Plasma N-Terminal Pro-Brain Natriuretic Peptide and Adrenomedullin

Prognostic Utility and Prediction of Benefit From Carvedilol in Chronic Ischemic Left Ventricular Dysfunction

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OBJECTIVES
We sought to assess plasma concentrations of the amino (N)-terminal portion of pro-brain natriuretic peptide (N-BNP) and adrenomedullin for prediction of adverse outcomes and responses to treatment in 297 patients with ischemic left ventricular (LV) dysfunction who were randomly assigned to receive carvedilol or placebo.

BACKGROUND
Although neurohormonal status has known prognostic significance in heart failure, the predictive power of either N-BNP or adrenomedullin in chronic ischemic LV dysfunction has not been previously reported.

METHODS
Plasma N-BNP and adrenomedullin were measured in 297 patients with chronic ischemic LV dysfunction before randomization to carvedilol or placebo, added to established treatment with a converting enzyme inhibitor and loop diuretic (with or without digoxin). The patients' clinical outcomes, including mortality and heart failure events, were recorded for 18 months.

RESULTS
Above-median N-BNP and adrenomedullin levels conferred increased risks (all p < 0.001) of mortality (risk ratios [95% confidence intervals]: 4.67 [2–10.9] and 3.92 [1.76–8.7], respectively) and hospital admission with heart failure (4.7 [2.2–10.3] and 2.4 [1.3–4.5], respectively). Both of these predicted death or heart failure independent of age, New York Heart Association functional class, LV ejection fraction, previous myocardial infarction or previous admission with heart failure. Carvedilol reduced the risk of death or heart failure in patients with above-median levels of N-BNP or adrenomedullin, or both, to rates not significantly different from those observed in patients with levels below the median value.

CONCLUSIONS
In patients with established ischemic LV dysfunction, plasma N-BNP and adrenomedullin are independent predictors of mortality and heart failure. Carvedilol reduced mortality and heart failure in patients with higher pretreatment plasma N-BNP and adrenomedullin.

Neurohormonal responses to cardiac impairment participate in the pathogenesis of heart failure (1–4). Plasma levels of neurohormonal factors offer an indication of cardiovascular prognosis (1,4–6) and can assist in the prediction of benefit from treatment. The cardiac peptide called brain natriuretic peptide (BNP) has received close attention as a cardiovascular marker (5,7–9). We have recently reported that the 76-amino acid residue amino (N)-terminal portion of pro-BNP (N-BNP) circulates in human plasma (10) and that levels are elevated in cardiac impairment (10,11). In normal subjects, mean plasma concentrations are similar to those of BNP (~10 pmol/liter), whereas in cardiac impairment, proportional and absolute increments above normal levels of N-BNP exceed those for BNP, suggesting that it may be the more discerning marker. We have demonstrated N-BNP to be an independent prognostic marker of mortality or decompensated heart failure after myocardial infarction (MI) (12).

Adrenomedullin is a 52-amino acid peptide originally isolated from pheochromocytoma cells (13). It has been detected in a range of tissues, including the heart, vasculature, kidney, adrenal medulla, lung, and brain (14). In experimental heart failure, adrenomedullin expression is increased and peptide levels rise, and administered peptide has powerful vasodilator effects, increases cardiac output and is natriuretic (15,16). Plasma concentrations are in the low picomolar range in healthy individuals, but they increase in those with hypertension and congestive heart failure (CHF) in proportion to the severity of the disease (17). In heart failure, plasma adrenomedullin is inversely related to left ventricular ejection fraction (LVEF) and is positively associated with left ventricular (LV) end-diastolic pressure (18,19). At least two reports have indicated that plasma adrenomedullin levels act as an indicator of prognosis after acute MI (12,20).

There are no published data relating plasma concentrations of either N-BNP or adrenomedullin to cardiovascular
prognosis in patients with established ischemic LV dysfunction. We hypothesized that plasma levels of either or both peptides would have independent prognostic utility in such patients and also assist in predicting any benefit from treatment with carvedilol. We report the results from 297 patients with ischemic LV impairment who were randomly assigned to treatment with carvedilol or placebo for CHF.

METHODS

The organization of the study and the effects of carvedilol on LV function, exercise tolerance, symptoms and morbidity and mortality rates have been reported (21,22). Twenty centers in Australia and New Zealand participated (see Appendix). The study was coordinated by the Clinical Trials Research Unit, University of Auckland, New Zealand. Neurohormonal assays and related analyses were conducted by the Christchurch Cardioendocrine Research Group, Christchurch School of Medicine, Christchurch, New Zealand. The study protocol was approved by the Ethics Committee at each participating center. All patients provided written, informed consent.

