

Biomarkers of Cardiovascular Stress and Incident Chronic Kidney Disease

Jennifer E. Ho,^{1,2,3*} Shih-Jen Hwang,^{1,2} Kai C. Wollert,⁴ Martin G. Larson,^{1,5} Susan Cheng,^{1,6} Tibor Kempf,⁴ Ramachandran S. Vasan,^{1,7} James L. Januzzi,⁸ Thomas J. Wang,^{1,9} and Caroline S. Fox^{1,2,10}

BACKGROUND: Growth differentiation factor-15 (GDF-15), soluble ST2 (sST2), and high-sensitivity troponin I (hsTnI) are emerging predictors of adverse clinical outcomes. We examined whether circulating concentrations are related to the development of kidney disease in the community.

METHODS: Plasma GDF-15, sST2, and hsTnI concentrations were measured in 2614 Framingham Offspring cohort participants (mean age 57 years, 54% women) at the sixth examination cycle (1995–1998). Associations of biomarkers with incident chronic kidney disease [CKD, eGFR <60 mL · min⁻¹ · (1.73 m²)⁻¹, n = 276], microalbuminuria (urinary albumin to creatinine ratio ≥25 mg/g in women and 17 mg/g in men, n = 191), and rapid decline in renal function [decline in eGFR ≥3 mL · min⁻¹ · (1.73 m²)⁻¹ per year, n = 237], were evaluated using multivariable logistic regression; *P* < 0.006 was considered statistically significant in primary analyses.

RESULTS: Participants were followed over a mean of 9.5 years. Higher plasma GDF-15 was associated with incident CKD [multivariable-adjusted odds ratio (OR) 1.9 per 1-U increase in log-GDF-15, 95% CI 1.6–2.3, *P* < 0.0001] and rapid decline in renal function (OR, 1.6; 95% CI, 1.3–1.8; *P* < 0.0001). GDF-15, sST2, and hsTnI had suggestive associations with incident microalbuminuria but did not meet the prespecified *P*-value threshold after multivariable adjustment. Adding plasma GDF-15 to clinical covariates improved risk prediction of incident CKD: the *c*-statistic increased from 0.826 to 0.845 (*P* = 0.0007), and categorical net reclassification was 6.3% (95% CI, 2.7–9.9%).

CONCLUSIONS: Higher circulating GDF-15 is associated with incident renal outcomes and improves risk prediction of incident CKD. These findings may provide insights into the mechanisms of renal injury.

© 2013 American Association for Clinical Chemistry

Over 83 million Americans have cardiovascular disease and over 26 million Americans have kidney disease (1). Additionally, worsening renal function may be both a risk factor for and a consequence of progressive cardiovascular disease (2, 3). Indeed, clinical studies suggest that impaired renal function is a risk factor for the development of cardiovascular disease and subsequent prognosis (2). Conversely, experimental evidence suggests that cardiac injury may in turn accelerate loss of renal function (3).

Although the underlying mechanisms leading to chronic kidney disease (CKD)¹¹ are multifactorial, immune and inflammatory responses are potential shared biologic pathways that can contribute to the worsening of both kidney function (4) and cardiovascular disease (5). Two novel cardiovascular biomarkers, growth differentiation factor-15 (GDF-15) and soluble ST2 (sST2), are upregulated in response to inflammation (6, 7) as well as cardiovascular stress (8, 9). In combination with troponin, which is released during myocardial necrosis, GDF-15 and sST2 may reflect potential insult to the cardiovascular system. Indeed, all 3 biomarkers, GDF-15, sST2, and troponin, have emerged as strong predictors of mortality in individuals with existing cardiovascular disease (10–12), as well as community-dwelling adults (13–15).

¹ Framingham Heart Study of the National Heart, Lung and Blood Institute and Boston University School of Medicine, Framingham, MA; ² Center for Population Studies, National Heart, Lung, and Blood Institute, Bethesda, MD; ³ Cardiovascular Medicine Section, Department of Medicine, Boston University Medical Center, Boston, MA; ⁴ Division of Molecular and Translational Cardiology, Department of Cardiology and Angiology, Hannover Medical School, Hannover, Germany; ⁵ Department of Mathematics and Statistics, Boston University, Boston, MA; ⁶ Division of Cardiology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; ⁷ Cardiology and Preventive Medicine, Department of Medicine, Boston University School of Medicine, Boston, MA; ⁸ Cardiology Division, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA; ⁹ Cardiovascular Medicine Division, Department of Medicine, Vanderbilt University;

¹⁰ Division of Metabolism and Diabetes, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

* Address correspondence to this author at: Cardiovascular Medicine Section, Boston University Medical Center, 88 East Newton St., C-818, Boston, MA 02118. Fax 617-638-8729; e-mail jennifer.ho@bmc.org.

Received March 7, 2013; accepted June 27, 2013.

