Plasma Amino-Terminal B-Type Natriuretic Peptide Measured by Elecsys 2010 Assay in a Trial of Hormone-guided Treatment for Heart Failure, Richard W. Troughton, Christopher M. Frampton, Timothy G. Yandle, Eric A. Espiner, Gary Nicholls, and Mark Richards* (Christchurch Cardioendocrine Research Group, Christchurch School of Medicine and Health Sciences, Christchurch, 8001 New Zealand; * address correspondence to this author at: Department of Medicine, Christchurch School of Medicine and Health Sciences, PO Box 4345, Christchurch, New Zealand; fax 64-3-364-1115, e-mail mark.richards@cdhb.govt.nz)

Plasma concentrations of the cardiac natriuretic peptides, atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), and their amino-terminal congeners (N-ANP and N-BNP) are secreted from the heart in response to intracardiac pressure and volume load. Plasma concentrations of these peptides thus reflect cardiac function. Plasma BNP and N-BNP in particular have shown a close relationship with the degree of cardiac decompensation, hemodynamic and imaging indicators of left ventricular dysfunction, and prognosis in acute coronary syndromes and in heart failure (1–3). Plasma peptide concentrations have potential application in the diagnosis of heart failure in acutely symptomatic patients and in titration of anti-heart failure therapy (4, 5). Guidelines published by the European Society of Cardiology for the diagnosis of heart failure now incorporate mention of measurement of plasma natriuretic peptides, and the most recent version of the American Heart Association/American College of Cardiology guidelines mention the potential for such measurements to assist diagnosis of heart failure, although their role is not yet fully established (6, 7).

The extensive background literature concerning the relationship between cardiac natriuretic peptides and cardiac function and prognosis has relied on an array of labor-intensive immunoassays (8–12) that have various degrees of validation (13). Widespread clinical application of such measurements requires well-standardized, rapid turnaround, well-supported assays with excellent quality control. We therefore compared a locally developed, labor-intensive RIA for N-BNP with a recently released, fully automated commercial assay and applied both measurements to plasma samples from our recently conducted trial on management of heart failure by titration of anti-heart failure therapy according to plasma N-BNP.

The methods and protocols used in this study have been published in full previously (5). All participants gave informed, signed consent, and the protocol was approved by the Ethics Committee (Canterbury) of the Ministry of Health. In brief, patients 35–85 years of age admitted to hospital or seen in the specialist cardiology outpatient clinic with decompensated heart failure with ejection fractions <40% were recruited to the study. All patients received follow-ups in a specialist heart failure clinic, and after initial assessment and stabilization were randomized in a double-blind manner to treatment guided by either plasma N-BNP or standardized clinical assessment. At every visit, patients were assessed clinically by an investigator blinded to group allocation and were assessed as being either compensated or decompensated according to a standardized clinical score. EDTA plasma was separated from blood samples and stored at −80 °C before assay for plasma N-BNP measurements. The treatment target in the clinically guided group was compensated heart failure according to the predetermined scoring system; the target in the N-BNP group was a peptide concentration <200 pmol/L. This threshold corresponds with BNP and N-BNP concentrations with high sensitivity and specificity for distinguishing dyspnea of cardiac from noncardiac etiology. If these targets were not achieved, drug treatment (including loop diuretics, converting enzyme inhibitors, spironolactone, beta blockers, digoxin, and vasodilators) was intensified according to a predetermined stepwise protocol.

Patients in either group not meeting treatment targets were reassessed at 2-week intervals, and treatment was intensified until targets were met (or therapeutic options were exhausted), at which point 3-month reviews were resumed. The primary clinical endpoint was cardiovascular events (cardiovascular death plus hospital admission for heart failure, acute coronary syndrome, cerebrovascular accident, peripheral vascular event, arrhythmia, or syncope plus any new outpatient episode of decompensated heart failure requiring an increase in medication). A secondary endpoint was the plasma N-BNP concentration.

Initially study samples were assayed by a competitive RIA for N-BNP developed and validated by the Christchurch Cardioendocrine Research Group (10). This assay was the first ever established for measurement of plasma N-BNP (10, 11). The detection limit of the assay (2 SD from zero) was 5.2 (0.6) pmol/L. The within-assay CVs were 11% at 24 pmol/L and 9.9% at 186 pmol/L. The reference interval is 5 pmol/L (97.5 percentile in 200 healthy electoral role individuals) with doubling of mean concentrations between 30 and 70 years of age and higher concentrations in women (30%) than in men.

