Cardiac Troponin T and C-Reactive Protein for Predicting Prognosis, Coronary Atherosclerosis, and Cardiomyopathy in Patients Undergoing Long-term Hemodialysis

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MORTALITY AMONG PATIENTS WITH END-STAGE RENAL DISEASE (ESRD) REMAINS AS HIGH AS 23% PER YEAR DESPITE ADVANCES IN DIALYSIS, WITH CARDIAC CAUSES ACCOUNTING FOR 40% TO 45% OF ALL DEATHS.1 BECAUSE THIS HIGH BURDEN OF DISEASE RESULTS FROM CORONARY ARTERY DISEASE (CAD) OR CARDIOMYOPATHY, CARDIAC RISK STRATIFICATION IS A KEY ISSUE IN THE CLINICAL MANAGEMENT OF THESE PATIENTS.

Inflammation, critical to the pathogenesis of atherosclerosis,2 can be demonstrated by elevation of serum C-reactive protein (CRP) levels in more than 70% of patients receiving hemodialysis.3,7 Furthermore, levels of cardiac troponin T (cTnT), a serum marker of myocardial infarction,8 can be elevated

Context Cardiac troponin T (cTnT) and C-reactive protein (CRP) are prognostic markers in acute coronary syndromes. However, for patients with end-stage renal disease (ESRD) the ability of combinations of these markers to predict outcomes, and their association with cardiac pathology, are unclear.

Objective To investigate the association between levels of cTnT and CRP and long-term risk of cardiac pathology and death in patients with ESRD.

Design, Setting, and Participants A prospective cohort study initiated February through June 1998 and enrolling 224 patients with ESRD from 5 hemodialysis centers in the Houston-Galveston region of Texas. Levels of cTnT and CRP were analyzed at study entry in patients without ischemic symptoms.

Main Outcome Measures All-cause mortality during a mean follow-up of 827 (range, 29-1327) days. Secondary outcomes in predefined substudies were coronary artery disease (CAD), decreased (<40%) left ventricular ejection fraction (LVEF), and presence of left ventricular hypertrophy (LVH).

Results One hundred seventeen (52%) patients died during follow-up. For levels of cTnT and CRP, progressively higher levels predicted increased risk of death compared with the lowest quartile (for cTnT quartile 2: unadjusted hazard ratio [HR], 2.2; 95% confidence interval [CI], 1.2-4.1; quartile 3: HR, 2.7; 95% CI, 1.5-4.9; quartile 4: HR, 3.0; 95% CI, 1.6-5.3. For CRP quartile 2: HR, 0.9; 95% CI, 0.5-1.6; quartile 3: HR, 1.8; 95% CI, 1.1-3.1; quartile 4: HR, 1.8; 95% CI, 1.1-3.2). Both cTnT and CRP remained independent predictors of death after adjusting for a number of potential confounders. The combination of cTnT and CRP results provided prognostic information when patients were divided into groups at or above and below the biomarker medians (high cTnT/high CRP levels vs low cTnT/low CRP levels for risk of death: HR, 2.5; 95% CI, 1.5-4.0). Elevated levels of cTnT, but not CRP, were strongly associated with diffuse CAD (n=67; 0%, 25%, 50%, and 62% prevalence of multivessel CAD across progressive cTnT quartiles, P<.001). An LVEF of 40% or less was identified in 4 (9%), 3 (8%), 10 (27%), and 7 (19%) of patients across cTnT quartiles (P=.07). No trend for cTnT levels was found among patients with LVH (P=.45); similarly, no trend for CRP was found among patients with LVH (P=.65) or an LVEF of 40% or less (P=.75).

Conclusions Among stable patients with ESRD, increasing levels of cTnT and CRP are associated with increased risk of death. Furthermore, higher levels of cTnT may identify patients with severe angiographic coronary disease.
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in 30% to 75% of these patients.7,9-14 When elevated, these biomarkers may be associated with increased all-cause mortality.3,4,6,7,10-14 However, little is known regarding the potential complementary roles of measuring levels of cTnT and CRP for predicting all-cause mortality, and the relationship between these biomarker levels and underlying cardiac pathology in stable patients with ESRD.