Previously published online at DOI: 10.1373/clinchem.2013.205716

¹¹ Nonstandard abbreviations: CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; hsTnI, high-sensitivity troponin I; IDI, integrated discrimination improvement; NRI, net reclassification improvement; OR, odds ratio; sST2, soluble ST2; UACR, urinary albumin to creatinine ratio.

Despite a growing body of evidence substantiating the role of GDF-15, sST2, and troponin in prognosticating cardiovascular disease, less is known about the potential association with the development of kidney disease. Previous studies have shown higher GDF-15 concentrations to be associated with progressive deterioration of kidney function and adverse prognosis in patients with existing kidney disease (16, 17). Cross-sectionally, higher sST2 concentrations appear to be associated with worse kidney function in study participants with cardiovascular disease (18). Increased circulating troponin concentrations are commonly observed in patients with CKD and are associated with progression to end-stage renal disease and death (19). Despite these observations, the role of these biomarkers in the development of incident renal disease in individuals without preexisting kidney disease is unknown. The association of each of these biomarkers of cardiovascular stress with kidney function may provide insights into potential mechanisms of renal injury, and the complex interaction of cardiovascular and renal disease. Given the limitations of serum creatinine in detecting early decreases in renal function, these biomarkers may also improve our ability to identify individuals at risk for the development of kidney disease. Thus, the purpose of this study was to examine the association of GDF-15, sST2, and high-sensitivity troponin I (hsTnI), with incident renal outcomes including CKD, microalbuminuria, and progressive decline in renal function.

Materials and Methods

STUDY SAMPLE

The Framingham Heart Study offspring cohort is a longitudinal community-based cohort that was recruited in 1971 and includes the children (and spouses' children) of the original cohort participants. Participants have undergone serial examinations, including routine questionnaires, a physical examination, anthropometry, and blood testing (20). Plasma GDF-15, sST2, and hsTnI concentrations were measured in frozen stored samples collected at the sixth examination (1995–1998). Of the 3532 attendees at the sixth examination, 2786 also attended the eighth examination (2005–2008). Sample characteristics of those who did and did not attend the follow-up examination are shown in Table 1 in the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol59/issue11>. A total of 132 participants were excluded because of missing creatinine data, 14 because of missing biomarker data, and 26 because of missing covariate data, leaving 2614 participants for this analysis. For each outcome, we further excluded participants with prevalent disease at the

sixth examination (164 with prevalent CKD for the incident CKD analyses and 698 with prevalent microalbuminuria or missing covariates that were additionally adjusted for in the microalbuminuria analyses). The study was approved by the institutional review board of Boston University Medical Center, and all participants provided written informed consent.

MEASUREMENTS AND DEFINITIONS OF RENAL OUTCOMES

We measured serum creatinine using the modified Jaffe method, calibrated as previously described (21). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula (22). CKD was defined as stage 3 CKD or higher [$\text{eGFR} < 60 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$]. A rapid decline in renal function was defined as a decline in $\text{eGFR} \geq 3 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ per year (23).

At the sixth and eighth examinations, spot morning urine samples were collected and initially frozen at -20°C and then transferred to -80°C . Urine albumin concentration was measured by using the Tinaquant immunoturbidimetric assay (Roche Diagnostics; interassay CV, 3.1%; intraassay CV, 2.1%), and urinary creatinine was measured by using the modified Jaffe method (Roche Diagnostics; interassay CV, 2.8%; intraassay CV, 2.1%). The urinary albumin-to-creatinine ratio (UACR) was used as a measure of urinary albumin excretion (24). Microalbuminuria was defined as a UACR $\geq 25 \text{ mg/g}$ in women and 17 mg/g in men (25). Dipstick proteinuria was assessed on spot urine samples (Ames Labstix).

BIOMARKER MEASUREMENTS

Citrated plasma samples were collected after an overnight fast and immediately centrifuged and stored at -80°C until assayed. Samples had not previously been thawed. Plasma GDF-15 concentrations were measured at a core laboratory (K.C. Wollert) with a precommercial, automated electrochemiluminescent immunoassay on a Cobas e 411 analyzer (Roche Diagnostics). The assay has a limit of detection below 10 ng/L, a linear measuring range up to 20 000 ng/L, with an intraassay CV of 0.8% and an interassay CV of 2.3% at low concentrations (1120 ng/L) and an intraassay CV of 1.1% and interassay CV of 1.0% at high concentrations (9031 ng/L). sST2 concentrations were measured at Critical Diagnostics with a high-sensitivity sandwich immunoassay (PresageTM ST2, Critical Diagnostics) (26), with a lower detection limit of 2 ng/mL and an interassay CV of 7.5% at low sST2 concentrations (25.6 ng/mL) and 6.0% at high concentrations (70.9 ng/mL). Reference limits for GDF-15 and sST2 concentrations have previously been described in participants of the Framingham offspring cohort (27, 28).

hsTnI concentrations were measured at Singulex with a high-sensitivity immunoassay for cardiac troponin I, utilizing novel, single-molecule counting technology (Erenna hsTnI, Singulex), with a limit of detection of 0.2 pg/mL, an analytical measurement range of 0.5–70 pg/mL (29), and an interassay CV of 10.0% at low hsTnI concentrations (4.7 ng/L) and 7.7% at high concentrations (19.0 ng/L). Plasma homocysteine, aldosterone, B-type natriuretic peptide (BNP), and cystatin C were measured as previously described (see the online Supplemental Methods).