Patients (n = 415) were recruited with chronic, stable heart failure caused by ischemic heart disease, LVEF <45% (measured by radionuclide ventriculography), and current New York Heart Association (NYHA) functional class II or III or previous functional class II–IV. Exclusion criteria included current functional class IV, heart rate <50 beats/min, sick sinus syndrome, second- or third-degree heart block, blood pressure <90 mm Hg systolic or >160/100 mm Hg diastolic, treadmill exercise duration <2 or >18 min (modified Naughton protocol), coronary event or procedure within four weeks, primary myocardial or valve disease, insulin-dependent diabetes, chronic airways disease, hepatic disease (serum transaminase >3 times normal), renal impairment (creatinine >250 µmol/liter) and life-threatening noncardiac disease or current treatment with a beta-blocker, beta-agonist or verapamil.

Patients tolerating 6.25 mg of carvedilol twice daily were randomly assigned, in a double-blinded manner, to receive continued carvedilol (titrated to a maximal dose of 25 mg twice daily) or matching placebo for a trial period of 18 months.

The study outcomes included all-cause mortality, death from worsening heart failure, episodes of worsening heart failure (defined as deterioration requiring an increase in nonstudy antihypertension treatment, an increase in NYHA functional class, hospital admission for worsening symptoms of heart failure or non-sudden death from progressive heart failure), hospital admission for worsening heart failure and hospital admission for acute coronary syndromes.

Neurohormonal sampling was conducted before randomization. Venous blood (EDTA) was collected (9 AM–12 PM) after intravenous cannulation of the patient, who had been seated for 30 min. The sample was separated within 20 min, and the plasma was stored at −80°C before transport, on dry ice, to the laboratory for assay. Assays for N-BNP and adrenomedullin were conducted according to our published methods (11,23). Inter-assay and intra-assay coefficients of variation fell within the range of 5% to 9%.

Statistics. Event rates were compared by chi-square tests, with risk ratios (RRs) (95% confidence intervals [CIs]), and Kaplan-Meier curves calculated for groups with hospital admission levels above and below the group median of individual neurohormonal factors and LVEF. Analyses were conducted separately for groups randomized to receive carvedilol and placebo, as well as for the total patient group. Cox proportional hazards analysis was used to study the two peptides and other potential indicators (e.g., treatment, NYHA functional class, LVEF, age, previous MI, previous hospital admission for heart failure), as well as the interaction of N-BNP with treatment (i.e., placebo or carvedilol), or adrenomedullin with treatment, or both, for independent prediction of clinical outcomes. The value p < 0.05 (two-tailed) indicated statistical significance.

RESULTS

Seventy percent of the patients were in NYHA functional class II or III and 30% were in class I at randomization (43% had previously been in class IV at some time). Eighty-eight percent had a previous MI, and 43% had a previous hospital admission for heart failure. Eight-five percent were receiving an angiotensin-converting enzyme (ACE) inhibitor; 75% took diuretics; and 38% took digoxin. The average LVEF at entry was 29%.

The effects of treatment on morbidity and mortality and the surrogate end points have been reported (21,22). Carvedilol improved LVEF without deterioration in symptoms or exercise capacity. The combined end point of death or hospital admission for any reason was reduced by 26% (104 patients receiving carvedilol vs. 131 patients receiving placebo; 95% CI −43% to −5%; 2p = 0.02). We have also previously reported important interactions between pre-randomization plasma levels of other hormones and the response to carvedilol (24). Carvedilol reduced mortality
Table 1. Event Rates According to Median Levels of Prognostic Markers

