A Multi-Marker Approach to Predict Incident CKD and Microalbuminuria


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ABSTRACT

Traditional risk factors do not adequately identify individuals at risk for CKD. We related a multi-marker panel consisting of the following seven circulating biomarkers to the incidence of CKD and microalbuminuria (MA) in 2345 participants who attended the sixth Framingham Offspring Study examination (1995 to 1998): C-reactive protein, aldosterone, renin, B-type natriuretic peptide (BNP), plasminogen-activator inhibitor type 1, fibrinogen, and homocysteine. We defined CKD at follow-up (2005 to 2008) as estimated GFR (eGFR) < 60 ml/min per 1.73 m²; we defined MA as urine albumin-to-creatinine ratio ≥25 (women) or 17 (men) mg/g on spot urine samples. We identified a parsimonious set of markers related to outcomes adjusting for standard risk factors and baseline renal function, and we assessed their incremental predictive utility. During a mean 9.5-year follow-up, 213 participants developed CKD and 186 developed MA. In multivariable logistic regression models, the multi-marker panel was associated with incident CKD (P < 0.001) and MA (P < 0.003). Serum homocysteine and aldosterone both were significantly associated with CKD incidence, and log-transformed aldosterone, BNP, and homocysteine were significantly associated with incident MA. Biomarkers improved risk prediction as measured by improvements in the c-statistics for both CKD and MA and by a 7% increase in net risk reclassification. In conclusion, circulating homocysteine, aldosterone, and BNP provide incremental information regarding risk for incident CKD and MA beyond traditional risk factors.


Chronic kidney disease (CKD) affects nearly 26 million adults in the United States, which is nearly 13% of the adult population.1 CKD is associated with metabolic abnormalities2 and bone disease3 and is also an important risk factor for peripheral vascular disease,4,5 cardiovascular disease (CVD),6–8 stroke,9 and all-cause mortality.9,10 Hypertension and diabetes are key risk factors for CKD11 but do not fully identify individuals at risk for developing CKD. A recently developed algorithm for predicting incident CKD risk showed suboptimal discrimination,12 underscoring the need to identify novel markers and use newer approaches for identifying high-risk individuals to prevent CKD in the community.

Serum creatinine, as used in most GFR esti-
Table 1. Baseline characteristics of study participants (n = 2345)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD) or Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.6 (8.9)</td>
</tr>
<tr>
<td>Women (% [n])</td>
<td>53.3 (1250)</td>
</tr>
<tr>
<td>Body mass index (kg/m^2)</td>
<td>27.9 (5.2)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>51.6 (16.0)</td>
</tr>
<tr>
<td>Current smoker (n [%])</td>
<td>13.9 (325)</td>
</tr>
<tr>
<td>Hypertension (% [n])</td>
<td>35.4 (830)</td>
</tr>
<tr>
<td>Diabetes [% [n]]</td>
<td>8.4 (197)</td>
</tr>
<tr>
<td>Estimated GFR (ml/min per 1.73 m^2)</td>
<td>95.1 (83.9)</td>
</tr>
<tr>
<td>UACR (mg/g)*</td>
<td>5.7 (2.6, 12.7)</td>
</tr>
<tr>
<td>Biomarker levels</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>1.8 (0.8, 4.4)</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>7.4 (4.0, 16.1)</td>
</tr>
<tr>
<td>aldosterone (mg/dl)</td>
<td>10.0 (7.0, 14.0)</td>
</tr>
<tr>
<td>renin (mU/L)</td>
<td>12.0 (7.0, 22.0)</td>
</tr>
<tr>
<td>fibrinogen (mg/dl)</td>
<td>327.0 (288.0, 376.0)</td>
</tr>
<tr>
<td>plasminogen-activator inhibitor type 1 (ng/ml)</td>
<td>22.2 (13.7, 33.3)</td>
</tr>
<tr>
<td>homocysteine (mmol/L)</td>
<td>8.8 (7.3, 10.5)</td>
</tr>
</tbody>
</table>

Data presented as mean (SD) for continuous variables and percentage for dichotomous variables unless otherwise indicated. *All biomarkers presented as median (q1, q3).