The comparison assay used was the Elecsys 2010 platform (Roche Diagnostics GmbH), which uses two polyclonal antibodies directed against the amino-terminal and mid-portion of the N-BNP molecule in a “sandwich” immunoassay configuration (14). In our recent experience over 8 months and 178 assay occasions, interassay CVs were 3.1% at 165 pmol/L and 3.8% at 563 pmol/L. We detected no significant change in results for samples refrozen (−80 °C) and reassayed over an 8-month period. Similar changes with respect to age and gender were observed as for the in-house RIA.
Patient demographics, baseline variables, follow-up duration, and clinical endpoints were compared between the two groups by independent t-tests, $\chi^2$, and Mann–Whitney U-tests as appropriate (SPSS for Windows, release 8.0.0; SPSS Inc.). A Poisson regression analysis (Statistics for Windows, Ver. 1.0; Analytic Software) was used to compare total cardiovascular event rates between groups with adjustment for differences in baseline variables. Changes in amino-terminal BNP concentrations were analyzed by ANOVA for repeated measures (SPSS). $P < 0.05$ was accepted as statistically significant. Results from the two separate assays were compared by paired t-test scatterplot correlation analysis in both arithmetic and natural log forms and by Bland–Altman plots. For individual patients, serial plasma N-BNP data were plotted according to both assays.

The results obtained with the Christchurch RIA have been published previously (5). Briefly, 69 patients were recruited, with 33 randomized to N-BNP guidance and 36 to clinically guided treatment. The median follow-up was 9.7 months in the N-BNP and 9.5 months in the clinical group ($P = 0.78$). Groups were matched for demographic and clinical features, left ventricular function, and functional status. N-BNP concentrations fell significantly below baseline in the hormone-guided group but not in the clinically managed patients. The primary combined clinical endpoint (cardiovascular death, hospital admission, and outpatient heart failure) was significantly lower in the BNP group than the clinical group (19 vs 54 events, respectively; $P = 0.02$). When analyzed as events per patient-year (0.7 vs 2.0), this was more significant ($P = 0.01$). Multivariate analysis by Poisson regression (including left ventricular ejection fraction, N-BNP concentration, age, New York Health Administration class, frusemide dose, angiotensin-converting enzyme inhibitor dose, heart rate, and systolic blood pressure as covariates) confirmed a highly significant ($\chi^2 = 14.2; P < 0.001$) difference between groups. Plasma samples were assayed at the time the study by the Christchurch RIA. Samples were thawed and reassayed by the Elecsys assay after variable storage intervals ($-80^\circ$C) between 1 and 2 years. A grand total of 249 samples from the 69 patients were available for re assay.

When we used natural log values (because of upwardly skewed data), the observed correlation gave an $r$ value of 0.95 and $r^2$ of 0.90 ($P < 0.001$). Table 1 lists the mean, median, SE, SD, minimum, maximum, and 25th and 75th percentiles in pmol/L for both the automated sandwich assay and the manual RIA. The median values were well matched. The Elecsys assay exhibited a more pronounced upward skew than the matched RIA values, and paired t-tests showed a highly significant difference between the absolute parallel values generated by the two assays ($P = 0.005$). This was confirmed by the Bland–Altman plot, which indicated progressive separation of the assay values at high immunoreactivities, particularly at concentrations beyond 500 pmol/L (Fig. 1A). In most cases of heart failure (90%), plasma N-BNP fell well below this value, and the results for the group as a whole, whether shown as serial mean (SE) values (Fig. 1B), as integrated means from the full study duration [292 (44) and 198 (41) pmol/L for clinical and hormone-guided groups by the Elecsys assay compared with 228 (26) and 167 (29) pmol/L, respectively, by the Christchurch RIA], or as parallel plots of serial values (from both assays) for individual patients, showed clinically useful agreement among assay results. Plasma N-BNP values by both assays showed good parallelism, and concentrations altered in response to changes in clinical status with upward movement during episodes of decompensation and decreases with intensification of therapy and return to a compensated state.