To address these issues, we undertook a prospective multicenter study of patients receiving long-term hemodialysis without symptoms of myocardial ischemia in whom follow-up outcomes, CAD, and left ventricular function and mass were assessed. Our goals were to compare the predictive values of cTnT and CRP levels for long-term risk of all-cause mortality and to investigate if these biomarkers are indicators of the severity of either coronary atherosclerosis demonstrated by angiography or of cardiomyopathy as manifested by left ventricular systolic dysfunction or hypertrophy determined by echocardiography.

METHODS
Study Design
The protocol was approved by the institutional review board at the University of Texas Medical Branch at Galveston, and written informed consent was obtained from each patient. The study was conducted in the Houston-Galveston region of Texas, and from February through June 1998 224 of 334 screened dialysis patients were prospectively enrolled at 5 centers, including 3 community-based dialysis sites located in suburban and semirural areas, and 2 university-based sites located in urban settings (range of enrollment per center was 29-70 patients). To be enrolled, patients had to have been undergoing hemodialysis for more than 30 days, be 18 years of age or older, and be free from any acute coronary event for more than 4 weeks. Of the 110 screened patients not included, 89 declined to participate and 21 consented but did not have blood drawn secondary to transfer (n=7), transplant (n=1), withdrawal of consent (n=7), poor compliance with dialysis (n=1), or death (n=5). The initial clinical evaluation included a history, physical examination, and a review of medical records for prior cardiac events. The adequacy of dialysis was estimated at study entry by a dialysis dose (Kt/V) greater than 1.2 calculated by the single-pool method.15 Levels of serum biomarkers were obtained immediately prior to the midweek dialysis at study entry.

Outcome End Points
Outcomes through October 2001 were determined by linking to data in the United States Renal Data System (USRDS) for deaths and kidney transplantation. The primary end point was all-cause mortality. Cause of death was determined from the USRDS, with cardiac etiologies defined as deaths related to myocardial infarction, congestive heart failure, cardiomyopathy, arrhythmia, and cardiac arrest.1 Patients were censored from further follow-up if they underwent kidney transplantation (n=25; mean [SD] days enrolled in the study, 604 [337]).

Blood Sampling and Analysis
Blood samples were collected without an anticoagulant and allowed to clot for at least 30 minutes, then centrifuged at 1000g for 12 minutes. The resulting serum was aliquoted, frozen, and maintained at –70°C. Samples were thawed within 6 months for measurements of cTnT levels using a third-generation assay (Troponin T STAT immunoassay, Elecsys 2010 system, Roche Diagnostics, Indianapolis, Ind) and then refrozen at –70°C within 24 hours. Rethawed samples were used to measure highsensitivity CRP (BN II analyzer, Dade Behring, Glasgow, Del). Routine clinical chemistry variables (including albumin and creatine kinase-MB) were analyzed by standardized methods.

Coronary Angiography
An angiographic substudy was undertaken to determine the prevalence and severity of CAD according to biomarker concentrations. Volunteers were recruited for angiography in the absence of coronary angiography within 2 years of study enrollment or vascular disease precluding access to the femoral arteries. The study paid the technical fees; professional fees were deferred. All patients without contraindications were provided an opportunity to volunteer during the first 10 months of the study.

Angiography was performed on offf-dialysis days using a low-osmolality nonionic contrast medium (iodixanol) and were interpreted independently by 2 experienced angiographers who were blinded to the levels of biomarkers. Differences were resolved by consensus.

High interobserver and intraobserver correlation for these readers has been shown.16 Coronary artery disease was defined as a 50% or greater lumen narrowing of a major epicardial artery or its branches. A left main stenosis of 50% or greater was regarded as equivalent to 2-vessel disease. In addition, we applied a CAD prognostic index that accounts for both the severity and location of coronary stenosis. For the analysis we identified patients with a score greater than 48; this score has been previously associated with a poor prognosis in patients with ischemic cardiomyopathy.17 For this index analysis we assumed a stenosis of 70% or greater was equivalent to 75% or greater.