RESULTS

Baseline Study Characteristics
At baseline, the sample was on average 56.6 years of age, 35.4% had hypertension, and 8.4% had diabetes. Median and first and third quartile cutoffs for all biomarkers are presented in Table 1; Supplementary Tables 1 and 2 show the median biomarkers at baseline according to presence versus absence of CKD or MA on follow-up.

Multi-marker Panel and Incident CKD
Over 9.5 years of follow-up, 213 participants (118 women) developed new-onset CKD. The multi-marker panel was associated with the development of CKD at follow-up (P = 0.0005). In particular, homocysteine (odds ratio [OR] per SD increment log homocysteine, 1.41; 95% confidence interval [CI], 1.20 to 1.65; Table 2) and aldosterone (OR per SD increment in log-aldosterone, 1.17; 95% CI, 1.002 to 1.36) were associated with incident CKD even after adjusting for multiple CKD risk factors including baseline estimated GFR (eGFR). Notably, baseline urinary albumin-to-creatinine ratio (UACR) was not significant in this model (P = 0.73).

The addition of log homocysteine to the prediction model increased the c-statistic for incident CKD from 0.810 (multivariable-adjusted model) to 0.822 (P = 0.0023 for the change). We also performed reclassification (Table 3); the net reclassification index was 6.9% (P = 0.0004), confirming the incremental predictive utility of plasma homocysteine and aldosterone for prediction of CKD incidence.

Multi-Marker Panel and Incident MA
Overall, 186 individuals (73 women) developed new-onset MA after 9.5 years of follow-up. The multi-marker panel was associated with incident MA (P = 0.003). Specifically, aldosterone, B-type natriuretic peptide (BNP), and homocysteine were associated with incident MA (Table 2). These biomarkers significantly increased the c-statistic from 0.732 (multivariable model including baseline UACR) to 0.748 (P = 0.003). The net reclassification index increased (6.9%, P = 0.007; Table 3), confirming the predictive utility of the biomarkers.

Biomarker Score
We created a biomarker score for all biomarkers that were statistically significant in the multivariable models evaluating the two outcomes of interest. Among individuals with either plasma total homocysteine or aldosterone above the median (homocysteine median, 10.61 mmol/L; aldosterone median, 14.00 mg/dl; n = 603), the OR for new-onset CKD was 2.07 (95% CI, 1.30 to 3.30; P = 0.002). When both markers were above the median, the OR was 2.39 (95% CI, 1.46 to 3.91; P =

Table 2. Association of the multi-marker panel and the individual biomarkers with incident CKD and MA

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>P</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident CKD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>entire panel</td>
<td>0.0005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>specific markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>homocysteine</td>
<td>&lt;0.0001</td>
<td>1.41</td>
<td>1.20 to 1.65</td>
</tr>
<tr>
<td>aldosterone</td>
<td>0.047</td>
<td>1.17</td>
<td>1.002 to 1.36</td>
</tr>
<tr>
<td>Incident microalbuminuria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>entire panel</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>specific markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aldosterone</td>
<td>0.017</td>
<td>1.23</td>
<td>1.04 to 1.46</td>
</tr>
<tr>
<td>BNP</td>
<td>0.0037</td>
<td>1.30</td>
<td>1.09 to 1.54</td>
</tr>
<tr>
<td>homocysteine</td>
<td>0.04</td>
<td>1.20</td>
<td>1.01 to 1.42</td>
</tr>
</tbody>
</table>

*Markers that are selected after backward elimination; presented per SD unit increase.
Microalbuminuria

Among participants without diabetes or CKD at baseline (n = 2148), 173 developed MA at follow-up. The multi-marker panel was associated with MA (OR = 3.36; 95% CI, 1.63 to 6.94; P = 0.0005). This relative risk for MA was higher than the OR associated with diabetes (OR, 1.87) in the same model.

For incident MA, among individuals with levels of homocysteine, BNP, and aldosterone all above their respective median values (homocysteine median, 10.61 mmol/L; BNP median, 15.9 pg/ml; aldosterone median, 13.00 mg/dl; n = 219), the OR for MA was 3.36 (95% CI, 1.63 to 6.94; P = 0.0001), which was higher than the relative risk estimate associated with diabetes (OR, 1.90) in the same model.

