SHORT REPORTS

Comparison of the clinical utility of atrial and B type natriuretic peptide measurement for the diagnosis of systolic dysfunction in a low-risk population

Gavin Galasko, Paul O Collinson, Sophie C Barnes, David Gaze, Arjivit Lahiri, Roxy Senior

J Clin Pathol 2007;**60**:570–572. doi: 10.1136/jcp.2005.034306

Background: Measurement of B type natriuretic peptide and its N terminal prohormone (NTproBNP) can now be performed routinely by automated high-throughput immunoassays. The study compared measurement of NTproBNP with measurement of N terminal pro-atrial natriuretic peptide (NTproANP) for detection of ventricular systolic dysfunction in primary care.

Methods: 734 subjects aged >45 years (349 men and 385 women, median age 58 years, range 45–89, interquartile range 51–67 years) from seven representative general practices attended for echocardiography with determination of ejection fraction and completed a questionnaire. Blood samples were collected into gel serum separation tubes (Becton–Dickinson, Franklin Lakes, New Jersey, USA), the serum separated and aliquots stored frozen at -70°C until analyses. Samples were analysed for NTproBNP (Roche Diagnostics, Lewes, UK; coefficient of variation (CV) 3.2–2.4%) and for NTproANP (Biomedica, Vienna, Austria; CV 5.6–10.1%). Echocardiography was used as the diagnostic “gold standard”, with ventricular systolic dysfunction defined as abnormal when there was an ejection fraction of $\leq 40\%$. Patients were dichotomised by ejection fraction from 50% to 30%, and receiver operating characteristic curves constructed and the area under the curve (AUC) compared.

Results: At 40% ejection fraction, NTproANP and NTproBNP showed AUCs of, respectively, 0.738 (0.601–0.875) and 0.973 (0.958–0.989), $p < 0.004$.

Conclusion: NTproBNP is superior to NTproANP for detection of systolic dysfunction.

Measurement of atrial natriuretic peptide (ANP) or the N terminal portion of its prohormone, N terminal pro-ANP (NTproANP), or measurement of B type natriuretic peptide (BNP) has been used for the detection of ventricular dysfunction in patients at high risk.^{1–3} The two peptides have different properties; ANP is stored in granules and is released in response to atrial stretch. BNP is not stored to any significant extent, but is dynamically transcribed and translated, with the rate of transcription determined by ventricular wall stretch. The two peptides might be expected to have different diagnostic efficiencies. Measurement of ANP or NTproANP is currently available only as a manual radioimmunoassay, or by enzyme-linked immunoassay on microtitre plates, which takes 1–2 days to complete. Neither method is suitable for routine clinical use, which requires the use of high-throughput automated systems to achieve rapid result return. The routine pathology laboratory can now perform measurement of the N terminal portion of the BNP prohormone, N terminal pro-BNP (NTproBNP). There are no published data comparing the diagnostic efficiency of NTproANP with the new automated NTproBNP assay. We

therefore compared NTproANP with the commercially available NTproBNP for the detection of clinically relevant ventricular dysfunction in the primary care setting using echocardiography as the gold standard diagnostic test.

METHODS

Ethical permission for the study was obtained from the local research ethics committees of both St George's and Northwick Park Hospitals. The study was performed in accordance with the Declaration of Helsinki. In all, 1392 subjects ≥ 45 years old without a diagnosis of known ventricular dysfunction were selected by list randomisation from the computer records of seven geographically and socioeconomically representative community practices in Harrow (North London, UK) and invited to attend. Of those invited, 734 (53%) attended (349 men and 385 women). The median age was 58 years (range 45–89, interquartile range 51–67 years). In total, 85 (11.6%) were receiving a diuretic, 60 (8.2%) an angiotensin-converting enzyme inhibitor and 10 (1.4%) an angiotensin 2 receptor blocker, with 126 (17.2%) receiving drugs with anti-failure actions and 214 (29.2%) receiving cardioactive drugs (excluding aspirin and statins).

Attending subjects answered a questionnaire, and underwent clinical assessment, spirometry, electrocardiography, echocardiography, and venesection for NTproANP and NTproBNP serum levels. Participants were assessed between January 2000 and December 2001. Spirometry included forced expiratory volume in one second (FEV_1), forced vital capacity and their ratio ($\text{FEV}_1\%$). Two-dimensional echocardiography was performed using a Sonos 4500 (Philips, Eindhoven, Netherlands). Left ventricular ejection fraction (LVEF) was calculated quantitatively using Simpson's apical biplane rule as the average of three readings. This technique has been recommended as the most accurate two-dimensional echocardiographic assessment of LVEF,⁴ and had previously been validated.⁵

Blood was taken after 5 min supine rest in parallel into routine biochemistry gel serum separation tubes and tubes using potassium EDTA as anticoagulant. The serum separation tubes were allowed to clot, centrifuged, and the serum separated. EDTA tubes were spun and the plasma separated. Serum and plasma were stored at -20°C for 24 h and then at -70°C until analysis. Samples were thawed before analysis, centrifuged and run. Previous studies had shown that both NTproANP and NTproBNP⁶ were stable at -20°C and through one freeze–thaw cycle.

