Multi-Biomarker Risk Stratification of N-Terminal Pro-B-Type Natriuretic Peptide, High-Sensitivity C-Reactive Protein, and Cardiac Troponin T and I in End-Stage Renal Disease for All-Cause Death

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Background: In patients with end-stage renal disease (ESRD), the ability of single and multiple biomarker monitoring to predict adverse outcomes has not been well established. This study determined the prognostic value of multiple biomarkers for all-cause death over 2 years in 399 ESRD patients.

Methods: The risk of all-cause death was determined by use of multiple biomarkers based on concentrations for a reference population (normal) and cutoffs based on tertile distributions in the ESRD group. Biomarkers studied included N-terminal pro-B-type natriuretic peptide (NT-proBNP), high-sensitivity C-reactive protein (hsCRP; Dade Behring and Roche assays), and cardiac troponin T (cTnT; Roche) and I (cTnI; Dade Behring and Beckman Coulter assays). Relative risks of death were estimated and survival curves computed.

Results: A total of 101 deaths occurred during 594 patient-years of follow-up. Increased NT-proBNP concentrations were not predictive of death on the basis of the normal cutoffs. However, tertile analysis of NT-proBNP was significantly predictive of death and had a ROC area under the curve equivalent to or better than any of the other biomarkers. Biomarkers independently predictive of survival were hsCRP (P < 0.001, either assay), cTnT (P < 0.05), and cTnI (Dade, P < 0.05). Two-year mortality rates were 6% (n = 45) with normal hsCRP, cTnI, and cTnT concentrations; 19% (n = 173) with increased hsCRP or cTnT and normal cTnI; 44% (n = 160) with both hsCRP and cTnT increased and normal cTnI; 61% (n = 21) with increased cTnI (Dade) or 47% (n = 74) with increased cTnI (Beckman) regardless of hsCRP or cTnT concentrations. Defined by the normal cutoffs, increased concentrations of biomarkers were present in various proportions of the 399 patients with ESRD: NT-proBNP, 99%; hsCRP, 46% (both Roche and Dade assays); cTnT, 85%; cTnI, 19% (Beckman assay) and 5% (Dade assay).

Conclusions: Although mechanisms likely vary for causation, increased plasma hsCRP, cTnT, and cTnI above the cutoffs for our reference (normal) population were all independently predictive of subsequent death in ESRD patients. Tertile analysis for NT-proBNP also demonstrated prognostic value.

Cardiac disease is the major cause of death in patients with end-stage renal disease (ESRD),4 accounting for ~45% of all deaths (1–4). In dialysis patients, ~20% of cardiac deaths are attributed to acute myocardial infarction (MI), with a 2-year all-cause mortality rate of 73% (1–3). Numerous investigations have recently monitored biomarkers, such as cardiac troponins and high-sensitivity C-reactive protein (hsCRP), in addition to clinical information, such as diabetes and hypertension, to assist in identifying patients with coronary artery disease (CAD) and in risk stratification of ESRD patients for both death and MI (5–13). Whether more aggressive treatment modalities for cardiovascular disease in these patients, based on biomarker findings, lead to improvements in...
outcomes needs to be explored by the medical community.

Recent guidelines endorsed by the European Society of Cardiology, the American College of Cardiology, and the American Heart Association state that cardiac troponin T (cTnT) and I (cTnI) are the preferred biomarkers for the detection of myocardial injury and diagnosis of MI (14–16). In addition, increased cardiac troponins have strong prognostic value, irrespective of the mechanism of injury, in acute coronary syndrome patients with or without renal insufficiency (17–19). Early pharmacologic intervention trials with low-molecular-weight heparin and with glycoprotein IIb/IIa inhibitors have demonstrated a significant decrease in risk of death and nonfatal MI in cardiac-troponin-positive acute coronary syndrome patients (20, 21). Whether aggressive interventional management in ESRD patients could provide improved clinical outcomes has not been studied. Previously, we demonstrated that increases in cTnT and cTnI in ESRD patients were associated with a two- to fivefold increase in risk of mortality at 2-year follow-up, with substantially greater numbers of patients having an increased cTnT vs cTnI (8).