CLINICAL ASSESSMENT

Participants underwent a comprehensive clinical assessment at the baseline examination. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or current antihypertensive drug treatment. Diabetes mellitus was defined as a fasting glucose ≥ 126 mg/dL (≥ 6.99 mmol/L) or the use of insulin or oral hypoglycemic medications. Current smoking was defined as smoking at least 1 cigarette per day over the past year. Total and HDL cholesterol concentrations were measured after an overnight fast. Cardiovascular events were adjudicated by a 3-physician panel after systematic review of outpatient and hospital medical records.

STATISTICAL ANALYSES

The association of each biomarker (GDF-15, sST2, and hsTnI) with each renal outcome (incident CKD, incident microalbuminuria, rapid decline in renal function) was modeled using multivariable logistic regression. Biomarkers were natural-log-transformed owing to right-skewed distributions. The primary models for incident CKD and rapid decline were adjusted for age, sex, baseline eGFR, hypertension, diabetes, and baseline proteinuria by dipstick; microalbuminuria analyses were adjusted for age, sex, hypertension, diabetes, BMI, smoking, baseline log-UACR, and HDL cholesterol. On the basis of previous analyses of predictors of incident kidney disease (30, 31), models were further adjusted for homocysteine and aldosterone in secondary analyses; additionally, microalbuminuria analyses were adjusted for BNP. To account for the number of biomarkers ($n = 3$) and kidney traits tested ($n = 3$), primary analysis results were considered significant at a corrected P -value threshold of $0.05/9 = 0.006$.

In secondary analyses, we excluded participants with baseline diabetes mellitus, hypertension, or prevalent coronary heart disease or heart failure from GDF-15 analyses. We also adjusted for cystatin C (measured at exam 7) in secondary analyses.

To assess the incremental benefit of each biomarker (if found significantly associated with renal function), the c -statistic was compared in models with

Table 1. Baseline characteristics of 2,614 Framingham Heart Study participants.

Clinical characteristic	
Age, years	57 (9)
Women, n (%)	1415 (54)
Systolic blood pressure, mmHg	127 (18)
Diastolic blood pressure, mmHg	75 (9)
Antihypertensive medications, n (%)	634 (24)
Diabetes, n (%)	195 (7)
Prevalent heart failure, n (%)	5 (0.2)
Prevalent coronary heart disease, n (%)	77 (3)
Body-mass index, kg/m ²	27.8 (5.1)
Current smoking, n (%)	364 (14)
Total cholesterol, mg/dl [mmol/L]	206 (38) [5.33 (0.98)]
HDL cholesterol, mg/dl [mmol/L]	52 (16) [1.34 (0.41)]
Renal function	
eGFR, mL · min ⁻¹ · (1.73 m ²) ⁻¹	87 (18)
UACR, median (25th–75th percentile)	5.9 (2.7–13.6)
Microalbuminuria by UACR, n (%)	338 (15)
Dipstick proteinuria, n (%)	454 (17)
Biomarkers (median, 25th–75th percentile)	
GDF-15, ng/L	983 (790–1261)
sST2, ng/mL	20.5 (16.3–25.5)
hsTnI, ng/L	1.28 (0.84–2.11)
Values are means (SD) unless otherwise indicated.	

traditional risk factors with and without the addition of the biomarker. We estimated the integrated discrimination improvement (IDI) and the category-free net reclassification improvement (NRI) metric (32). Category-based NRI metrics were calculated using the same empirical groupings of risk as previously reported (low, 0% to <3%; intermediate, 3% to 6%; and high, >6% 8-year risk of CKD or microalbuminuria) (30). Analyses were conducted with SAS version 9.2 for Windows.

Results

The study sample included 2614 participants of the Framingham Offspring cohort with a mean age of 57 years, and slightly more than half were women. Thirty-seven percent had hypertension, 7% had diabetes, and 3% had a history of coronary heart disease at baseline. Median and first/third quartile concentrations for each biomarker are displayed in Table 1. Baseline renal function parameters by subsequent outcome are displayed in online Supplemental Table 2.

Table 2. Association of cardiovascular biomarkers with incident CKD.