Echocardiography
Echocardiograms were prospectively obtained at 1 of 3 points during the first 18 months of the study: just prior to dialysis, at the time of angiography for patients volunteering for the procedure, or as part of a clinically indicated study. Echocardiograms were interpreted without knowledge of biomarker concentrations. Left ventricular volumes were quantified using the Simpson biplane formula. Left ventricular mass was calculated using the area-length method18 and indexed for body surface area. Left ventricular hypertrophy (LVH) was defined as left ventricular mass index greater than 125 g/m².19

Statistical Analysis
Continuous clinical variables are reported as medians and interquartile
ranges or as means and SDs. Kruskal-Wallis tests and Fisher exact tests were used to assess between-group differences for continuous and categorical data, respectively. For the relationships of biomarker quartiles with multivessel CAD, CAD prognostic index, and echocardiographic parameters, the existence of trends was explored using the Cochran-Armitage test for categorical variables and Spearman rank correlation analysis for continuous variables. Logistic regression was used to test if levels of cTnT were an independent predictor of multivessel CAD. The stability of this estimate was tested using the method of Hosmer and Lemeshow. Prevalence ratios were calculated from the odds ratios using the method of Zhang and Yu.

Kaplan-Meier estimates were generated to describe outcome time-course for quartiles of biomarker levels and cTnT/CRP combinations, with each biomarker dichotomized at its median. The log-rank test was used to compare differences.

Cox regression models for all-cause mortality were used to calculate the hazard ratio (HR) of progressively higher quartiles of biomarker levels, cTnT/CRP combinations, and to determine the effects of baseline cTnT and CRP levels after adjustment for clinical variables. Various transformations (ie, none, inverse, square root, square, quadratic, quartiles) for each biomarker were explored for best goodness-of-fit in individual Cox models, adjusting each for age. The transformations selected in this way were: for cTnT, transformation to quartiles; for CRP, no transformation. In addition to biomarker levels, the following clinical variables were considered in Cox regression models: age; white race; sex; length of time receiving dialysis; history of smoking, coronary disease, or diabetes; levels of albumin; Kt/V; and body surface area. Interactions between levels of cTnT and CRP and each potential confounder were explored in Cox models that included 1 biomarker and the interaction of the confounder being tested with that biomarker. Stepwise Cox models were created that forced cTnT and CRP into the model and selected among the confounders to create a final model that was identical for P-to-enter values between .05 and .25 and for P-to-remove values of .05 or .10. The assumption of proportionality was tested using the method described by SAS. The test for trend in the HR involved the inclusion of a time-dependent interaction term. The stability of point estimates of HRs was verified using 1000 bootstrap resampling analyses. The final Cox model was run for each sample, and the lower 2.5% confidence limits on the HRs for all independent variables were calculated.

Two-sided P values less than .05 were considered statistically significant. Analyses were performed using SAS version 8.02 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics and Outcomes

Baseline clinical and biochemical characteristics of the study population are shown in Table 1. No patients were lost to follow-up. After a mean follow-up period of 827 days (SD, 430 days; range, 29-1327 days), 117 patients (52%) died (62 [53%] cardiac, 33 [28%] noncardiac, and 22 [19%] of uncertain etiology). Etiologies of noncardiac deaths included infection/sepsis (43%), cerebrovascular accident (21%), malignancy (14%), and other causes (22%).

Risk Gradient Across Biomarker Quartiles

The distribution of all-cause mortality over time by quartiles of cTnT and CRP levels is shown in Kaplan-Meier plots in Figure 1. Separation of the biomarker quartiles curves occurred initially within the first months of follow-up and typically continued to widen during the next 3.5 years for both biomarkers. Unadjusted Cox regression analyses are summarized in Table 2. For levels of cTnT and CRP, progressively higher levels predicted increased risk of death compared with the lowest quartile.