<table>
<thead>
<tr>
<th>End Point</th>
<th>A</th>
<th>B</th>
<th>RR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality (n = 35)</td>
<td></td>
<td></td>
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<tr>
<td>N-BNP (pmol/liter)</td>
<td>29/151 (19.2)</td>
<td>6/146 (4.1)</td>
<td>4.67 (2–10.9)</td>
<td>0.00005</td>
</tr>
<tr>
<td>ADM (pmol/liter)</td>
<td>28/150 (18.7)</td>
<td>7/147 (4.8)</td>
<td>3.92 (1.7–8.7)</td>
<td>0.0002</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>24/146 (16.4)</td>
<td>11/151 (7.3)</td>
<td>2.26 (1.1–4.4)</td>
<td>0.0144</td>
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<tr>
<td>Heart failure mortality (n = 24)</td>
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<tr>
<td>N-BNP</td>
<td>23/151 (15.2)</td>
<td>1/145 (0.7)</td>
<td>22.1 (3–16.1)</td>
<td>0.000001</td>
</tr>
<tr>
<td>ADM</td>
<td>20/150 (13.3)</td>
<td>4/146 (2.7)</td>
<td>4.87 (1.7–13.9)</td>
<td>0.0008</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>17/129 (13.2)</td>
<td>7/150 (4.7)</td>
<td>2.5 (1.1–5.8)</td>
<td>0.0279</td>
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<tr>
<td>Admission with heart failure (n = 41)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-BNP</td>
<td>34/151 (22.5)</td>
<td>7/146 (4.8)</td>
<td>4.7 (2.2–10.3)</td>
<td>0.00001</td>
</tr>
<tr>
<td>ADM</td>
<td>29/150 (19.3)</td>
<td>12/147 (8.2)</td>
<td>2.4 (1.3–4.5)</td>
<td>0.0053</td>
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<tr>
<td>LVEF (%)</td>
<td>23/146 (15.8)</td>
<td>18/151 (11.9)</td>
<td>0.8 (0.4–1.3)</td>
<td>0.338</td>
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<tr>
<td>Worsening heart failure (n = 108)</td>
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<tr>
<td>N-BNP</td>
<td>72/151 (47.7)</td>
<td>38/146 (26)</td>
<td>1.83 (1.3–2.5)</td>
<td>0.0002</td>
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<tr>
<td>ADM</td>
<td>69/150 (46.0)</td>
<td>41/147 (27.9)</td>
<td>1.65 (1.2–2.3)</td>
<td>0.001</td>
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<tr>
<td>LVEF (%)</td>
<td>53/151 (35.1)</td>
<td>57/146 (39.0)</td>
<td>1.11 (0.83–1.5)</td>
<td>0.48</td>
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<tr>
<td>Admission with acute coronary syndrome (n = 45)</td>
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<tr>
<td>N-BNP</td>
<td>22/151 (14.6)</td>
<td>23/146 (15.8)</td>
<td>0.9 (0.5–1.6)</td>
<td>0.776</td>
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<tr>
<td>ADM</td>
<td>26/150 (17.3)</td>
<td>19/147 (12.9)</td>
<td>1.34 (0.8–2.3)</td>
<td>0.289</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>27/151 (17.9)</td>
<td>18/146 (12.3)</td>
<td>0.7 (0.4–1.2)</td>
<td>0.182</td>
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</table>

In column A, event rates are separately listed for patients with above-median N-BNP (line 1), above-median ADM (line 2) and below-median LVEF (line 3). In column B, event rates are separately listed for patients with below-median N-BNP (line 1), above-median ADM (line 2) and above-median LVEF (line 2) values. Median values (and 25th and 75th percentiles) for N-BNP, ADM and LVEF were 99 (54–179) pmol/liter; 9.8 (7.9–12.9) pmol/liter and 29% (22–35%), respectively.

ADM = adrenomedullin; CI = confidence interval; E = number of events; LVEF = left ventricular ejection fraction; N-BNP = amino (N)-terminal pro-brain natriuretic peptide; RR = risk ratio; T = total in subgroup.

rates and heart failure in those with higher pre-treatment BNP levels, but lesser activation of plasma norepinephrine.

After our initial neurohormonal substudy (24), sufficient plasma (EDTA) stored appropriately (−80°C) remained available for measurements of N-BNP and adrenomedullin in 297 (72%) of the original 415 study participants. A comparison of these 297 subjects with the remaining 118 subjects revealed no statistically significant differences in any demographic, LV imaging, functional or neurohormonal indicator or in the frequency of any clinical outcome. Hence, findings within this majority subgroup (72%) are representative of the study group as a whole.

**Neurohormonal markers of morbidity and mortality.** Table 1 indicates event rates for the designated study outcomes according to median levels of N-BNP, adrenomedullin and LVEF. Pre-randomization plasma levels of either peptide that fell above the median value for the group were more powerfully predictive than LVEF and indicated a significantly increased risk (irrespective of whether carvedilol or placebo was subsequently administered) for all-cause mortality, death due to CHF, hospital admission due to heart failure and worsening heart failure, but not for hospital admission due to a new acute coronary syndrome. Kaplan-Meier analyses corroborated these findings, with examples given in Figures 1 and 2 illustrating significant separation of event-free survival curves for all-cause mortality (Fig. 1) and hospital admission due to worsening heart failure (Fig. 2).