Outcome	Biomarker	Multivariable model	
		OR ^a (95% CI)	<i>P</i> ^b
Incident CKD ^c (n = 276)	GDF-15	1.91 (1.61–2.26)	<0.0001
	sST2	1.15 (1.00–1.33)	0.05
	hsTnI	1.16 (1.01–1.33)	0.03
Microalbuminuria ^d (n = 191)	GDF-15	1.25 (1.03–1.52)	0.02
	sST2	1.24 (1.06–1.45)	0.007
	hsTnI	1.22 (1.06–1.41)	0.007
Rapid decline in renal function ^c (n = 237)	GDF-15	1.55 (1.31–1.83)	<0.0001
	sST2	1.17 (1.00–1.36)	0.05
	hsTnI	1.09 (0.94–1.26)	0.24

^a OR is per 1-unit increase in the log-transformed biomarker, where a 1 SD change is 0.43 log-units for GDF-15, 0.36 log-units for sST2, and 0.75 log-units for hsTnI.
^b Corrected *P*-value threshold = 0.006.
^c Adjusted for age, sex, baseline eGFR, hypertension, diabetes, and dipstick proteinuria.
^d Adjusted for age, sex, hypertension, diabetes, body-mass index, smoking, log-UACR, and HDL cholesterol.

BIOMARKERS AND INCIDENT CKD

Over 9.5 years of follow-up, 276 participants (140 women) developed incident CKD, defined as an eGFR $<60 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$. Each 1-U increase in log-GDF-15 was associated with a nearly 2-fold increased odds of incident CKD [multivariable-adjusted odds ratio (OR) per unit increase log-GDF-15, 1.91; 95% CI, 1.61–2.26; $P < 0.0001$] (Table 2). Neither sST2 nor hsTnI was associated with incident CKD at the prespecified threshold for statistical significance after we accounted for clinical covariates ($P = 0.05$ and $P = 0.03$, respectively).

Multivariable-adjusted ORs of incident CKD by quartiles of GDF-15, sST2, and hsTnI (Fig. 1A) showed a significant increase in CKD risk across GDF-15 (P for trend <0.0001). Compared with the lowest quartile, the upper GDF-15 quartile was associated with a 5.65-fold increased odds (95% CI, 2.97–10.75; $P < 0.0001$) of incident CKD. The number of incident CKD events by biomarker quartiles is shown in online Supplemental Table 3.

BIOMARKERS AND INCIDENT MICROALBUMINURIA

A total of 191 participants (78 women) developed incident microalbuminuria after 9.5 years of follow-up, defined as a UACR $\geq 25 \text{ mg/g}$ in women and $\geq 17 \text{ mg/g}$ in men. All 3 biomarkers had suggestive associations with incident microalbuminuria but did not meet statistical significance after multivariable adjustment, when the corrected *P*-value threshold was considered ($P = 0.02$ for GDF-15; $P = 0.007$ for both sST2 and hsTnI) (Table 2).

The risk of incident microalbuminuria by quartiles of GDF-15, sST2, and hsTnI is displayed in Fig. 1B,

and shows trends toward increasing risk of microalbuminuria across sST2 and hsTnI quartiles (P for trend = 0.005 for both). The number of incident microalbuminuria events by biomarker quartiles is shown in online Supplemental Table 3.

