)	9.78 (6.63, 14.43)	125.98 (73.06, 217.22)

Data are shown as adjusted hazard ratios (95% confidence intervals). All hazard ratios adjusted for age, race, sex, and total cholesterol, history of diabetes, hypertension, smoking, body mass index, and C-reactive protein eGFR, estimated GFR; ACR, albumin/creatinine ratio.

increased risk even in isolation (both $eGFR_{cystatin}$ and ACR normal) compared with those without CKD; however, if additional markers were abnormal, risk was higher. In contrast, $eGFR_{cystatin}$ 30–59 was associated with increased risk of all outcomes even if the other two markers were normal compared with no CKD (Figure 1B).

Risk Prediction

Risk classification for all outcomes improved when ACR was added to $eGFR_{creatinine}$, and it improved further when $eGFR_{cystatin}$ was added to the model with both $eGFR_{creatinine}$ and ACR in addition to demographics and traditional cardiovascular risk factors (Table 4). For CHD and ESRD, the *C* statistic did not show a significant improvement of risk prediction with the addition of either ACR to $eGFR_{creatinine}$ or $eGFR_{cystatin}$ to both $eGFR_{creatinine}$ and ACR; however, cNRI was significantly positive suggesting improvement of reclassification. For other outcomes, both the *C* statistic and cNRI supported improvement of risk prediction with the addition of either ACR to $eGFR_{creatinine}$ or $eGFR_{cystatin}$ to both $eGFR_{creatinine}$ and ACR (Table 4).

Discussion

Our study showed increased risk of mortality and cardiovascular and kidney outcomes with reduced $eGFR_{creatinine}$ and $eGFR_{cystatin}$ as well as with elevated ACR, independently of each other. In our cohort, 42% of those with $eGFR_{creatinine}$ <60 had normal ACR and normal $eGFR_{cystatin}$, and this group was not observed to be at increased risk for any of the nonkidney outcomes compared with those with no CKD. This suggests that $eGFR_{cystatin}$ and ACR can be used as confirmatory markers for future risk in those with $eGFR_{creatinine}$ <60. Furthermore, in those with $eGFR_{creatinine}$ <60 and normal ACR, $eGFR_{cystatin}$ provided additional useful prognostic information, and identified those who were actually not at increased risk. Importantly, $eGFR_{cystatin}$ and ACR were also useful when we focused on individuals with moderately decreased $eGFR_{creatinine}$ of 30–59. In contrast, for kidney outcomes, low $eGFR_{creatinine}$ conferred to increased risk similar to $eGFR_{cystatin}$. We also observed that risk prediction for all outcomes improved with the addition of either ACR to $eGFR_{creatinine}$ or addition of $eGFR_{cystatin}$ to both $eGFR_{creatinine}$ and ACR.

Our findings are consistent with the existing evidence that $eGFR_{cystatin}$ can be a useful prognostic marker in individuals with CKD. A study with data from the Multi-Ethnic Study of Atherosclerosis (MESA) and the Cardiovascular Health Study (CHS) showed that in those with CKD, $eGFR_{cystatin}$ identifies individuals at higher risk of complications. In MESA, they found that risk of mortality was hazard ratio [HR], 0.80 (0.50, 1.26 95% confidence interval [CI]) in those