The incidence and prevalence of heart failure are steadily increasing, and this common and lethal syndrome will constitute one of the epidemics of the 21st century (6, 7). Treatment is becoming increasingly complex as at least five classes of drugs have demonstrated efficacy (15–19). The complexity of available treatments and the need for a broader range of medical and para medical professionals with greater and lesser expertise in heart failure to engage in its management signal a need for a test that will guide in diagnosis, prognosis, and adjustment of treatment. BNP and N-BNP are currently the strongest candidates to fulfill this role. Widespread application of such measurements requires well-standardized, well-validated, rapid turnaround, well-quality-controlled, robust, and affordable measurement technologies. In the current study we have demonstrated improved outcomes in heart failure patients managed according to plasma N-BNP concentrations rather than standardized clinical assessment and have demonstrated useful parallelism between our originally validated RIA, used at the time the clinical study was performed, and the Elecsys 2010 automated immunoassay, now commercially distributed. The concordance of these two independently developed immunoassays is striking in the context of the existing spectrum of published assays for N-BNP, among which reported values for healthy individuals vary over 100-fold, from 1 pmol/L (median) to 247 pmol/L (mean) (20, 21). As with other assays (with various configurations and methodologies) for ANP and BNP, both N-BNP assays are influenced by gender (concentrations are higher in women), age, and renal function (data not

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<th>Table 1. Descriptive statistics comparing 249 results from 69 patients for N-BNP measured by both the Christchurch RIA and Elecsys 2010.</th>
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<td><strong>Christchurch RIA, pmol/L (n = 249)</strong></td>
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$^a$ By paired t-testing, the means differ significantly ($P = 0.005$)
shown). Our data do not allow any definitive conclusion as to whether these factors modify the results of one assay more than the other, and these issues require fuller assessment for individual assays.

The cut-point used in the current study for titration of anti-heart failure treatment was 200 pmol/L, and if the Elecsys 2010 assay had been used in the original clinical trial, this would have produced discordance in the decision to alter or not alter treatment for only 11 of 249 such decisions. This reflects the very close agreement of the absolute values produced by the two assays over the clinically commonplace range of N-BNP observed in mild to moderate heart failure (up to ~500 pmol/L). It is important to realize that cutoff values and test performance will depend not only on individual assays but also on the population and purpose to which the test is applied. Different optimum values with different sensitivities, specificities, positive and negative predictive

![Bland–Altman plot for the two methods (A), and comparison of hormone-guided (dashed line) and non-hormone-guided (solid line) treatment of heart failure (B).](image)

(A), the Bland–Altman plot of the mean (natural log) of RIA and automated assay results (horizontal axis) against the difference between the results (vertical axis) indicates a significant relationship ($P < 0.0001$), with automated assay values exceeding RIA readings at high peptide concentrations. The solid line is the regression line. (B), mean (SE; error bars) N-BNP concentrations obtained with the Elecsys (left) and manual RIA (right) methods.
values, and overall accuracies will be observed in the diagnosis in acutely decompensated heart failure when compared with use of titrating treatment in established chronic heart failure (as in the current trial) or with population screening for asymptomatic ventricular impairment. All of these potential applications remain under investigation at present, and appropriate cut-points will emerge from large and varied cohorts that are beyond the scope of the current report.

The reason for the discordance between the two assays at higher concentrations cannot be conclusively defined from our current data. Our competitive RIA uses a single polyclonal antibody directed to the extreme amino-terminal region of N-BNP. This terminus is subject to some degradation in vivo, potentially yielding lower values than those observed with the Elecsys assay, which uses antibodies directed to possibly more stable epitopes. The RIA is calibrated with a truncated peptide comprising the first 21 amino acids of N-BNP 1–76, whereas the Elecsys assay uses the full 76-amino acid peptide, and this may potentially lead to increasing divergence of measured values over the range of the two assays. The Elecsys results agree with our previous findings, suggesting that it is a reliable assay and should allow routine use of plasma N-BNP assays worldwide.

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References

Maternal Glucocorticoid Supplementation and S100B Protein Concentrations in Cord Blood and Urine of Preterm Infants, Diego Gazzolo,1 Maria Kornacka,2 Matteo Bruschettini,1 Mario Lituania,1 Lia Giovannini,1 Giovanni Serra,1 Urszula Majewska,2 and Fabrizio Michetti3* (1 Department of Pediatrics and Obstetrics, G Gaslini Children’s University Hospital, I-00168 Rome, Italy; * author for correspondence: fax 39-0630154813, e-mail fabrizio.michetti@rm.unicatt.it)

Maternal glucocorticoid (GC) supplementation is widely used for the prevention of lung immaturity (1,2), but its possible harmful effects on other organs, including the central nervous system (CNS), are still a matter of debate (3–9).

S100B, which is present mainly in the nervous system (10) and has a short half-life (11), is regarded as a useful marker of brain injury, although at physiologic concentrations it may act as a cytokine with a neurotrophic effect (10,12–19). S100B concentrations in cord blood or urine