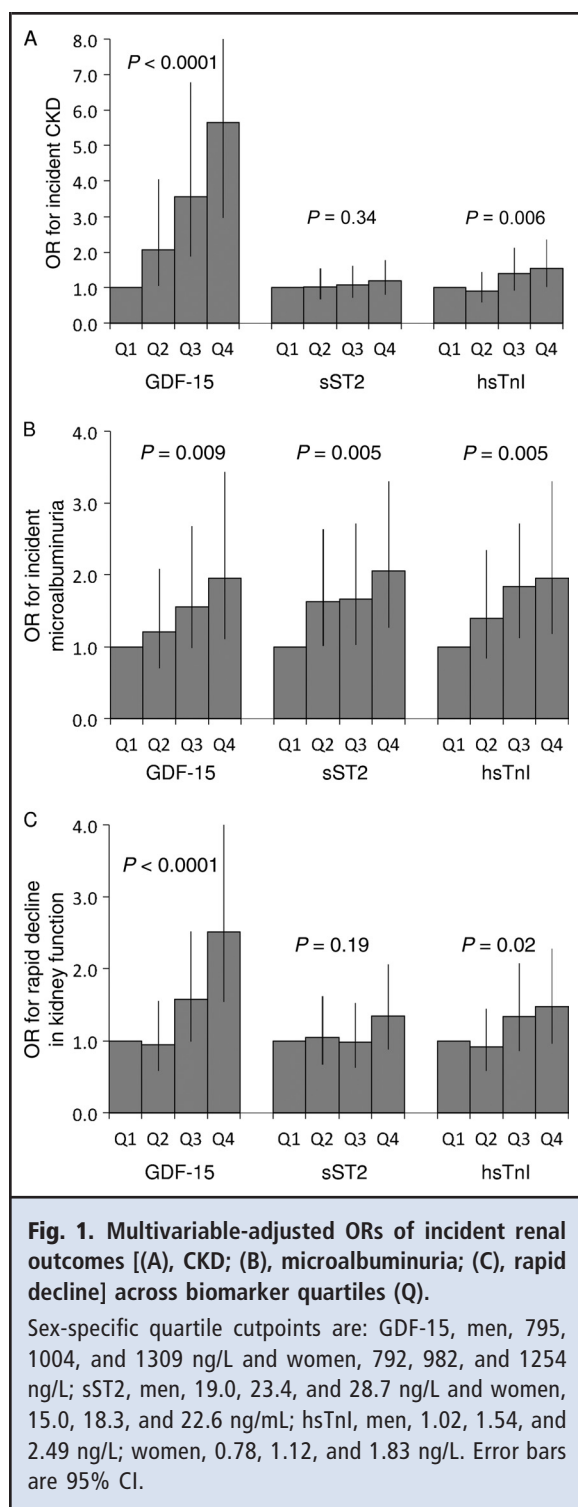
BIOMARKERS AND RAPID DECLINE IN RENAL FUNCTION

Over 9.5 years of follow-up, 237 participants (133 women) experienced a rapid decline in kidney function, defined as a decline in eGFR $\geq 3 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ per year. GDF-15 was associated with rapid decline (OR per unit increase log-GDF, 1.55; 95% CI, 1.31–1.83; $P < 0.0001$) (Table 2). Neither sST2 nor hsTnI were associated with a rapid decline in eGFR after multivariable adjustment.

Multivariable-adjusted ORs of rapid decline in renal function by quartiles of GDF-15, sST2, and hsTnI are displayed in Fig. 1C. Increasing GDF-15 quartiles were associated with a higher risk of rapid decline in renal function (P for trend <0.0001). Specifically, the upper GDF-15 quartile was associated with a 2.51-fold increased odds (95% CI, 1.54–4.09; $P = 0.0002$) of rapid decline compared with the lowest quartile. The numbers of rapid decline events are shown by biomarker quartile in online Supplemental Table 3.

GDF-15 IN THE PREDICTION OF KIDNEY DISEASE

We next evaluated the incremental predictive value of GDF-15 when added to a base model of established clinical CKD risk factors. The addition of GDF-15 to a clinical model predicting incident CKD resulted in a statistically significant increase in the *c*-statistic (from 0.826 to 0.845, $P = 0.0007$) (Table 3) and also led to



modest but significant improvements in reclassification [categorical NRI, 6.3%; 95% CI 2.7–9.9% (see online Supplemental Table 4) category-free NRI 43.5%, 95% CI, 31.1–55.9%].

Table 3. GDF-15 in the prediction of incident CKD.

	Incident CKD
<i>c</i> -Statistic (95% CI)	
Clinical model ^a	0.826 (0.801 to 0.850)
Clinical model + GDF-15	0.845 (0.822 to 0.868)
<i>P</i> value (clinical model vs clinical model + GDF-15)	0.0007
IDI (95% CI)	0.034 (0.021 to 0.047)
Categorical NRI ^b (95% CI) (clinical model vs clinical model + GDF-15)	6.3% (2.7% to 9.9%)
Category-free NRI (95% CI) (clinical model vs clinical model + GDF-15)	43.5% (31.1% to 55.9%)

^a Includes age, sex, baseline eGFR, hypertension, diabetes, and dipstick proteinuria.
^b Categorical NRI with thresholds at 3% and 6% of predicted risk to change classification.

SECONDARY ANALYSES

After accounting for the 3 biomarkers simultaneously, in addition to previously established biomarkers associated with renal outcomes (aldosterone and homocysteine for incident CKD and rapid decline in renal function; aldosterone, homocysteine, and BNP for microalbuminuria) (30, 31), GDF-15 remained a significant predictor of incident CKD (OR per 1-U increase in log-GDF15, 1.72; 95% CI, 1.44–2.07; $P < 0.0001$) and rapid decline in renal function (OR per unit increase log-GDF-15, 1.34; 95% CI, 1.11–1.62; $P = 0.002$). These results were not materially different after additional adjustment for BNP. After we adjusted for the previously established biomarkers, GDF-15, sST2, and hsTnI were not significant predictors of incident microalbuminuria ($P = 0.13, 0.04$, and 0.03 , respectively).

After additional adjustment for cystatin C, GDF-15 remained an independent predictor of incident CKD (multivariable-adjusted OR per unit increase log-GDF-15, 1.45; 95% CI, 1.21–1.75; $P < 0.0001$), whereas the association with rapid decline in renal function was attenuated but remained significant (OR per unit increase log-GDF-15, 1.23; 95% CI, 1.01–1.49; $P = 0.04$).