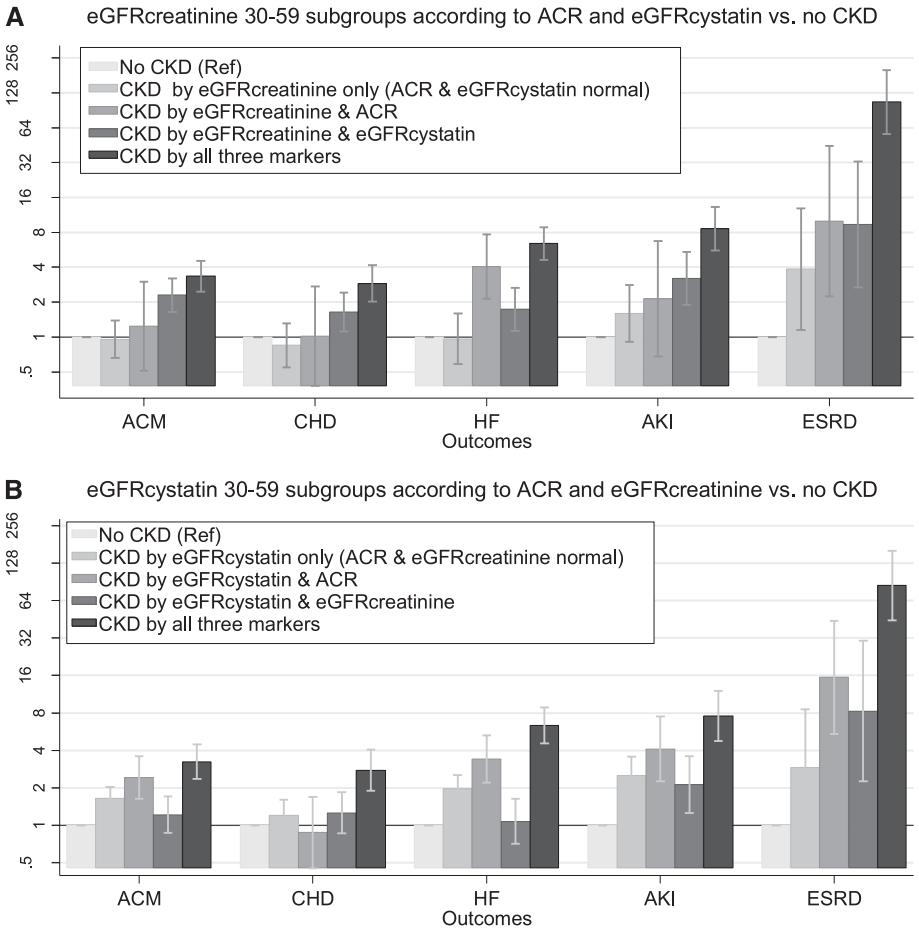

Figure 1. | eGFR Category 30–59 ml/min per 1.73 m^2 (A) $eGFR_{creatinine}$ 30–59 category versus no CKD by any marker (reference). (B) $eGFR_{cystatin}$ 30–59 category versus no CKD by any marker (reference). All hazard ratios are adjusted for age, race, sex, total cholesterol, history of diabetes, hypertension, smoking, body mass index, and C-reactive protein. In addition, hazard ratios for $eGFR_{cystatin}$ (fourth bar for each outcome) in A are further adjusted for $eGFR_{creatinine}$. Hazard ratios for $eGFR_{creatinine}$ (fourth bar) in B are further adjusted for $eGFR_{cystatin}$. Reference is no CKD by any marker (n=7950); 4% (n=21) of those with $eGFR_{creatinine}$ 30–59 have $eGFR_{cystatin}$ <30. eGFR, estimated GFR; ACR, albumin/creatinine ratio; ACM, all-cause mortality; CHD, coronary heart disease; HF, heart failure.

with CKD by $eGFR_{creatinine}$ only and HR, 3.23 (1.84, 5.67 95% CI) in those with CKD by $eGFR_{cystatin}$ only compared with those with eGFR >60 by both $eGFR_{creatinine}$ and $eGFR_{cystatin}$. In CHS, similar results were observed suggesting that among those with CKD by $eGFR_{creatinine}$, risk of complications is limited to those with abnormal $eGFR_{cystatin}$. However, for ESRD, CKD by $eGFR_{creatinine}$ alone was associated with increased risk compared with eGFR>60; (HR, 2.60; 1.00, 6.75 95% CI) (26). Our study also confirms findings from REGARDS (13) and extends it to cardiovascular outcomes and AKI that cystatin C can be used as a confirmatory marker in those with CKD by $eGFR_{creatinine}$ with normal ACR. In this group, cystatin C confirms the increased risk if it is abnormal and assures lower risk if it is normal. We believe that this approach would be most practical in those with $eGFR_{creatinine}$ 30–59 with normal ACR (<30) because very few individuals with $eGFR_{creatinine}$ <30 move up to >60 by $eGFR_{cystatin}$ (in our cohort, no one with $eGFR_{creatinine}$ <30 moved up to >60 by $eGFR_{cystatin}$).

Our findings are also consistent with the existing evidence that $eGFR_{creatinine}$ is associated with increased risk of ESRD independently of $eGFR_{cystatin}$ and ACR (13,26). We found that CKD by $eGFR_{creatinine}$ alone was associated with increased risk of kidney outcomes, which is contrary to what we observed for nonkidney outcomes. This association between low $eGFR_{creatinine}$ and kidney outcomes may reflect the fact that, in clinical practice, serum creatinine is predominantly used to track kidney disease progression or injury. Nevertheless, even for kidney outcomes, $eGFR_{cystatin}$ and ACR provided useful prognostic information similar to mortality and cardiovascular outcomes. In those with CKD by $eGFR_{creatinine}$, if $eGFR_{cystatin}$ or ACR or both were also abnormal, risk prediction improved significantly. On the other hand, if we only measure $eGFR_{creatinine}$ and ignore ACR and $eGFR_{cystatin}$, we misclassify a large proportion of individuals into the higher risk category when their actual risk is several folds lower if the other two markers are not in the CKD range. Although we observed that for ESRD the C statistic was very high (*i.e.*, >0.9 with $eGFR_{creatinine}$ and traditional risk factors), there was a slight nonsignificant further improvement in C statistic with the addition of ACR or both ACR and $eGFR_{cystatin}$. However, better discrimination was more evident when we used cNRI, which