In backward elimination, of the biomarker panel, aldosterone, BNP, and homocysteine are significant. Incremental predictive utility of biomarkers for incident CKD, MA C-statistic, reclassification p-value (NRI, IDI), calibration statistic* (χ², P)

<table>
<thead>
<tr>
<th></th>
<th>C-statistic</th>
<th>NRI</th>
<th>Relative IDI</th>
<th>Calibration Statistic* (χ², P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>model 1: age-sex</td>
<td>0.776</td>
<td></td>
<td></td>
<td>8.20 (P = 0.41)</td>
</tr>
<tr>
<td>model 2: age-sex-MV</td>
<td>0.810</td>
<td></td>
<td></td>
<td>2.98 (P = 0.94)</td>
</tr>
<tr>
<td>model 3: age-sex-MV-biomarker</td>
<td>0.822</td>
<td>6.9% (P = 0.0004)</td>
<td>0.013 (P = 0.004)</td>
<td>3.48 (P = 0.90)</td>
</tr>
<tr>
<td>P value comparing model 2 to model 3</td>
<td>P = 0.0023</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>model 1: age-sex</td>
<td>0.694</td>
<td></td>
<td></td>
<td>19.5 (P = 0.01)</td>
</tr>
<tr>
<td>model 2: age-sex-MV</td>
<td>0.732</td>
<td></td>
<td></td>
<td>10.7 (P = 0.22)</td>
</tr>
<tr>
<td>model 3: age-sex-MV-biomarker</td>
<td>0.748</td>
<td>6.9% (P = 0.0007)</td>
<td>0.012 (P = 0.005)</td>
<td>9.44 (P = 0.31)</td>
</tr>
<tr>
<td>P value comparing model 2 to model 3</td>
<td>P = 0.003</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Well-calibrated models have nonsignificant P value.

In backward elimination, of the biomarker panel, homocysteine and aldosterone are significant.

In backward elimination, of the biomarker panel, aldosterone, BNP, and homocysteine are significant.

For incident MA, among individuals with levels of homocysteine, BNP, and aldosterone all above their respective median values (homocysteine median, 10.61 mmol/L; BNP median, 15.9 pg/ml; aldosterone median, 13.00 mg/dl; n = 219), the OR for MA was 3.36 (95% CI, 1.63 to 6.94; P = 0.0001), which was higher than the relative risk estimate associated with diabetes (OR, 1.90) in the same model.

**Discussion**

**Principal Findings**

In our study of incident CKD and new-onset MA in a community-based sample, we observed that a panel of seven biomarkers selected to be representative of several distinct biologic pathways was associated with the development of CKD and MA. We identified a smaller set of biomarkers associated with these outcomes that incrementally enhanced prediction of risk as judged by improvement in the model c-statistic and risk reclassification. These data suggest that newer biomarkers may improve our ability to identify individuals at greater risk for the development of renal disease (CKD or MA) up to 10 years before its clinical onset. More importantly, these findings identify potential pathways that may be involved in the pathogenesis of CKD and MA.

In the Context of the Current Literature

These findings contrast with the efforts to use biomarkers to improve risk prediction for CVD,14,15 which have generally not yielded improvements in risk prediction, although some important exceptions have been noted in women16 and elderly men.17 The better incremental predictive utility of newer biomarkers for CKD and MA may be because of several key factors. First, risk factors for CVD are well known, most are known to be modifiable, and multiple well-validated risk-algorithms exist to aid in risk prediction.18 For CKD, the primary risk factors are less well character-
ized; indeed, hypertension and diabetes (two key risk factors) are unable to adequately discriminate those who eventually develop CKD. Whereas the c-statistic for the basic clinical model for CKD (0.810, this study) is higher than that reported for CHD (0.688 to 0.760), this is likely because we included baseline eGFR in our prediction model, which is also used to define the CKD outcome. Second, it is possible that the coronary heart disease phenotype is more complex from a pathophysiologic perspective compared with CKD, which is defined biochemically using a serum-based measurement (creatinine) as in this study; it is conceivable that a trait defined based on one measure may be easier to predict with blood-based biomarkers.