Multivariate analysis (Cox proportional hazards) indicated that, of the eight putative predictors, both N-BNP and adrenomedullin were significant predictors of all outcomes (with the exception of hospital admission with an acute coronary syndrome), independent of treatment, NYHA functional class, LVEF, previous MI, previous hospital admission with heart failure or age. Furthermore, for the prospectively defined end points of all-cause mortality (n = 35), death from CHF (n = 24), admission with heart failure (n = 41) and worsening heart failure (n = 108), there were statistically significant, independently predictive interactions of treatment with N-terminal BNP (p = 0.016, 0.003, 0.002 and 0.001, respectively) and treatment with plasma adrenomedullin (p = 0.004, 0.019, 0.049 and 0.024, respectively).

A key interaction is illustrated in Figure 3, where event-free survival curves for hospital admission with cardiac failure are shown for patients with pre-randomization N-BNP and adrenomedullin levels above and below the median value, subclassified according to allocation to the placebo or carvedilol group. In patients with supramedian levels of either peptide who were randomly assigned to placebo, the rate of admission with heart failure was high relative to patients with submedian levels (adjusted RRs [95% CIs]: 8.32 [2.3–29.8], p < 0.001 and 2.81 [1.1–7.1], p < 0.05 for N-BNP and adrenomedullin, respectively). In contrast, in such patients receiving carvedilol (after an initial period of ~300 days during which events tended to be increased), the cumulative risk of this end point was halved (Fig. 3) and did not differ significantly from that of patients...
with inframedian peptide levels (adjusted RRs [95% CIs]: 2.72 [0.8–9.1], \( p = 0.106 \) and 1.48 [0.5–4.2], \( p = 0.471 \)). All-cause mortality, death secondary to CHF and worsening heart failure were similarly significantly ameliorated by carvedilol in the supramedian hormone subgroups.

**DISCUSSION**

The current study is the first to demonstrate, to the best of our knowledge, the ability of plasma concentrations of N-BNP and adrenomedullin to independently predict all-cause mortality and heart failure in patients with established ischemic LV dysfunction. Both peptides had greater predictive power for mortality and heart failure, compared with LVEF, whether considered in univariate (Table 1) or multivariate analyses. Furthermore, this is the first report of the ability of these peptides to predict benefit from treatment with carvedilol.

**Carvedilol and N-BNP in heart failure.** Recent trials of carvedilol and other beta-blockers in heart failure indicate that these are an important advance in therapy for a common lethal condition (21,22,25–27). However, appropriate patient selection and prediction of benefit remain uncertain. The current report extends our previous demonstration of prediction of benefit from carvedilol in those patients with higher pre-treatment plasma BNP levels, but lesser activation of norepinephrine (24). Plasma BNP and

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**Figure 1.** All-cause mortality (death) survival curves for patients with pre-randomization plasma adrenomedullin (ADM, left) and N-BNP (right) above (group A, solid line) and below (group B, dashed line) the group median value. †\( p < 0.001 \).

**Figure 2.** Event-free survival for hospital admission with CHF in patients with pre-randomization plasma adrenomedullin (ADM, left) and N-BNP (right) above (group A, solid line) and below (group B, dashed line) the group median value. **\( p < 0.01 \). †\( p < 0.001 \).
N-BNP are very closely (r = 0.90) correlated (12), and the current report indicates that N-BNP is a comparable or better prognostic marker than BNP, which was superior to atrial natriuretic peptide (ANP), plasma catecholamines and arginine vasopressin in the Australia-New Zealand Heart Failure Study cohort (24). N-terminal BNP circulates at higher levels than its C-terminal congener, has a longer half-life and is less likely to be perturbed by acute stimuli. Plasma levels of N-BNP rise more steeply for a given degree of cardiac impairment, compared with BNP. For these reasons, N-BNP may be somewhat more reliable than BNP-32 as a prognostic marker. Plasma N-BNP and its C-terminal congener are derived from LV secretion in response to ventricular wall stress. It is therefore not surprising that these levels are more closely related to concurrent LV function and prognosis, compared with other circulating neurohormonal markers.

The mechanism underlying the predictive power of N-BNP for a benefit from carvedilol is unknown. Beta-blockers may downregulate natriuretic peptide clearance receptors (28), and it is possible that some of the benefit from carvedilol is derived from enhancing plasma ANP or BNP levels, or both, with promotion of natriuresis, vasodilation and relative suppression of the sympathetic and renin-angiotensin-aldosterone systems. Beta-blocker-induced enhancement of natriuretic peptide levels may be proportional to baseline peptide concentrations. Hence, the relationship between pre-treatment N-BNP and the likelihood of benefit from carvedilol may simply reflect its close correlation with the bioactive species BNP and, to a lesser extent, ANP.