B-Type natriuretic peptides (BNPs) and renal function have been shown to be prognostic indicators of survival in patients with congestive heart failure (CHF) (5, 6, 22, 23). Renal function correlates weakly with the measurement of BNP and may influence the cutoff concentration used for the final diagnosis of CHF (24). However, measurement of the N-terminal pro-B-type natriuretic peptide (NT-proBNP) appears to be strongly influenced by renal insufficiency, with dialysis patients with ESRD having increased concentrations (22, 24). Furthermore, inflammation is known to play an essential role in the pathogenesis of coronary heart disease. Increased markers of inflammatory activity, particularly hsCRP, are associated with increased risk of death in healthy individuals, in patients with acute coronary syndrome, and in patients with impaired renal function (7, 9–13, 22, 25).

No studies to date have examined the relationship between multiple cardiac biomarkers and mortality risk in ESRD in a large group of patients with ESRD. Using a subset of the ESRD patient cohort described previously (8), our primary purpose in the current study was to determine mortality risk associated with increased NT-proBNP and hsCRP concentrations. Secondarily, we assessed the independent contributions for mortality risk of these biomarkers along with cTnT and cTnI measured by two different assays. Finally, we describe the prevalence of increased biomarkers in the ESRD group.

Materials and Methods
We enrolled 399 ESRD patients, treated by chronic intermittent hemodialysis for at least 30 days (Monday, Wednesday, Friday or Tuesday, Thursday, and Saturday) at outpatient dialysis units throughout Minneapolis-St. Paul, MN, from April 1998 to March 1999. This study had received previous Institutional Review Board approval. Patient demographics, past medical histories, and follow-up data were obtained by chart review by personnel unaware of the cardiac biomarker results. A predialysis blood (heparinized plasma) sample was obtained from each patient. The patients studied were a subgroup of the original ESRD patient database reported previously (8) for whom adequate sample volumes (frozen at −70 °C) remained for additional biomarker analysis. In-house data have demonstrated cTnI and cTnT stability over 6 years. According to each assay manufacturer, hsCRP and NT-proBNP have been reported stable frozen for 1 year, but no data in the literature address stability for periods >1 year. All biomarkers were measured according to the manufacturers’ guidelines.

NT-proBNP was measured on the Elecsys 2010 analyzer (Elecsys proBNP Immunoassay; Roche Diagnostics) (26). Cutoffs used were the 97.5th percentiles (normal) from normal blood donors taken from the manufacturer’s package insert: ages <75 years, 125 ng/L; ≥75 years, 450 ng/L. Total imprecision at 355 ng/L was 2.9%. BNP was not measured because heparin plasma was not a suitable specimen for analysis by any BNP assay.

hsCRP was measured by both the Roche/Hitachi Tina-quant (Latex) assay (Hitachi 917; Roche Diagnostics) and the high-sensitivity flex reagent assay (Dimension; Dade Behring) (27). For both hsCRP assays, a normal cutoff of <1.0 mg/L, based on American Heart Association guidelines, was used for risk assessments (9). Imprecision was <6% for both assays at <1.0 mg/L.

cTnT (Elecsys Troponin T STAT Immunoassay, third generation; Roche Diagnostics) was measured on the Roche Elecsys 2010 analyzer (8, 28). The manufacturer’s stated detection limit is <0.01 μg/L. The lowest concentration to attain a CV of 10% was 0.03 μg/L, as determined in our laboratory. The 99th percentile of a reference population (n = 680) as determined in our laboratory was 0.01 μg/L. Total imprecision was 7.0% at 0.07 μg/L.