To test if combinations of the 2 biomarker levels were complementary for predicting death, patients were divided into 4 groups on the basis of whether their biomarker levels fell at or above (high) or below (low) the medians for cTnT and CRP. The results of the analyses are shown in Table 2 and Figure 2. There was a 2.5-fold (95% CI, 1.3-4.0) increased risk of death over the study period in patients having high levels of both cTnT and CRP compared with those having low levels of both. For patients with high levels of only 1 marker, the risk of death was between that for those with low or high levels of both biomarkers, although it was not significantly different from that for patients with low levels of both markers. Among patients with low levels of CRP, low and high levels of cTnT did not differentiate a risk of a cardiac cause of death (63% vs 42% respectively; age-adjusted HR, 0.53; 95% CI, 0.23-1.25; P = .22). Among patients with high levels of CRP the presence of

| Table 1. Baseline Clinical Characteristics and Serum Biomarker Levels in Patients With End-Stage Renal Disease |
|-----------------|-------------------|-------------------|
| Patients        | Age, median (IQR), y | Men, No. (%)     |
| (N = 224)       | (IQR)              | 121 (54)          |
|                 | 62 (50-72)         |                   |
| Race, No. (%)   |                   | Hispanic 49 (21)  |
| White           | 86 (38)            |
| Black           | 86 (38)            |
| Hispanic        |                   |
|                 | 49 (21)            |
| Clinical characteristics | No. (%) |                   |
| Diabetes, No. (%) | 107 (48)          |                   |
| Smoking, No. (%) | 47 (21)           |                   |
| Known coronary disease, No. (%) | 80 (36) |                   |
| Blood pressure, median (IQR), mm Hg | 99 (88-108) | |
| Years receiving dialysis, median (IQR) | 2.1 (0.8-3.9) | |
| Kt/V, median (IQR) | 1.6 (1.4-1.8) | |
| Body surface area, median (IQR), m² | 1.80 (1.65-1.96) | |
| Biomarker levels, median (IQR) |       |                   |
| Creatine kinase-MB, ng/mL | 2.2 (1.5-3.4) | |
| cTnT, ng/mL | 0.003 (0.028-0.116) | |
| CRP, mg/L | 8.2 (3.9-21.6) | |
| Albumin, g/dL | 2.8 (3.6-4.1) | |
| Hematocrit, % | 35.4 (32.3-38.5) | |
| Cardiovascular drugs, No. (%) |  |                   |
| Aspirin | 60 (27) | |
| β-Blockers | 40 (18) | |
| Calcium channel blockers | 123 (56) | |
| Nitrates | 60 (27) | |
| ACE inhibitors/ A-II antagonists | 56 (25) | |

Abbreviations: ACE, angiotensin-converting enzyme; A-II, angiotensin II; CRP, C-reactive protein; cTnT, cardiac troponin T; IQR, interquartile range; Kt/V, dialysis dose.
low or high levels of cTnT trended toward discriminating those at risk for cardiac death (33% vs 65% respectively; age-adjusted HR, 2.08; 95% confidence interval [CI], 0.90-4.84; P=.03).

Nearly half (49%) of all cardiac deaths occurred in patients with high levels of both cTnT and CRP. Risk of death determined by each biomarker level was stratified separately by presence or absence of a history of CAD and by white race. No interaction was detected between these 2 factors and the biomarkers for prediction of death.

A multivariable model was developed to identify if levels of cTnT and CRP remained independent predictors of all-cause mortality. Levels of CRP considered as a continuous variable and high levels of cTnT (fourth quartile) both remained independent predictors of all-cause mortality along with age, white race, history of diabetes, and body surface area. No important interaction was detected between any of the clinical risk factors and either biomarker.

Coronary Angiographic Findings
Sixty-seven patients volunteered for coronary angiography. No significant clinical differences were found between patients who underwent angiography and the remaining study patients, other than a lower age and a modestly lower Kt/V in the former group (mean [SD], 57 [13].