**Adrenomedullin in heart failure.** In contrast to the cardiac peptides, plasma adrenomedullin has a multi-tissue origin, being derived not only from the vasculature and heart but also from other organs (14). Hence, the mechanisms underlying its relationship to cardiac function and cardiovascular prognosis are less obvious. However, cardiac expression of adrenomedullin is increased in experimental ischemic heart failure (29), and Tsuruda et al. (30) have demonstrated enhanced adrenomedullin production by mechanical stretching of cultured cardiomyocytes. Hence, it is possible that with progressive cardiac impairment, plasma adrenomedullin is progressively more reflective of cardiac production of the peptide. Alternatively (or in addition), peripheral and cardiac production of adrenomedullin may rise in parallel as heart failure progresses, explaining the relationship of venous plasma concentrations of adrenomedullin with cardiac function and prognosis. Stimuli shown to augment adrenomedullin production from vascular cells in vitro, which may be present at elevated tissue or plasma levels in heart failure, include interleukin-1, tumor necrosis factor, angiotensin II, endothelin I, adrenaline and aldosterone (31). However, it remains unclear why plasma adrenomedullin levels should be predictive of a benefit from carvedilol.

Notably, a reduction in hospital admissions for heart failure in patients with high peptide levels receiving carvedilol was not apparent until 300 days after randomization. Before this point, the events tended to be more frequent in this subgroup (Fig. 3). The current data do not have sufficient power to formally assess for significant crossover of the survival curves. However, the possibility of early adverse effects of beta-adrenergic blockade on heart failure admissions of patients with neurohormonal activation cannot be ruled out. In our previous report in the Australia-New Zealand Heart Failure Neurohormonal Substudy (24), the
same finding was apparent for C-terminal BNP with respect to hospital admission with heart failure, but not for all-cause mortality. Hence, if a genuine, slight excess of early heart failure events is triggered by carvedilol in this high-risk neurohormonal group, it is not at the cost of concurrent, early, increased mortality.

Other markers in heart failure. The current data extend our previous findings (24) relating pre-treatment neurohormonal status with response to carvedilol. Previous reports have related neurohormonal status and response with converting enzyme inhibition. Swedberg et al. (32) reported that the mortality benefit from enalapril in patients with NYHA functional class IV heart failure was enhanced in those with plasma catecholamines, angiotensin II, aldosterone and ANP levels above the group median value. In the Survival And Ventricular Enlargement (SAVE) trial (33), plasma renin activity predicted an increased efficacy of captopril in reducing one-year mortality rates, and enalapril gave greater survival benefit in patients with higher norepinephrine and renin levels in the Vasodilator Heart Failure Trial (V-HeFT II) (4). The relationship of neurohormonal status with treatment effects on rates of worsening heart failure was not addressed in that report. The COoperative North Scandinavian ENalapril SUrvival Study (CONSENSUS), SAVE and V-HeFT II did not provide data on BNP, N-BNP or adrenomedullin. Therefore, available reports indicate that elevated pre-treatment renin, norepinephrine and cardiac peptide levels are positively associated with a benefit from ACE inhibitor therapy in heart failure. The current report indicates that N-BNP and adrenomedullin should be added to ANP and BNP as positive predictors of benefit when a beta-blocker is added.

The conditions of the current study preclude conclusions about whether or not plasma N-BNP or adrenomedullin could predict a benefit from beta-blockers, without a previous introduction of ACE inhibitor treatment, or from other antihypertensive therapies (e.g., endopeptidase inhibitors, angiotensin II receptor blockers, endothelin antagonists), and these questions should be addressed in future randomized trials of heart failure therapy. Furthermore, our results cannot be extended to other patient populations. Subgroup analyses entail a loss of statistical power and these questions should be addressed in future randomized trials of heart failure therapy. Moreover, the current report requires confirmation. The relative (or potential complementary) strength of N-BNP and adrenomedullin compared with (or combined with) other circulating markers of cardiac dysfunction (the interleukins, for example), which were not measured in our study, cannot be assessed in this report.

Conclusions. The current findings indicate that plasma N-BNP and adrenomedullin levels in patients with established ischemic LV dysfunction receiving standard therapy with ACE inhibitors and loop diuretics (with or without digoxin) are independently predictive of mortality and heart failure. Elevated plasma levels of both of these peptides predict a long-term benefit from introduction of carvedilol.

APPENDIX

Australia-New Zealand Heart Failure Research Collaborative Group


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REFERENCES