cTnI was measured on the Dimension analyzer (second-generation assay; Dade Behring) (8, 28). The manufacturer’s stated detection limit is 0.04 μg/L. The lowest concentration to attain a 10% CV was 0.1 μg/L. The 99th percentile of a reference population (n = 680; normal) as determined in our laboratory was 0.07 μg/L. However, because all results <0.1 μg/L were reported as <0.1 μg/L, we have used <0.1 μg/L as the 99th percentile cutoff. Total imprecision was 8.5% at 0.6 μg/L. cTnI was also measured by the Beckman Access method (AccuTnI, second generation; Beckman Coulter) (28). The manufacturer’s stated detection limit is 0.01 μg/L. The lowest concentration to attain a 10% CV was 0.06 μg/L. The 99th percentile of a reference population (n = 680; normal) determined in our laboratory was 0.04 μg/L.

Differences in cumulative 2-year survival rates were compared between patients with increased and normal cardiac biomarker concentrations. Exposure was computed from date of blood draw until date of death with
censoring first for length of time interval of interest (2 years), renal transplant, transfer of patient to another dialysis facility, or regaining renal function. Unadjusted and adjusted relative risks (RRs) of death and 95% confidence intervals were estimated by use of Cox proportional hazard models. Adjusted RRs were estimated after first fitting models with variables identified in previous analyses as independent risk factors, including diabetes, age, predraw history of CAD, and time since initial dialysis (8). Survival curves were computed by the Kaplan–Meier method and compared among risk stratification groups using the log-rank statistic. Agreement between hsCRP assays and between cTnI assays was summarized as percentage agreement as well as the \( \kappa \) statistic, which accounts for chance agreement. ROC curve analysis was performed to determine the differences or similarities between areas under the curves for NT-proBNP, hsCRP, cTnI, and cTnT. All tests were two-sided, and statistical significance was accepted at the 0.05 level. Analyses were done with SPSS PC software.

### Results

The clinical characteristics and demographic information available for the 399 ESRD patients are summarized in Table 1. Just over one half of patients were male (58%) and Caucasian (59%), and the mean patient age was 61 years. Diabetes and a history of CAD were found in 46% and 30% of patients, respectively. The median (range) number of years on dialysis was 2.0 years (0.1–22 years). Median patient follow-up was 1.7 patient-years (range, 41 days to 2 years), with a total of 101 deaths occurring during 594 patient-years of follow-up. Patient exposure was censored for renal transplant (n = 19), discontinuation of hemodialysis because of regaining of renal function (n = 1), and transfer of patient to another renal dialysis unit (n = 19).

NT-proBNP was not predictive of mortality based on the normal cutoffs; 99% of patients had an increased concentration. NT-proBNP based on tertiles, however, was predictive of survival (Fig. 1). Two-year mortality rates based on NT-proBNP in the top tertile and hsCRP (Roche assay) \( \geq 1 \text{ mg/L} \) were the following: neither increased, 14% (n = 169); either increased, 35% (n = 142); both increased, 54% (n = 88). Substituting the Dade hsCRP assay for the Roche assay gave similar findings (data not shown).

Patients with hsCRP concentrations \( \geq 1 \text{ mg/L} \) (Roche assay) had increased mortality (Fig. 2). The 2-year mortality rate for those with hsCRP in the 1–3 mg/L range was not significantly different \((P = 0.2)\) from the rate for those with hsCRP \( > 3 \text{ mg/L} \). Findings for the Dade hsCRP assay were similar (data not shown). Two-year mortality for patients with normal hsCRP concentrations was 18% compared with 44% for those with increased concentrations \( \geq 1 \text{ mg/L} \) \((P < 0.001)\). After adjustment for risk factors

### Table 1. Clinical characteristics and demographics of 399 ESRD patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (range) age, years</td>
<td>61 (19–93)</td>
</tr>
<tr>
<td>Males, %</td>
<td>58</td>
</tr>
<tr>
<td>Ethnicity, %</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>59</td>
</tr>
<tr>
<td>Black</td>
<td>26</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2</td>
</tr>
<tr>
<td>Asian</td>
<td>7</td>
</tr>
<tr>
<td>Native American/Other</td>
<td>5</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>46</td>
</tr>
<tr>
<td>History of CAD, %</td>
<td>30</td>
</tr>
<tr>
<td>History of MI, %</td>
<td>15</td>
</tr>
<tr>
<td>Years of dialysis, median (range)</td>
<td>2.0 (0.1–22)</td>
</tr>
</tbody>
</table>

Fig. 1. Kaplan–Meier survival curves by baseline NT-proBNP tertiles. The total numbers of patients at risk at baseline and after 1 and 2 years are shown at the bottom of the graph.

