Modulation of Novel Cardiorenal and Inflammatory Biomarkers by Intravenous Nitroglycerin and Nesiritide in Acute Decompensated Heart Failure

An Exploratory Study

Sheryl L. Chow, PharmD; Stephen A. O’Barr, PhD; Jessica Peng, PharmD; Eric Chew, PA-C; Firooz Pak, MD; Ryan Quist, PhD; Paryus Patel, MD; J. Herbert Patterson, PharmD; J. Thomas Heywood, MD

Background—Modulation of novel cardiorenal and inflammatory markers may provide insight into the disease process and outcomes of patients with acute decompensated heart failure.

Methods and Results—In this open-labeled, prospective, randomized study, 89 patients received either nesiritide (NES) or nitroglycerin (NTG) infusion by standard protocol. The serum or plasma concentrations of cystatin-C and inflammatory markers (high-sensitivity C-reactive protein, tumor necrosis factor-α, transforming growth factor-β1, and interleukin-6) were measured in 66 patients with acute decompensated heart failure at baseline and during drug infusion. Mean baseline values for demographics were not significantly different between NTG and NES groups; however, baseline inflammatory markers were elevated on admission. In NES compared with NTG groups, lower cystatin-C (1449 versus 2739 ng/mL, \(P < 0.05\)) and IL-6 (25 versus 50 pg/mL, \(P < 0.05\)) were observed. There were no significant differences in concentrations of high-sensitivity C-reactive protein, tumor necrosis factor-α, and transforming growth factor-β1 between groups over time.

Conclusions—The differential modulation effects of cystatin-C and interleukin-6 but not other inflammatory markers, in response to NES compared with NTG therapy, may provide important implications for vasodilator therapy. Further studies are warranted to confirm these findings.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00842023.

Key Words: natriuretic peptides • renal failure • biomarkers • inflammation • nitroglycerin • heart failure

Acute decompensated heart failure (ADHF) is often accompanied by renal impairment in addition to deterioration of heart failure. In these clinical conditions, neurohormonal activation and an increase in inflammation have been observed. Proinflammatory cytokines are imputed to impair myocardial function and accelerate heart failure progression in addition to other deleterious effects on the heart. Proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and high-sensitivity CRP (hsCRP) are associated with a greater propensity for heart failure. Cytokines have also been of important prognostic significance in ADHF, in which an inverse relationship between high levels of circulating IL-6 and TNF-α with survival was reported. However, whether modulation of inflammatory cytokines can occur in response to pharmacological therapy and whether any such change is associated with different clinical outcomes after discharge after an acute episode of ADHF is uncertain.

Clinical Perspective on p 455

Nesiritide (B-type natriuretic peptide, NES) and nitroglycerin (NTG) are the primary options for vasodilator therapy in ADHF. Although their beneficial hemodynamic effects are well established in ADHF, their effect on markers of renal function and inflammation are not well understood. Previous in vitro studies found that B-type natriuretic peptide (BNP) was associated with a reduction in transforming growth factor-β1 (TGF-β1, locally released cytokine promoting fibrosis and hypertrophy); however, there is a paucity of clinical data related to its effect on inflammatory response in patients with ADHF.
Serum creatinine as a marker of renal function has been previously investigated in several studies. In retrospective analyses, NES was found to elevate serum creatinine in comparison to control, and this effect appeared to be related to long-term infusion. Other studies have shown neutral effects on serum creatinine from NES infusion when dosed moderately, but novel markers of renal dysfunction have not been investigated in this setting. Cystatin-C is a protease inhibitor and a more sensitive endogenous marker of renal function. Analysis of kidney damage using a serum cystatin-C as a renal biomarker may better predict changes in glomerular filtration rate and may provide a better indicator to evaluate the renal effects of NES.

A primary study (Assessment of Biomarkers and Cardiorenal Syndrome with Acute Decompensated Heart Failure Vasodilator Therapy, ABC-HFT) on the effect of vasodilators on serum creatinine, neurohormones, and clinical outcomes was reported elsewhere. In view of the potential prognostic significance of inflammatory cytokines and the need for further insight regarding the effects of vasodilators on renal function, we also carried out this exploratory study to further investigate the effect of NES versus NTG on inflammatory markers (hsCRP, IL-6, TNF-α, and TGF-β) and cystatin-C.