Table 2. Prognostic Value of cTnT and CRP Levels at Study Entry for Predicting All-Cause Mortality in Patients With End-Stage Renal Disease*

<table>
<thead>
<tr>
<th>Quartiles of Biomarker Level</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnT level, ng/mL</td>
<td></td>
</tr>
<tr>
<td>Patients, No.</td>
<td>57</td>
</tr>
<tr>
<td>All-cause mortality, No. (%)</td>
<td>16 (28)</td>
</tr>
<tr>
<td>Unadjusted HR (95% CI)</td>
<td>2.2 (1.2-4.1)</td>
</tr>
<tr>
<td>Adjusted HR (95% CI)</td>
<td>1.6 (0.9-3.0)</td>
</tr>
<tr>
<td>CRP level, mg/L</td>
<td>3.89</td>
</tr>
<tr>
<td>Patients, No.</td>
<td>51</td>
</tr>
<tr>
<td>All-cause mortality, No. (%)</td>
<td>22 (43)</td>
</tr>
<tr>
<td>Unadjusted HR (95% CI)</td>
<td>0.9 (0.5-1.6)</td>
</tr>
</tbody>
</table>

*P values are for comparison of the risk of events with that for patients in quartile 1. Adjusted HRs were adjusted for age and sex.

Abbreviations: CI, confidence interval; CRP, C-reactive protein; cTnT, cardiac troponin T; HR, hazard ratio.

Figure 1. Temporal Distribution of All-Cause Mortality Among Patients Undergoing End-Stage Renal Hemodialysis, Based on Quartiles of cTnT and CRP Levels

**Table 3.** Multivariable Model for All-Cause Mortality

<table>
<thead>
<tr>
<th>Biomarker Level Above (High) or Below (Low) the Median</th>
<th>Low CRP/ Low cTnT</th>
<th>Low CRP/ High cTnT</th>
<th>High CRP/ Low cTnT</th>
<th>High CRP/ High cTnT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, No.</td>
<td>53</td>
<td>50</td>
<td>44</td>
<td>59</td>
</tr>
<tr>
<td>All-cause mortality, No. (%)</td>
<td>19 (36)</td>
<td>24 (48)</td>
<td>21 (48)</td>
<td>43 (73)</td>
</tr>
<tr>
<td>Unadjusted HR (95% CI)</td>
<td>1.2 (0.7-2.1)</td>
<td>1.3 (0.8-2.4)</td>
<td>2.5 (1.5-4.0)</td>
<td></td>
</tr>
<tr>
<td>Adjusted HR (95% CI)</td>
<td>1.4 (0.8-2.4)</td>
<td>1.5 (0.9-2.7)</td>
<td>2.4 (1.5-3.8)</td>
<td>1.8 (1.1-3.2)</td>
</tr>
</tbody>
</table>

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patients with LVH (P=.65) or an LVEF of 40% or less (P=.75).

**COMMENT**

This study provides insight into the association between elevated levels of cTnT with coronary atherosclerosis and the long-term prognostic importance for biomarkers of myocardial damage and inflammation in patients receiving long-term hemodialysis. An angiographic substudy found that an elevated level of cTnT is a marker of extensive CAD, as manifested by a 3.7-fold higher PR of multivessel CAD in patients with high levels of cTnT vs patients with low levels. This finding provides pathophysiological support to our main finding that elevated levels of cTnT were associated with an increased long-term risk of all-cause mortality.

Our results also indicate that measurement of inflammation in a population of patients receiving long-term hemodialysis, as determined by level of CRP, was an independent predictor of death. Moreover, the presence of a high level of CRP along with a high level of cTnT conveyed the highest risk of death.

**cTnT Elevation**

Proposed mechanisms of cTnT level elevation in patients receiving hemodialysis have included silent ischemic injury and an apoptotic process. We found that even small elevations of cTnT concentration, at levels lower than those traditionally used for the diagnosis of acute coronary syndromes, are associated with an increased likelihood of multivessel CAD in stable patients with ESRD. The fact that elevated levels of cTnT retain their predictive value for death in patients with and without known CAD, even after adjustment for other cardiac risk factors including white race, suggests the common nature of coronary atherosclerosis in the population of patients with ESRD. In this setting where the timing of troponin release is unknown, elevation of cTnT level, as a marker of diffuse multivessel CAD, fits...
with current thought that many acute coronary events, with associated plaque rupture and microembolization, are clinically silent.\textsuperscript{25}

It has been proposed that elevation of levels of cTnT in patients receiving dialysis may result from nonischemic cardiomyopathy or microvascular disease in the setting of LVH.\textsuperscript{26,27} Our echocardiographic findings demonstrated no correlation between left ventricular mass and elevated cTnT level, possibly because LVH is present in the majority.\textsuperscript{28} In addition, cTnT did not appear to correlate with either depressed LVEF or hypertrophy in patients without angiographically validated CAD. These findings highlight the need for further investigation using more sensitive techniques in patients without angiographic CAD to detect coronary atherosclerosis and myocardial ischemia.