Fig. 2. Kaplan–Meier survival curves by baseline hsCRP tertiles (Roche assay). The numbers of patients at risk at baseline and after 1 and 2 years for each group are shown at the bottom of the graph. Curves obtained with the Dade hsCRP assay were not significantly different (data not shown).
Biomarkers based on normal cutoffs independently predictive of survival were hsCRP ($P < 0.001$ for either the Roche or Dade assay), cTnT ($P < 0.05$), and cTnI ($P < 0.05$ for either the Dade or Beckman assay). Two-year mortality rates based on the Roche hsCRP assay and Dade cTnI assay were 6% with normal cTnI, normal cTnT, and normal hsCRP; 19% with normal cTnI and either increased cTnT or hsCRP; 44% with normal cTnI, increased cTnT, and increased hsCRP; 61% ($n = 21$) with increased cTnI (Dade) regardless of hsCRP or cTnT status $61\%$ (Fig. 3); and 47% ($n = 74$) with increased cTnI (Beckman) regardless of hsCRP or cTnT status.

The 2-year cumulative survivals by cTnT tertiles are shown in Fig. 4. The 2-year mortalities were 14% ($n = 139$) with cTnT <0.03 μg/L; 36% ($n = 129$) with cTnT 0.03–0.074 μg/L; and 41% ($n = 131$) with cTnT <0.074 μg/L ($P < 0.001$). The RRs (95% confidence intervals) for the second and third tertiles were 2.4 (1.4–4.3) and 3.2 (1.9–5.6), respectively ($P < 0.0001$ vs first tertile). Similar tertile analyses were not possible for the two cTnI assays because the large majority of concentrations were less than each assay’s detection limit.

ROC curve analysis for the multiple biomarkers as a predictor of subsequent death demonstrated the following areas under the curve: Roche NT-proBNP, 0.655; Dade cTnI, 0.538; Beckman cTnI, 0.601; Dade hsCRP, 0.654; and Roche hsCRP, 0.649.

The prevalence of increased biomarker concentrations varied by biomarker, from 5% of patients for the Dade cTnI to 99% for NT-proBNP (Table 2). A substantially greater number of patients had an increased cTnT (85%) compared with hsCRP by either assay (46%) or cTnI by either method (19% for Beckman; 5% for Dade). Percentage agreement between the Beckman and Dade assays for increased cTnI was 85% ($\kappa = 0.89$).

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### Table 2. Univariate and adjusted RRs for death by cardiac biomarker concentrations at baseline (n = 399).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Patients with increased concentrations, %</th>
<th>2-year cumulative mortality, %</th>
<th>Univariate RR (95% CI)</th>
<th>$P$</th>
<th>Adjusted RR (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CtnT &gt;0.01 μg/L</td>
<td>85</td>
<td>Normal: 11</td>
<td>Increased: 33</td>
<td>3.3 (1.3–8.1)</td>
<td>0.002</td>
<td>2.8 (1.1–6.8)</td>
</tr>
<tr>
<td>Dade cTn ≥0.1 μg/L</td>
<td>5</td>
<td>28</td>
<td>61</td>
<td>2.7 (1.5–4.9)</td>
<td>0.005</td>
<td>2.7 (1.5–5.0)</td>
</tr>
<tr>
<td>Beckman cTn &gt;0.04 μg/L</td>
<td>19</td>
<td>26</td>
<td>47</td>
<td>1.9 (1.3–3.0)</td>
<td>0.004</td>
<td>1.8 (1.1–2.7)</td>
</tr>
<tr>
<td>Roche hsCRP ≥1 mg/L</td>
<td>46</td>
<td>18</td>
<td>44</td>
<td>2.8 (1.9–4.3)</td>
<td>&lt;0.001</td>
<td>2.5 (1.7–3.8)</td>
</tr>
<tr>
<td>Dade hsCRP ≥1 mg/L</td>
<td>46</td>
<td>18</td>
<td>44</td>
<td>2.8 (1.8–4.2)</td>
<td>&lt;0.001</td>
<td>2.5 (1.6–3.8)</td>
</tr>
<tr>
<td>NT-proBNP,a ng/L</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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*a CI, confidence interval.