Methods

The ABC-HFT was conducted from July 1, 2006, to July 1, 2008, at Centinela Hospital Medical Center and was previously described. The protocol was approved by both the University and participating hospital human subjects committee. Patients who presented to the Emergency Department with the diagnosis of ADHF or admitted directly to the hospital within 24 hours with admission BNP levels >500 pg/mL were evaluated. Those who met the study criteria and provided consent were randomly assigned to either intravenous NES (2 mg/kg bolus followed by 0.01 mg/kg per minute infusion for at least 48 hours) plus standard treatment (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, spironolactone, β-blockers, and bolus diuretics) or intravenous NTG for 48 hours (10 μg/min initial starting dose titrated every 5 minutes until symptom relief, systolic blood pressure ≤90 mm Hg, or up to a maximum rate of 200 μg/min) plus standard treatment. Survival status and readmission rates were assessed at 3 and 6 months after hospital discharge.

Only patients with complete sets of investigational samples were evaluated for this study. Patients were excluded if 1 or more of the investigational laboratory values were missing or if any blood draws were not obtained within 30 minutes of the scheduled collection time. To compare the effect of NES versus NTG treatment, changes in cystatin-C and inflammatory markers were assessed at baseline and at 24 and 48 hours.

The standard renal function markers obtained and described in the primary study were compared with cystatin-C (highly sensitive endogenous freely filtered protease inhibitor). Inflammatory biomarkers obtained included TNF-α, IL-6, TGF-β, and hsCRP. All these markers were collected at the time of admission and during vasodilator therapy.

The routine blood chemistry and hematology tests were performed at the Centinela-Freeman Medical Center laboratory or Quest Diagnostics laboratory (Inglewood, CA). The plasma concentrations of inflammatory markers and cystatin-C were determined using the following standard ELISA assay kits: TGF-β1 (R&D Systems; normal range, 36 to 74 pg/mL), hsCRP (Life Diagnostics; normal range, 0 to 5 mg/L), IL-6 (R&D Systems; normal range, 0 to 14 pg/mL), TNF-α (R&D Systems, normal range: 10 to 50 pg/mL), and Cystatin C (R&D Systems; normal range, 500 to 1257 ng/mL). All measured concentrations were above the minimum detectable levels and were within intra-assay and interassay precision ranges.

Statistical Analysis

Data analysis was conducted using SPSS 16.0 (Chicago, IL). The treatment group assignments were blinded to the statistician before and during statistical analysis. Continuous variables were evaluated for normality using Shapiro-Wilk test with graphical methods to confirm findings. Baseline characteristics and biomarkers between the 2 treatment groups were compared using Student t test and χ² analysis. Univariate analyses were also conducted to identify differences associated with changes in serum creatinine, biomarkers, and outcomes. To compare differences in biomarkers (cystatin-C, hsCRP, TNF-α, TGF-β1, and IL-6) between the 2 treatment groups for the primary end point of the study, continuous variables were analyzed using repeated-measures multivariate analysis of variance (MANOVA). The treatment-by-time interaction was also analyzed in the MANOVA model. Partial and complete data sets of 85 patients were further examined using a random mixed effects model. On the basis of expected effect size f(v) of 0.50 and power of 0.80, an estimated sample size of 36 patients was required to detect changes in biomarkers between groups. All analyses were conducted at the 2-tailed level and the significance level was set at α=0.05.

Results

Six hundred twenty patients were screened and 89 were included in the ABC-HFT primary analysis. Sixty-six of the 89 matched the inclusion/exclusion criteria and had complete sets of investigational samples for this exploratory study. Except for age, all patient baseline characteristic were similar in the patient cohort used for this analysis compared with those who were excluded. Samples from 23 patients were either missing or considered uninterpretable. In 13 patients, blood samples were unobtainable because of patient refusal, unavailability, or poor venous access. Samples from 10 patients were also considered uninterpretable because they were drawn >30 minutes from the scheduled collection times. The mean baseline values were similar between the 2 groups (NTG versus NES), as shown in Table 1. All patients were scheduled to receive 48 hours of vasodilator therapy; the mean ± SD infusion duration was 45±10 hours for NES and 46±7 hours for NTG (mean maximal dose, 141 μg/min). The mean furosemide equivalent doses between the NES and NTG groups were similar during the 48-hour treatment period (180.6 versus 186.3 mg, P=0.82), and no significant differences in net fluid balance were observed after the 48 hours of infusion (−1645.7 mL versus −2399.5 mL, P=0.39). A total of 6 patients included in the current study had symptomatic hypotension (3 patients in each group). No differences in outcomes were observed at 6 months between the 2 treatment groups, as reported elsewhere. In the cohort of patients qualified for this biomarker study, there were 11 patient deaths (6 with NTG, 5 with NES), 45 readmissions (23 with NTG, 22 with NES), and 47 patients with the composite end point of death or readmissions (25 with NTG, 22 with NES) at 6 months.