Potential Mechanisms
Homocysteine has been implicated in the pathogenesis of coronary heart disease, although this is now debated given that homocysteine lowering is not effective in reducing CVD risk. Although CKD is known to be associated with elevated homocysteine levels, reducing homocysteine levels in CKD does not prevent vascular events or improve survival. This study extends the current literature that suggests that elevated homocysteine may be a risk factor or a risk marker of future CKD. The observed association may arise because plasma homocysteine tracks with reduced filtration more than GFR estimated by transforming equations. However, this is less likely, because we used the same eGFR measure at baseline and follow-up. It is also possible that homocysteine may be more reflective of true as compared with eGFR. Even so, the notion that homocysteine can improve our ability to detect who is at risk for CKD in the absence of gold standard measurements of GFR remains an important goal. Homocysteine may also be a risk marker for the development of CKD or along the causal pathway itself from risk factors to CKD. Observational epidemiologic research cannot distinguish between these two possibilities.

In addition, we identified BNP and aldosterone as biomarkers that additionally predicted CKD and incident MA. BNP is a hormone that comprises the natriuretic peptide family, is involved in volume and BP maintenance, and is cleared by the kidneys. Thus, it is possible that early reduced clearance of BNP could account for our findings; however, our models did account for baseline eGFR. The natriuretic peptides have been implicated in renal disease in several experimental studies where they seem to protect against mesangial fibrosis, glomerular hypertrophy, and glomerular hyperfiltration. Thus, higher BNP levels may also reflect compensatory responses to limit subclinical renal disease. BNP levels have also been correlated positively with MA cross-sectionally in some clinical reports.

There are multiple potential mechanisms whereby aldosterone may lead to altered kidney function, including inflammation and fibrosis. Rats with hypertension that drank saline-infused water had reduced albuminuria on treatment with spironolactone. In humans, angiotensin-converting enzyme inhibitors reduce the progression of CKD and proteinuria.

Implications
There are several implications from this study. First, our study provides proof of concept that select newer biomarkers may improve our ability to predict individuals at risk for CKD. Equally important is the need to elucidate the biologic mechanisms underlying the observed associations. Thus, further research is necessary to study whether homocysteine is along the causal pathway for the development of CKD and whether lowering homocysteine can reduce the development of CKD. In addition, additional research is warranted to evaluate whether targeting the BNP and aldosterone pathways can reduce the risk of CKD and MA. Our results will require replication and testing in clinical trial settings, as well as in cost-effectiveness settings, before being brought into the clinical arena.

Strengths and Limitations
Strengths include our well-characterized sample, with adequate number of incident CKD and MA events. We evaluated a moderate-sized multi-marker panel, and our multivariable analyses adjusted for several key and well-characterized covariates. Some limitations require mention. Our sample was white, limiting the generalizability to other ethnicities. We were constrained by biomarkers available at the baseline examination. For instance, we did not measure cystatin C, an alternative marker of CKD, at that examination. However, some recent studies do not suggest that cystatin C is superior to creatinine-based measures of renal function in estimating GFR. We considered reduced GFR separately from MA. Although CKD and MA are along a spectrum of renal disease, ample evidence suggests that the overlap and pathogenesis are disparate enough to be considered separately. Those excluded from our analysis tended to have more adverse risk factor profiles at baseline and may have biased our findings toward the null. Finally, we do not have a gold standard measure of GFR, given the unavoidable constraints in any large epidemiologic population-based study.

CONCLUSIONS
Circulating homocysteine and select neurohormonal biomarkers incrementally predicted incidence of CKD and MA in our large community-based sample. Additional studies are warranted to confirm our findings and to elucidate the underlying pathophysiologic basis for these associations.

CONCISE METHODS

Study Sample
Participants for this study were drawn from The Framingham Offspring Study, which began in 1971 with the enrollment of 5124
women and men. Participants have undergone examinations approximately every 4 years; this has been described elsewhere. Inclusion in this study included offspring cohort participants who participated in an examination during the sixth examination cycle (1995 to 1998; referred to as baseline for this study) and returned for their eighth quadrennial examination (2005 to 2008). Of the total 3532 attendees to the sixth examination cycle, 2786 also attended the eighth examination. For analyses of incident CKD, 33 were excluded because of missing serum creatinine data, 183 for CKD at exam 6, 142 for missing biomarker data, 74 for missing eGFR, and 9 for missing covariates, resulting in a final sample size of 2345 individuals free of CKD. For analyses of incident MA, 491 were excluded because of missing UACR values, 345 because of MA at exam 6, 127 because of missing biomarker data, and 1 because of missing covariates, resulting in a final study sample of 1822 participants free of MA. Those excluded from the incident CKD analysis tended to be older, had lower HDL levels, were more likely to be smokers, had higher rates of diabetes and hypertension, and had lower baseline eGFR. Those excluded from the incident MA analysis tended to be older, had lower HDL levels, were more likely to be smokers, had higher rates of diabetes and hypertension, and had higher baseline UACR levels.