b Adjusted for age, predraw history of CAD, and time since initial dialysis (<1 year, 1–5 years, >5 years).

c Cutoffs: <75 years, 125 ng/L; ≥75 years, 450 ng/L; those with concentrations below the cutoffs were too few ($n = 3$) to allow meaningful analysis.
Discussion

This is the first study to evaluate long-term survival in a large ESRD patient cohort based on predialysis simultaneous measurements of multiple cardiac biomarkers, including NT-proBNP, hsCRP, cTnT, and cTnl concentrations using evidence-based reference cutoff concentrations used in clinical practice and clinical trials and with tertile cutoffs (9,14–26). Our findings demonstrate the independent predictive power of both individual biomarkers and a combination of multiple biomarkers, including hsCRP, cTnT, and cTnI, as powerful tools for assessing all-cause death over 2 years in a large ESRD patient cohort (Fig. 3). ROC area under the curve analyses did not distinguish any particular biomarker or cutoff concentration as optimal for risk stratification purposes. Increased NT-proBNP concentrations were not predictive of death based on the normal cutoffs. However, tertile analysis of NT-proBNP demonstrated prognostic value.

Increased concentrations of hsCRP and both cTnT and cTnl detected in outpatient dialysis patients were independently predictive of all-cause mortality. Although the cardiac troponin findings in this study are not unique, their description was important in validating the patient data set and in validating previous observations when comparing results with hsCRP and NT-proBNP. Studies have now demonstrated in the ESRD population that significant angiographic findings are linked to increases in cardiac troponins (29–31).

Studies have suggested that BNP and NT-proBNP are important in regulation of blood pressure and electrolyte and volume homeostasis (32). In addition, NT-proBNP and BNP have emerged as biomarkers for altered myocardial function and structure. NT-proBNP and BNP also have shown to be predictors of mortality and drug efficacy in patients with heart failure (23,33). Studies have shown that increasing mortality in chronic renal failure is most frequently caused by CHF, coronary heart disease, or both (6). Furthermore, CHF in ESRD is associated with left ventricular hypertrophy, which is also an independent determinant of overall mortality in ESRD patients (5,6). Several reports have confirmed that BNP concentrations are increased in ESRD dialysis patients and that BNP concentrations are higher in ESRD patients who died from cardiovascular events than in those who survived longer than 15 months (5,6). Interestingly, at least one study has shown that BNP concentrations were not significantly increased in ESRD dialysis patients without left ventricular hypertrophy compared with controls, suggesting that increased BNP not associated with renal dysfunction (5). In the present study, however, 99% of NT-proBNP concentrations were increased above the normal cutoffs. This finding is likely explained by the predominant renal clearance mechanisms of NT-proBNP vs BNP (22,24) and further explains the poor prognostic ability of NT-proBNP to discriminate all-cause mortality between normal and increased concentrations. However, tertile analysis (Fig. 1) did show significant differences between groups, with increased values in the second and third tertiles providing confidence for use in risk stratification in ESRD vs the first tertile. Thus, our findings complement those of Clerico et al. (5), who reported that NT-proBNP is of limited independent prognostic use, being unable to detect acute changes in preload during dialysis, and less useful than BNP as a marker of left ventricular hypertrophy and cardiac impairment in ESRD. Alone, NT-proBNP was not a useful marker for mortality prediction in ESRD unless tertile analysis was used. Additional studies that directly compare BNP and NT-proBNP are needed to improve the understanding of the role of renal insufficiency and circulatory natriuretic peptides. Independent assessment of BNP for mortality risk assessment is also necessary because one should not extrapolate the findings for NT-proBNP to BNP.