Renal Function Markers and Inflammatory Cytokines

The overall result was significant (MANOVA analysis; P=0.005) among the cystatin-C and inflammatory markers, indicating that over time there were differences between the NES and NTG groups. The specific effects of these markers in response to NES and NTG are summarized.
Ethnicity, %

<table>
<thead>
<tr>
<th></th>
<th>NTG (n=30)</th>
<th>NES (n=36)</th>
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<tbody>
<tr>
<td>African American</td>
<td>22 (73)</td>
<td>27 (75)</td>
</tr>
<tr>
<td>White</td>
<td>7 (23)</td>
<td>6 (17)</td>
</tr>
<tr>
<td>Other</td>
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<td>3 (8)</td>
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Ejection fraction, mean, %

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<tr>
<th></th>
<th>NTG (n=30)</th>
<th>NES (n=36)</th>
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<tbody>
<tr>
<td>36±17</td>
<td>34±19</td>
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</table>

MAP, mean, mm Hg

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<thead>
<tr>
<th></th>
<th>NTG (n=30)</th>
<th>NES (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>92±19</td>
<td>92±21</td>
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</table>

NYHA functional class

<table>
<thead>
<tr>
<th>Class</th>
<th>NTG (n=30)</th>
<th>NES (n=36)</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>II</td>
<td>4 (13)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>III</td>
<td>8 (27)</td>
<td>10 (28)</td>
</tr>
<tr>
<td>IV</td>
<td>17 (57)</td>
<td>24 (67)</td>
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</tbody>
</table>

BNP baseline, mean, pg/mL

<table>
<thead>
<tr>
<th></th>
<th>NTG (n=30)</th>
<th>NES (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1733±1152</td>
<td>1550±1094</td>
<td></td>
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</table>

Serum creatinine, mean, mg/dL

<table>
<thead>
<tr>
<th></th>
<th>NTG (n=30)</th>
<th>NES (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4±0.4</td>
<td>1.3±0.4</td>
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</table>

Pulmonary edema, %

<table>
<thead>
<tr>
<th></th>
<th>NTG (n=30)</th>
<th>NES (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27 (90)</td>
<td>30 (83)</td>
<td></td>
</tr>
</tbody>
</table>

Comorbidities, %

<table>
<thead>
<tr>
<th>Condition</th>
<th>NTG (n=30)</th>
<th>NES (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD</td>
<td>11 (37)</td>
<td>14 (39)</td>
</tr>
<tr>
<td>DM</td>
<td>12 (40)</td>
<td>16 (44)</td>
</tr>
<tr>
<td>HTN</td>
<td>23 (77)</td>
<td>24 (67)</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; NYHA, New York Heart Association; CAD, coronary artery disease; DM, diabetes mellitus; and HTN, hypertension. No significant differences between groups.

Renal Function Markers

Although serum creatinine, creatinine clearance, and blood urea nitrogen were not affected, there was a significant increase (worsening) of cystatin-C concentrations with NTG infusion at 24 and 48 hours when compared with baseline, but not with NES infusion (Table 2). There was also a significant difference in the cystatin-C concentrations between these 2 treatment groups at 24 and 48 hours in the NTG compared with the NES arm, respectively. The overall between-group comparisons over time for cystatin-C were also significant through multivariate time-by-treatment interaction (P=0.027).

Inflammatory Cytokines

All 4 cytokines were elevated at baseline, and these values were not significantly different between the 2 treatment groups (Figure). IL-6 concentrations were significantly lower in the NES group at 48 hours compared with the NTG group. Among the cytokines, IL-6 concentrations were significantly reduced in response to NES therapy over time, based on MANOVA (P=0.036).

The changes in hsCRP concentrations showed a borderline significance (P=0.058) over time and between the groups. Neither TGF-β nor TNF-α showed any appreciable effect at 24 or 48 hours between the 2 treatment groups.