Table 4. *C* statistic and cNRI for each outcome

		C Statistic	*P* Value[a]	cNRI (95% CI)
All-cause mortality	$eGFR_{creatinine}$	0.727		
	+ ACR	0.732	0.001	23.6 (18.9, 28.4)
	+ $eGFR_{cystatin}$	0.737	0.001	11.9 (6.1, 17.6)
Coronary heart disease	$eGFR_{creatinine}$	0.712		
	+ ACR	0.713	0.09	12.0 (6.8, 17.3)
	+ $eGFR_{cystatin}$	0.716	0.11	17.3 (10.8, 23.7)
Heart failure	$eGFR_{creatinine}$	0.761		
	+ ACR	0.770	<0.001	18.7 (12.2, 25.2)
	+ $eGFR_{cystatin}$	0.778	0.001	14.0 (6.8, 21.3)
AKI	$eGFR_{creatinine}$	0.754		
	+ ACR	0.766	0.003	39.5 (31.8, 47.2)
	+ $eGFR_{cystatin}$	0.780	0.001	12.3 (2.8, 21.8)
ESRD	$eGFR_{creatinine}$	0.907		
	+ ACR	0.916	0.08	32.4 (14.0, 50.8)
	+ $eGFR_{cystatin}$	0.920	0.38	45.7 (28.6, 62.8)

Baseline model includes age, race, sex, and total cholesterol, history of diabetes, hypertension, smoking, body mass index, and C-reactive protein. + ACR, baseline model + $eGFR_{creatinine}$ + ACR; + $eGFR_{cystatin}$, baseline model + $eGFR_{creatinine}$ + ACR + $eGFR_{cystatin}$. cNRI, continuous net reclassification index; 95% CI, 95% confidence interval; eGFR, estimated GFR; ACR, albumin/creatinine ratio.
[a]*P* value comparing Harrell's *C* statistic from the above row.

is a more sensitive indicator of discrimination. In clinical practice, this improved discrimination translates into more focused efforts on higher-risk patient population potentially resulting in improved care. We also observed that $eGFR_{cystatin}<60$ is associated with increased risk of all outcomes even in isolation; however, its clinical use as a first line marker would involve testing a large number of individuals, which may not be practical given costs until we have studies on cost-benefit analysis.

Major strengths of our study include a well established large general population cohort with very a high follow-up rate. In the ARIC study, all measurements were done in a standardized fashion with high reliability, and data on all major risk factors were also collected in a standardized manner. To our knowledge, this is the first study that has used the latest GFR equations for both creatinine and cystatin C. Some of the limitations of our study include the following: First, we only had a single measurement of creatinine, cystatin C, and ACR that is subject to measurement error. However, these errors are likely to be random and so our estimates are likely to be attenuated. Second, this is an observational study and although we controlled for several potential confounders, residual confounding may still be present. We also acknowledge that some of our categories were small and thus power may have been limited. It is also important to note that both kidney outcomes, AKI and ESRD, were based on ICD diagnostic codes. Although these codes have been validated in prior studies and perform reasonably well, we expect to see some degree of random misclassification that may result in our estimates being somewhat conservative. Finally, although ARIC is a general population cohort and findings from this study are generalizable to the middle- to older-age populations in the United States, we acknowledge that the prevalence of CKD, in particular more advanced CKD is lower in ARIC than reports from the National Health and Nutrition Examination Survey suggest (27). Therefore, eGFR <60 may mainly represent stage 3a CKD, and thus our results for dichotomized kidney categories may underestimate their effect on the entire population.

In conclusion, our study suggests increased risk of mortality and cardiovascular and kidney outcomes independently with $eGFR_{creatinine}$, $eGFR_{cystatin}$, and ACR. In those with CKD by $eGFR_{creatinine}$, $eGFR_{cystatin}$ may be useful for confirming increased risk of CKD-related outcomes. We believe that this triple-marker approach not only improves risk classification but also provides reassurance to a large group of individuals with stage 3 CKD, resulting in referrals and workup appropriately corresponding to clinical risk and potentially reducing health care costs. It should however be noted that cystatin C is also not a perfect maker and may be affected by factors such as inflammation and body size. However, overall cystatin C is less biased than creatinine, and its ability to predict future risk significantly better than creatinine makes it a superior marker than creatinine. We propose that cystatin C should be useful in clinical practice as a confirmatory marker for evaluating clinical risk in those with stage 3 CKD based on serum creatinine, particularly when albuminuria is not elevated.

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Disclosures

None.

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