It has been shown that in uremic patients, mortality from cardiovascular disease is substantially higher than in the general population. Advancements in the understanding of the pathogenesis of atherosclerotic vascular disease now suggest a central contribution of inflammation to morbidity and mortality (9,11), and hsCRP, a circulating marker of inflammation, has been closely linked through epidemiologic data to increased cardiovascular disease (9). hsCRP has been shown to be increased in a substantial proportion of ESRD patients and associated with clinical outcomes, including all-cause death. Over the past decade, numerous studies have also demonstrated that hsCRP is increased in substantial numbers of ESRD patients without apparent clinical reason (9). The presence of increased hsCRP confirms the existence of a chronically activated acute-phase response in the ESRD population. Our current results confirm that hsCRP (using two different assays) is a strong predictor of 2-year mortality and an independent predictor of survival. hsCRP was also the most powerful cardiac biomarker in our data for predicting all-cause death when compared with NT-proBNP, cTnT, and cTnl. These data support the link between hsCRP and mortality in the ESRD population, with hsCRP possibly acting as a measure of atherosclerosis. We suggest that hsCRP should be considered in the routine investigation of ESRD patients possibly alone or in conjunction with cTnT. Overall, our findings complement the role of hsCRP as a valuable tool for identifying patients at risk of cardiovascular events in primary prevention and its use in the treatment of acute coronary syndromes (25).

Our current study also demonstrates differences between cTnl assays, but not hsCRP assays. On the basis of the normal cutoff concentrations used in this study, agreement between the Dade and Beckman cTnI concentrations was fair at best (κ = 0.32). Partly, this may be attributable to our limitation of using of a cutoff of <0.1 μg/L (not 0.07 μg/L) for the Dade assay. Furthermore, the Beckman Access assay likely detects a larger number.
of individuals with cTnI increases because of improved performance at the low end of the analytical range (34). Agreement between the Dade and Roche hsCRP assays was very good ($\kappa = 0.89$).

The findings of our current study substantiate differences identified between cTnI and cTnT assays. On the basis of our normal cutoff concentrations, 85% ($n = 341$) of cTnT vs only 5% or 19% ($n = 21$ and 74 for the Dade and Beckman assays, respectively) of cTnI concentrations were increased. Furthermore, we demonstrate significant 2-year cumulative mortality differences by cTnT tertiles (Fig. 4). The lowest tertile concentration, 0.03 μg/L, was equivalent to the lowest concentration to attain a 10% CV, similar to the lowest quartile concentration of <0.029 μg/L observed by deFilippi et al. (29) for predicting prognosis in ESRD patients with a similar RR of ~2.0. Because of the large number of cTnT concentrations undetectable by both assays, tertile analyses for cTnI were not performed. Regardless of the mechanisms of myocardial injury and the cTnI/cTnT differences found in ESRD patients, our current findings continue to substantiate and add to the growing literature demonstrating the prognostic power of cardiac troponin testing for predicting mortality in ESRD patients (8, 31).

We do recognize several limitations of our patient data set, including incomplete information pertaining to clinical histories for hypertension and smoking, serum creatinine and lipid concentrations, a history of CHF, left ventricular hypertrophy, and lack of electrocardiographic and angiographic data. We did not examine whether there is an interaction between medication and cardiac troponin risk, nor did we determine the influence of a history of CHF, left ventricular hypertrophy, and lipid concentrations, a history of CHF, left ventricular hypertrophy, and lack of electrocardiographic and angiographic data. We did not determine whether an increased cardiac biomarker concentration has a favorable socioeconomic impact on ESRD patient management or outcomes.

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