Analyses of all 85 patients with partial and complete data sets further corroborate these findings. The main treatment effect in response to NES compared with NTG was significant for both cystatin-C (P=0.03) and IL-6 (P=0.05). The treatment-by-time interaction was also significant between groups (P=0.05 and P=0.03, respectively). Although a treatment-by-time interaction was found with hsCRP (P=0.03), the main treatment effect was not statistically significant (P=0.83). There were no appreciable differences with either TNF-α or TGF-β.

Discussion

Modulation of Renal Markers in Response to Vasodilator Therapy in ADHF

A relevant clinical concern of vasodilator therapy in ADHF is its effect on renal function. The results of our prospective, randomized study showed that both NES and NTG did not worsen renal impairment over 48-hour infusions as assessed by standard markers of renal function (serum creatinine and creatinine clearance). In contrast, a significant worsening in cystatin-C concentration over time in ADHF patients treated with NTG but not NES was observed in our current exploratory study. This observed difference in cystatin-C appeared to be directly related to the vasodilators themselves rather than difference in diuretic exposure, given that furosemide equivalent doses were similar between the 2 groups at baseline and during the treatment period.

The differential effect of cystatin-C concentration in response to NTG compared with NES is of significant therapeutic interest. Cystatin-C has been reported to be an independent predictor of cardiac mortality in patients with ADHF with normal to moderately impaired renal function, with the highest quartile of cystatin-C showing the greatest relative risk of cardiac death. In addition, elevated cystatin-C concentrations were associated

### Table 2. Renal Function Markers at Specified Time Points

<table>
<thead>
<tr>
<th>Renal Function Marker</th>
<th>Baseline</th>
<th>At 24 Hours</th>
<th>At 48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NTG (n=44)</td>
<td>NES (n=45)</td>
<td>NTG (n=44)</td>
</tr>
<tr>
<td>Scr, mg/dL&lt;sup&gt;12&lt;/sup&gt;</td>
<td>1.3±0.4</td>
<td>1.3±0.4</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;k&lt;/sub&gt;&lt;sub&gt;cr&lt;/sub&gt;, mL/min&lt;sup&gt;12&lt;/sup&gt;</td>
<td>52.5±25.5</td>
<td>51.5±16.7</td>
<td>50.9±25.4</td>
</tr>
<tr>
<td>Cystatin-C&lt;sup&gt;*&lt;/sup&gt;, ng/mL</td>
<td>2026±1814</td>
<td>1332±1308</td>
<td>3108±3761††</td>
</tr>
</tbody>
</table>

Scr indicates serum creatinine; Cl<sub>k</sub><sub>cr</sub>, creatinine clearance.

Data are presented as mean±SD. Normal cystatin-C levels are 500 to 1257 ng/mL.

<sup>*</sup>P<0.05, between-group comparisons over time, MANOVA time-by-treatment interaction.

†<sup>P</sup><0.05 compared with baseline.

‡<sup>P</sup><0.05 compared with NES; Student t test.
with worsened prognosis at 12 months in the presence of normal creatinine and creatinine clearance values.\textsuperscript{16} Thus, cystatin-C appears to be a better predictor than serum creatinine or creatinine clearance for cardiac prognosis in ADHF patients, and its modulation may be of importance to long-term outcome. The results of the present study appear to confirm the potential utility of cystatin-C and support the benefit of vasodilator therapy in ADHF. Thus, our observed differential effect of NES compared with NTG on cystatin-C concentration may provide important clinical implication. Further prospective studies are warranted to confirm this important finding.

Our data appear to support the use of cystatin-C as a potential useful marker of glomerular filtration in comparison to serum creatinine or creatinine clearance. Cystatin-C is known to be an endogenous, freely filtered protease inhibitor that is produced by all cells. Because it does not undergo tubular secretion or significant extrarenal elimination, it is dependent on filtration by the glomerulus. Thus, it may be considered a highly sensitive marker for glomerular filtration rate and is capable of detecting small changes in glomerular filtration rate compared with serum creatinine and creatinine clearance. Furthermore, cystatin-C provides an additional advantage as a marker of renal function because its measurement is largely unaffected by sex, age, or body mass index.\textsuperscript{17–19}

Modulation of Inflammatory Cytokines in Response to Vasodilator Therapy in ADHF

In our study, all inflammatory cytokines were observed to be elevated on admission, confirming previous observations\textsuperscript{5,20–24} that acute heart failure is an active inflammatory process that may potentiate further left ventricular remodeling in a stress-cytokine-stress pattern of injury.