In sensitivity analyses excluding participants with baseline diabetes, hypertension, and coronary heart disease/heart failure, GDF-15 remained a significant predictor of incident CKD and rapid decline in renal function (see online Supplemental Table 5).

Discussion

Our findings demonstrate that GDF-15 is associated with incident CKD and rapid decline in renal function

in participants from the Framingham Heart Study. The addition of GDF-15 to clinical covariates also resulted in improvement in the prediction of incident CKD, including the *c*-statistic and risk reclassification metrics. This suggests that GDF-15 may be useful in predicting the development of CKD, years before the clinical onset of disease.

GDF-15 is a stress-responsive member of the transforming growth factor- β cytokine superfamily (33). In the kidney, GDF-15 is expressed weakly along the entire nephron, and it is markedly upregulated in the outer medullary collecting duct in response to metabolic acidosis and potassium depletion, where it appears to trigger compensatory proliferation of acid-secreting collecting duct cells (34). Despite potential cardio- and renal-protective effects shown in experimental studies, higher GDF-15 concentrations have been associated with adverse prognosis in patients with cardiovascular disease (10), as well as in individuals in the community (13, 15). Higher GDF-15 concentrations are also associated with increased mortality in hemodialysis patients and in patients with diabetic nephropathy (16, 17). In the latter group, increased GDF-15 concentrations were associated with a greater decline in estimated glomerular filtration rate and higher progression to end-stage renal disease (17). Our study extends these findings to a population-based sample, where we demonstrated a strong association with incident kidney disease. In addition, our findings establish the association of GDF-15 and incident kidney disease in a population with early CKD, for which associations are less likely to be confounded by other metabolic derangements and reduced filtration, which accompany more advanced renal disease.

Whether GDF-15 actively contributes to the development of CKD owing to its association with vascular dysfunction or via direct kidney effects remains to be investigated. In an experimental model of diabetic renal injury, increases in urinary GDF-15 were associated with a proximal tubule injury marker (35). Renal GDF-15 expression also appears to be upregulated in response to metabolic acidosis (34) and kidney injury (36). Outside of the kidney, GDF-15 is expressed in various tissue types, including cardiomyocytes (8), in response to oxidative and/or metabolic stress and inflammation. In the community, increased concentrations of GDF-15 are associated with endothelial dysfunction and other measures of subclinical cardiovascular disease (37). Further studies are needed to clarify whether GDF-15 is a marker or a causal mediator of cardiovascular and renal disease.

Our findings suggest that GDF-15 may be useful in the prediction of incident CKD. The addition of GDF-15 to the clinical model resulted in a modest increment in the *c*-statistic and increases in NRI that were

comparable to improvements seen with a multimarker panel consisting of aldosterone, BNP, and homocysteine in the same population (30). Biomarkers may be informative in CKD risk prediction, because the diagnosis of CKD is made on the basis of biochemical data. Future studies are needed to validate our findings in additional large cohorts, and to evaluate the clinical utility of using GDF-15 in CKD prevention efforts.

We also observed nominal associations of GDF-15, sST2, hsTnI, and incident microalbuminuria, although these associations did not persist after we accounted for multiple hypothesis testing or adjusted for previously studied biomarkers. ST2 is a member of the interleukin-1 receptor family, whose production in cardiomyocytes is upregulated in response to mechanical stress (11). Like GDF-15, ST2 and its ligand IL-33 are expressed in endothelial cells in response to inflammatory cytokines (6), and the ST2/IL-33 pathway appears to be a contributor to endothelial dysfunction and early atherosclerosis (38). Vascular dysfunction is known to precede the development of microalbuminuria (39) and may be one of the plausible mechanisms by which increased GDF-15 and sST2 are relevant to kidney injury. Increases in circulating troponins are observed commonly in patients with CKD and are associated with increased risk of kidney disease progression (19). In experimental studies, myocardial damage is directly associated with the development of proteinuria and focal glomerulosclerosis (3), suggesting that the association of circulating troponin and kidney damage is related to cardiac injury, rather than diminished clearance (19). Further studies will be needed to firmly establish any association of sST2, hsTnI, and kidney outcomes.

There are several limitations to our study that deserve mention. Kidney function was estimated on the basis of a single creatinine and urinary albumin measurement, rather than persistent changes over time, which may have resulted in misclassification of renal outcomes. Blood samples were frozen and stored until analysis, and sample degradation over time may have influenced results. Our results may have also been influenced by survivor bias, as only individuals who attended both initial and follow-up examinations for assessment of renal function were included. Thus, sicker individuals at greater risk for developing kidney disease may not have been captured. However, both of these potential limitations would have likely biased our results toward the null. Our sample was restricted to a predominantly white population of European ancestry, and generalization of our findings to other ethnicities is unclear.