NTproANP was measured using a microtitre plate ELISA assay (Biomedica, Vienna, Austria) that uses two immunoaffinity-purified polyclonal antibodies directed at residues 10–19

Abbreviations: ANP, atrial natriuretic peptide; BNP, B type natriuretic peptide; LVEF, left ventricular ejection fraction; NTproANP, N terminal pro-atrial natriuretic peptide; NTproBNP, N terminal prohormone

and 85–90 of the NTproANP molecule. The percentage coefficient of variation(%CV) of the assay is 5.6–10.1%, from 824 pmol/l (8701.4 ng/l) to 2117 pmol/l (22 355.5 ng/l), with an analytical range of 50–5000 pmol/l (528–52 800 ng/l). NTproBNP was measured in serum on the Elecsys 2010 system (Roche Diagnostics, Lewes, UK). The assay is an electrochemiluminescent sandwich immunoassay that uses two polyclonal antibodies directed at residues 1–21 and 39–50 of the NTproBNP molecule. The %CV of the assay is 3.2–2.4%, from 20.7 pmol/l (175 mg/l) to 585.5 pmol/l (4962 mg/l), with an analytical range of 0.6–4138.6 pmol/l (5–35 000 ng/l).

Statistical methods

Diagnostic efficiency was compared by construction of receiver operator characteristic curves and comparison of the area under the curve (AUC) by the Hanley and McNeil method,⁷ using NTproANP and NTproBNP as the continuous variables and LVEF as the dichotomous variable. An LVEF from $\leq 50\%$ to $\leq 30\%$ in 5% intervals was used for the analysis in order to cover the range from probable ventricular function ($\leq 50\%$) to definite abnormality ($\leq 30\%$). A value of $\leq 40\%$ is normally

Figure 1 Patient participation in the study. EF, ejection fraction; NTproANP, N terminal pro-atrial natriuretic peptide; NTproBNP, N terminal prohormone.

Figure 2 Comparison of the receiver operating characteristic curves for N terminal pro-atrial natriuretic peptide (NTproANP) and N terminal prohormone (NTproBNP) at a left ventricular ejection fraction of $\leq 40\%$. Area under the curve for NTproANP was 0.738 (CI, 0.601 to 0.875) and that for NTproBNP was 0.973 (CI, 0.958 to 0.989).

considered to be abnormal, and was used for determination of decision thresholds.

RESULTS

Figure 1 summarises the recruitment to the study, and table 1 shows the AUCs and comparison data. NTproBNP was superior to NTproANP at all values of LVEF. Using the $\leq 40\%$ point (fig 2), the 90% sensitivity cut-off for NTproANP was 775 pmol/l (8184 ng/l) and that for NTproBNP 33.6 pmol/l (285 ng/l). When the population was divided according to sex and reanalysed, the cut-off points obtained for men and women were not significantly different, owing to reduction in sample size.

DISCUSSION

This is the first study to compare NTproANP with a routinely available NTproBNP assay in a low-risk primary care population. NTproBNP seems to be superior to NTproANP for the detection of ventricular systolic dysfunction as defined (as reduced ejection fraction at all values of ejection fraction from 50% to 30%). The results obtained are qualitatively similar to those obtained from research assays that used more selected patient sets with a higher prior prevalence of abnormal systolic function, although the AUCs in our study were higher.^{1–3} This may represent the effect of the automated analytical system compared with a semiautomated system, although CVs were comparable, or they may be due to the patient population. Comparison of a semiautomated NTproANP method with a semiautomated NTproBNP method gave similar AUCs, although NTproBNP was considered superior.⁸ Even in studies using research assays for measurement of NTproBNP in the primary care population, comparable AUCs were observed.^{9–10} The differences are therefore likely to be due to the patient group examined. The differences in absolute magnitude of the natriuretic peptide values between this and other studies represent the use of different assay systems. Both tests met the criteria for clinical utility, with an AUC for the ROC curve of >0.7 ,¹¹ but NTproBNP was significantly better in this clinical group. NTproBNP is stable for routine use and analytically robust.⁶ It was clinically superior to NTproANP and had an AUC >0.9 . In conclusion, measurement of B type natriuretic peptide as NTproBNP using this system is preferred to measurement of A type natriuretic peptide as NTproANP for detection of systolic

Table 1 Comparison of areas under the curve for receiver operating characteristic curves using ejection fractions from 50% to 40% for NTproANP and NTproBNP

Ejection fraction cut-off (%)	Normal	Abnormal	NTproANP AUC (confidence interval)	NTproBNP AUC (confidence interval)	p Value
≤ 50	633	38	0.697 (0.613 to 0.782)	0.804 (0.719 to 0.889)	0.012
≤ 45	646	25	0.739 (0.633 to 0.846)	0.895 (0.822 to 0.968)	0.001
≤ 40	656	15	0.738 (0.601 to 0.875)	0.973 (0.958 to 0.989)	0.001
≤ 35	660	11	0.705 (0.524 to 0.886)	0.974 (0.955 to 0.993)	0.002
≤ 30	665	6	0.692 (0.474 to 0.910)	0.977 (0.958 to 0.996)	0.009

AUC, area under curve; NTproANP, N terminal pro-atrial natriuretic peptide; NTproBNP, N terminal pro-hormone.

dysfunction by natriuretic peptide measurement. Whether measurement of NTproBNP is superior to BNP remains to be determined.

Authors' affiliations

Gavin Galasko, Arjiv Lahiri, Roxy Senior, Department of Cardiovascular Medicine, Northwick Park Hospital, Harrow, Middlesex, UK

Paul O Collinson, Sophie C Barnes, David Gaze, Department of Chemical Pathology, St George's Hospital, London, UK

Funding: This study was funded by the Northwick Park Hospital Cardiac Research Fund and a grant from West London Research Network (WeReN). We thank Roche diagnostics for the gift of the NTproBNP reagents.

Competing interests: None.

Correspondence to: Dr P O Collinson, Department of Chemical Pathology, St George's Hospital, 2nd Floor, Jenner Wing, Blackshaw Road, London SW17 0QT, UK; paul.collinson@stgeorges.nhs.uk

Accepted 3 March 2006

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