This study was approved by the institutional review boards of the Boston University Medical Center, and all subjects provided written informed consent.

**Measurements and Definitions of Renal Outcomes**

eGFR was used to estimate kidney function using the simplified Modification of Diet in Renal Disease Study Equation. CKD was defined as GFR < 60 ml/min per 1.73 m²; throughout the paper, CKD refers to stage 3 CKD or lower. Serum creatinine was measured using the modified Jaffe method. Because variations in serum creatinine can occur across laboratories, we calibrated our levels using a two-step nation. For analyses of incident MA, 491 were excluded because of missing UACR values, 345 because of MA at exam 6, 127 because of missing biomarker data, and 1 because of missing covariates, resulting in a final study sample of 1822 participants free of MA. Those excluded from the incident CKD analysis tended to be older, had lower HDL levels, were more likely to be smokers, had higher rates of diabetes and hypertension, and had lower baseline eGFR. Those excluded from the incident MA analysis tended to be older, had lower HDL levels, were more likely to be smokers, had higher rates of diabetes and hypertension, and had higher baseline UACR levels.

This study was approved by the institutional review boards of the Boston University Medical Center, and all subjects provided written informed consent.

**Statistical Analysis**

Distributions of markers were calculated using the median and first and third quartile cutoffs. Markers were natural log-transformed and gender-standardized to a mean of 0 and SD of 1 to normalize their skewed distributions and to account for gender-related differences. For analyses of incident CKD and MA (separate analyses for each of these outcomes), three models were estimated: (1) model 1, which included only age and gender as covariates; (2) model 2, which included age, gender, systolic BP, hypertension treatment, smoking, body mass index, HDL, diabetes, baseline eGFR (for incident CKD), or baseline log UACR (for incident MA); and (3) model 3, which additionally included the entire biomarker panel. In model 3, if the entire biomarker panel was statistically significant, we used backward elimination to identify a parsimonious set of biomarkers that remained associated with incident CKD or incident MA. This strategy reduces the extent of multiple testing compared with relating each biomarker individually to the two outcomes of interest.

Once the select biomarkers associated with outcomes were determined, we used several different indices to determine their incremental predictive utility for identifying individuals at risk for the development of CKD or MA. For this purpose, we compared models with biomarkers to models without the biomarkers (but with all other covariates) to assess the increment in the model c-statistic (comparing the proportion of people at risk reclassified appropriately [risk reclassification]), the integrated discrimination index, and calibration indices. Because risk reclassification requires categorization of longitudinal risk (in this instance, 8-year risk of CKD or MA), we empirically (in the absence of an accepted grouping of risk) defined low-, intermediate-, and high-risk categories as 0 to <3, 3 to 6, and >6% for both outcomes, respectively.

We created biomarker scores using the median cutoffpoint of the
biomarkers associated with the outcomes. We compared the risk of outcomes (CKD and MA) in individuals with biomarker levels above the median cutpoint with that in participants with biomarker concentrations below the median cutpoint. These analyses complemented the analyses of biomarkers as log-transformed continuous variables.

We conducted additional analyses in which we related the multivariable panel to incidence of outcome events (CKD and MA, separate analyses for each): individuals without diabetes mellitus and in non-hypertensive participants. These analyses mirrored the primary analyses (if the panel was associated with outcomes, backward elimination was used to identify select biomarkers related with these outcomes).

The SAS statistical software version 8.1 was used for all statistical tests. Statistical significance was defined as two-tailed $P < 0.05$.

ACKNOWLEDGMENTS

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DISCLOSURES

None.

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See related editorial, “Prediction Modeling to Assess the Prognostic Significance of a Biomarker Panel,” on pages 2017–2019. Supplemental information for this article is available online at http://www.jasn.org/.