Elevated IL-6 levels have been identified previously as a predictor of worsened outcomes and long-term mortality,\textsuperscript{5,25} and thus modulation of the elevated IL-6 concentration in ADHF may benefit long-term outcome. Our observed differential effect of reduction in IL-6 concentrations (at 24 and 48 hours) in response to NES versus elevation of IL-6 concentrations in response to NTG may be of important therapeutic implication. However, future prospective studies with larger patient populations are warranted to confirm such findings.

The specific mechanism associated the observed IL-6 change in our present study is unclear and requires further investigation. Previous studies have shown that IL-6 concentration could be augmented in the cardiomyocytes through the stimulation of catecholamines.\textsuperscript{26,27} Transient elevation in arterial epinephrine levels have been demonstrated with intravenous NTG, which could lead to activation of cytokines.\textsuperscript{28} Conversely, NES has been shown to reduce plasma norepinephrine levels,\textsuperscript{29} which may lead to a reduction in plasma IL-6 concentration over time, as observed in our study.

No significant difference was observed with other markers. However, NES did appear to improve hsCRP. Whether larger numbers of patients and longer infusion times would produce significant improvement of hsCRP is unknown. Despite potential modulation of hsCRP, the clinical significance is
uncertain. Reductions in hsCRP have not been shown to improve patient outcomes.30

TGF-β and TNF-α were less affected by our vasodilator therapy. Although BNP was previously found to antagonize TGF-β in cardiac fibroblasts,31 such effect may not have produced changes on circulating concentrations observed within the time frame of the present study. TNF-α was not significantly reduced by either vasodilator therapy for reasons probably related to its variability of production in heart failure.32–35 Studies investigating inflammatory response to treatments for ADHF are limited31; however, modulation of inflammatory mediators have been reported to occur in chronic heart failure and have produced differential effects. Carvedilol has been found to significantly reduce IL-6 but not TNF-α after 4 to 7 weeks of treatment.35 Other studies have shown reductions in hsCRP, TNF-α receptors, and IL-6 after 1 year of statin therapy;36 however, significant anti-inflammatory effects were not observed with short-term therapy.37 Such observation appears to be consistent with some of the markers in our present short-term study in ADHF patients.

Study Limitations
Because the treatment assignment was unblinded, bias from the knowledge of the treatment could affect the outcomes of the study. However, the measured concentration of cystatin-C and inflammatory markers in the present study are unlikely to be affected because these assays were performed without the knowledge of treatment identity. Although our present sample size found a significant relationship between the 2 treatment groups in their modulation of certain cytokines, such finding is based on a total of 66 patients and should be interpreted cautiously. Furthermore, in view of moderate duration of vasodilator therapy and limited time frame in which the markers were obtained, the results of the present study cannot be extrapolated to long-term effects.

Conclusions
NTG but not NES significantly increased (worsened) the plasma concentrations of cystatin-C, a potential sensitive marker of glomerular filtration. Furthermore, NTG infusion resulted in an increase, whereas NES a decrease, of the inflammatory IL-6 concentrations. These observed differential modulation effects of cystatin-C and IL-6 in response to NES versus NTG may have important therapeutic implications if our preliminary results of these 2 markers for ADHF are substantiated. Further studies with large patient populations are warranted to determine the relationship of biomarker response to clinical outcomes.

Acknowledgments
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Disclosures
Drs Chow, Pak, Patel, and Chew were previously on the Scios speakers bureau but are no longer active. Dr Patterson was previously on the Scios speakers bureau and consultant/advisory board and is a recipient of a Scios Grant. Dr Heywood was previously on the Scios speakers bureau and consultant/advisory board.

References


**CLINICAL PERSPECTIVE**

To date, there are only a few published prospective, randomized studies investigating biomarkers in acute decompensated heart failure therapy and a paucity of data evaluating the impact of vasodilators on renal and inflammatory markers in this population. In this open-labeled, randomized, biomarker study, patients diagnosed with acute decompensated heart failure therapy received 48 hours of intravenous nitroglycerin or nesiritide therapy. Treatment groups were assessed for inflammatory markers (interleukin-6, high-sensitivity C-reactive protein, tumor necrosis factor-α, and transforming growth factor-β) in addition to cystatin-C, as a novel marker of renal function. Nesiritide reduced circulating levels of interleukin-6 and cystatin-C; however, other markers of renal function were not significantly affected. Elevated concentrations of interleukin-6 and cystatin-C may portend worse outcomes in patients with heart failure, and reducing these levels by way of vasodilator therapy may be of important therapeutic value.
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