**CRP Elevation**

Despite the near ubiquitous presence of elevated levels, CRP retained an independent association with all-cause mortality consistent with several,\textsuperscript{3,4,6} but not all,\textsuperscript{3,7} previous findings in patients with ESRD. Studies of patients receiving dialysis often show striking elevations of this marker of systemic inflammation.\textsuperscript{3,7} Our use of the high-sensitivity assay for CRP detected values above the 80th percentile of the general population (>4.19 mg/L, highest risk group) in more than 70% of our study patients.\textsuperscript{29} Unlike the general population, in which low levels of CRP elevation may reflect chronic vascular inflammation and increased risk of vascular events, patients receiving dialysis are subject to multiple nonvascular inflammatory stimuli, including chronic infections and the dialysis process itself.\textsuperscript{30} Therefore, given the multiple etiologies of CRP level elevation in these patients, it should not be surprising that prediction of risk of cardiac death is complemented by simultaneous biochemical evidence of myocardial injury. This risk appears graded, with the spectrum of risk continuing to increase at CRP levels exceeding those found to predict risk in the general population.\textsuperscript{31} One hypothesis for this finding is that, while many patients with ESRD and a large burden of coronary atherosclerosis are identified by elevated levels of cTnT resulting from clinically silent plaque ruptures, ultimately it is the systemic inflammatory milieu that raises the risk of triggering a fatal cardiac event. The absence of an association between elevated levels of CRP and angiographic evidence of multivessel CAD in patients receiving hemodialysis is consistent with the poor correlation that exists between angiographic findings and results of electron-beam computed tomography with levels of CRP in clinically stable patients in the general population.\textsuperscript{32,33} Based on the frequent presence of elevated levels of cTnT and their association with diffuse CAD, stratification of risk might best compare hemodialysis patients without ischemic syndromes with acute coronary syndrome patients without renal disease. In such patients, elevated levels of CRP also provide additional long-term risk prediction independent of cTnT.\textsuperscript{34}

**Limitations**

Selection of patients with ESRD but without ischemic symptoms to undergo coronary angiography would ideally be randomized. However, such a...
strategy could have introduced a selection bias into this prospective outcomes study by intentionally enrolling only patients who were also willing to undergo angiography. Reliance on volunteers potentially improves the generalizability of the findings, particularly compared with series of dialysis patients referred for clinical indications.

Echocardiography comprised a second complementary substudy for evaluating the pathophysiology of biomarker elevation. The diverse and distant sites of this multicenter study limited the rapid acquisition of high-quality echocardiograms. Despite the large number of echocardiograms analyzed, bias due to missing data and temporal changes in left ventricular function or mass cannot be excluded.

Determination of cause of death using death notifications completed by clinicians (as used by the USRDS) may be inaccurate.35 However, when the USRDS-determined cause of death was compared with the adjudicated end points of a large prospective clinical trial, the classification of death as cardiac compared favorably whereas the type of cardiac death correlated poorly between the two.36 Therefore, we did not comment on the type of cardiac death identified by high levels of biomarkers.

Conclusions
For patients with ESRD but without ischemic symptoms undergoing hemodialysis, randomly assessed levels of cTnT and CRP independently identify patients at risk of death, and the combination of the 2 levels identify patients at particularly high risk. Furthermore, small elevations of cTnT level predict a markedly increased risk of multivessel coronary atherosclerosis. Taken together, these findings identify a potential role for these markers to be incorporated into future diagnostic and therapeutic strategies aimed at the earlier detection and management of clinically silent, but high-risk, diffuse CAD.

Author Contributions: Study concept and design; obtained funding: deFilippi. Acquisition of data: deFilippi, Rosano, Tibbler, Spenger.

REFERENCES