In summary, we found that GDF-15 was associated with new-onset kidney disease and rapid decline in renal function in the community. The addition of

GDF-15 to established risk factors resulted in improvements in CKD risk prediction. This result suggests that GDF-15 may help to identify individuals at high risk for developing CKD and, in turn, may aid in targeting preventive efforts in the future.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors' Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Employment or Leadership: None declared.

Consultant or Advisory Role: K.C. Wollert, Roche Diagnostics; J.L. Januzzi, Critical Diagnostics.

Stock Ownership: None declared.

Honoraria: K.C. Wollert, Roche Diagnostics.

Research Funding: The urinary albumin excretion assays were supported by a grant from the American Diabetes Association (Dr. James Meigs, not a co-author on this paper) and by donation of reagents by Roche Diagnostics, Inc. (Framingham Heart Study); J.E. Ho, American Heart Association, the National Institutes of Health (1K23-HL116780); K.C. Wollert, Roche Diagnostics and the German Ministry of Education and Research (BMBF, BioChancePlus); S. Cheng, the Ellison Foundation; J.L. Januzzi, the DeSanctis Clinical Scholar Endowment, Roche Diagnostics, and Critical Diagnostics. The National Heart, Lung and Blood Institute's Framingham Heart Study (contract no. N01-HC-25195 to Boston University). Measurement of sST2 was performed by Critical Diagnostics, Inc; GDF-15 assays were provided by Roche Diagnostics, Inc; measurement of hsTnI was performed by Singulex, Inc (T.J. Wang, J.L. Januzzi, R.S. Vasan, and K.C. Wollert).

Expert Testimony: K.C. Wollert, Roche Diagnostics.

Patents: T. Kempf, patent number EP1884777. T. Kempf and K.C. Wollert are named as co-inventors on a patent for the use of GDF-15 for cardiovascular applications, and have a contract with Roche Diagnostics for the development of a GDF-15 assay (European patent 2047275B1).

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References

- Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, et al. Heart disease and stroke statistics—2013 update: a report from the American Heart Association. *Circulation* 2013; 127:e6–245.
- Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 2003;108:2154–69.
- van Dokkum RP, Eijkelkamp WB, Kluppel AC, Henning RH, van Goor H, Citgez M, et al. Myocardial infarction enhances progressive renal damage in an experimental model for cardio-renal interaction. *J Am Soc Nephrol* 2004;15: 3103–10.
- Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G, Cholesterol and Recurrent Events (CARE) Trial Investigators. Biomarkers of inflammation and progression of chronic kidney disease. *Kidney Int* 2005;68:237–45.
- Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135–43.
- Bartunek J, Delrue L, Van Durme F, Muller O, Casselman F, De Wiest B, et al. Nonmyocardial production of ST2 protein in human hypertrophy and failure is related to diastolic load. *J Am Coll Cardiol* 2008;52:2166–74.
- Secchiero P, Corallini F, Gonelli A, Dell'Eva R, Vitale M, Capitani S, et al. Antiangiogenic activity of the MDM2 antagonist nutlin-3. *Circ Res* 2007; 100:61–9.
- Kempf T, Eden M, Strelau J, Naguib M, Willenbockel C, Tongers J, et al. The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. *Circ Res* 2006;98: 351–60.
- Weinberg EO, Shimp M, De Keulenaer GW, MacGillivray C, Tominaga S, Solomon SD, et al. Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. *Circulation* 2002;106: 2961–6.
- Wollert KC, Kempf T, Peter T, Olofsson S, James S, Johnston N, et al. Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome. *Circulation* 2007;115:962–71.
- Weinberg EO, Shimp M, Hurwitz S, Tominaga S, Rouleau JL, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation* 2003;107:721–6.
- Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, et al. A sensitive cardiac troponin T assay in stable coronary artery disease. *N Engl J Med* 2009;361:2538–47.
- Daniels LB, Clopton P, Laughlin GA, Maisel AS, Barrett-Connor E. Growth-differentiation factor-15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: the Rancho Bernardo Study. *Circulation* 2011;123: 2101–10.
- Saunders JT, Nambi V, de Lemos JA, Chambless LE, Virani SS, Boerwinkle E, et al. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the atherosclerosis risk in communities study. *Circulation* 2011;123:1367–76.
- Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, et al. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation* 2012;126: 1596–604.
- Breit SN, Carrero JJ, Tsai VW, Yagoutifam N, Luo W, Kuffner T, et al. Macrophage inhibitory cytokine-1 (MIC-1/GDF15) and mortality in end-stage renal disease. *Nephrol Dial Transplant* 2011;27:70–5.
- Lajer M, Jorsal A, Tarnow L, Parving HH, Rossing P. Plasma growth differentiation factor-15 independently predicts all-cause and cardiovascular mortality as well as deterioration of kidney function in type 1 diabetic patients with nephropathy. *Diabetes Care* 2010;33:1567–72.
- Januzzi JL Jr, Peacock WF, Maisel AS, Chae CU, Jesse RL, Baggish AL, et al. Measurement of the interleukin family member ST2 in patients with acute dyspnea: results from the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study. *J Am Coll Cardiol* 2007;50:607–13.
- Desai AS, Toto R, Jarolim P, Uno H, Eckardt KU, Kewalramani R, et al. Association between cardiac biomarkers and the development of ESRD in patients with type 2 diabetes mellitus, anemia, and CKD. *Am J Kidney Dis* 2011;58:717–28.
- Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. *Am J Epidemiol* 1979;110:281–90.
- Fox CS, Larson MG, Leip EP, Culleton B, Wilson PWF, Levy D. Predictors of new-onset kidney disease in a community-based population. *JAMA* 2004;291:844–50.
- Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150:604–12.
- Shlipak MG, Katz R, Kestenbaum B, Fried LF, Siscovick D, Sarnak MJ. Clinical and subclinical cardiovascular disease and kidney function decline in the elderly. *Atherosclerosis* 2009;204: 298–303.
- Nathan DM, Rosenbaum C, Protasowicki VD. Single-void urine samples can be used to estimate quantitative microalbuminuria. *Diabetes Care* 1987;10:414–8.
- National Kidney Foundation. *K/DOQI clinical*

- practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002;39(2 Suppl 1):S1–266.
26. Dieplinger B, Januzzi JL Jr, Steinmair M, Gabriel C, Poelz W, Haltmayer M, Mueller T. Analytical and clinical evaluation of a novel high-sensitivity assay for measurement of soluble ST2 in human plasma—the Presage ST2 assay. *Clin Chim Acta* 2009;409:33–40.
 27. Coglianese EE, Larson MG, Vasan RS, Ho JE, Ghorbani A, McCabe EL, et al. Distribution and clinical correlates of the interleukin receptor family member soluble ST2 in the Framingham Heart Study. *Clin Chem* 2012;58:1673–81.
 28. Ho JE, Mahajan A, Chen MH, Larson MG, McCabe EL, Ghorbani A, et al. Clinical and genetic correlates of growth differentiation factor 15 in the community. *Clin Chem* 2012;58:1582–91.
 29. Todd J, Freese B, Lu A, Held D, Morey J, Livingston R, Goix P. Ultrasensitive flow-based immunoassays using single-molecule counting. *Clin Chem* 2007;53:1990–5.
 30. Fox CS, Gona P, Larson MG, Selhub J, Tofler G, Hwang SJ, et al. A multi-marker approach to predict incident CKD and microalbuminuria. *J Am Soc Nephrol* 2010;21:2143–9.
 31. O’Seaghdha CM, Hwang S-J, Upadhyay A, Meigs JB, Fox CS. Predictors of incident albuminuria in the Framingham Offspring cohort. *Am J Kidney Dis* 2010;56:852–60.
 32. Pencina MJ, D’Agostino RB Sr, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med* 2011;30:11–21.
 33. Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc Natl Acad Sci USA* 1997;94:11514–9.
 34. Duong Van Huyen JP, Cheval L, Bloch-Faure M, Belair MF, Heudes D, Bruneval P, Doucet A. GDF15 triggers homeostatic proliferation of acid-secreting collecting duct cells. *J Am Soc Nephrol* 2008;19:1965–74.
 35. Simonson MS, Tikkin M, Debanne SM, Rahman M, Berger B, Hricik D, Ismail-Beigi F. The renal transcriptome of db/db mice identifies putative urinary biomarker proteins in patients with type 2 diabetes: a pilot study. *Am J Physiol Renal Physiol* 2012;302:F820–9.
 36. Zimmers TA, Jin X, Hsiao EC, McGrath SA, Esquela AF, Koniaris LG. Growth differentiation factor-15/macrophage inhibitory cytokine-1 induction after kidney and lung injury. *Shock* 2005;23:543–8.
 37. Rohatgi A, Patel P, Das SR, Ayers CR, Khera A, Martinez-Rumayor A, et al. Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: observations from the Dallas Heart Study. *Clin Chem* 2012;58:172–82.
 38. Demyanets S, Konya V, Kastl SP, Kaun C, Rauscher S, Niessner A, et al. Interleukin-33 induces expression of adhesion molecules and inflammatory activation in human endothelial cells and in human atherosclerotic plaques. *Arterioscler Thromb Vasc Biol* 2011;31:2080–9.
 39. Stehouwer CD, Fischer HR, van Kuikj AW, Polak BC, Donker AJ. Endothelial dysfunction precedes development of microalbuminuria in IDDM. *Diabetes* 1995;44:561–4.