TECHNIQUE

Stability of plasma concentrations of N and C terminal atrial natriuretic peptides at room temperature

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Abstract

Background—Plasma concentrations of atrial natriuretic peptide (ANP) are increased in patients with ventricular dysfunction and could have a diagnostic role in heart failure. ANP may be unstable after collection, however, limiting any practical diagnostic role.

Methods—Blood samples were obtained from 18 patients with various conditions. Aliquots were either processed optimally or kept as blood or plasma at room temperature for 6–72 h before processing.

Results—Concentrations of C-terminal ANP were lower in specimens kept as blood for 24 and 72 h (mean difference from control –43% and –76%, respectively, ($P < 0.001$) but N-terminal ANP (extracted) seemed to be stable under all conditions studied (–2% at 24 h and –7% at 72 h, not significant).

Conclusions—N-terminal ANP (extracted) is stable and potentially has a role in the diagnosis of heart failure in routine clinical practice.

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Keywords: atrial natriuretic peptide; stability; heart failure; diagnosis

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Recent reports have highlighted the inadequacy of a diagnosis of mild to moderate heart failure made by clinical means alone.^{1,2} This had led to the suggestion that all patients in whom heart failure is suspected should have an echocardiogram. However, the availability of echocardiography, although wide, is still limited compared with the large number of patients in whom the diagnosis of heart failure needs to be confirmed or excluded. Either echocardiography must be made more widely available or alternative diagnostic tests are required.

A strong candidate as an alternative test for the presence of, though not the cause of, heart failure is atrial natriuretic peptide (ANP).^{3,4} N-terminal ANP may be a superior diagnostic tool to the biologically active C-terminal ANP, because of its longer plasma half life and hence higher and more easily detectable plasma concentration.³ Some investigators, however, have found that ANP is unstable even under rigor-

ous conditions of collection and storage.⁵ If this is true then ANP has limited application as a diagnostic tool as the speed with which the samples would need to be processed and stored would be a major disincentive to routine use. Accordingly we measured the stability of N- and C-terminal ANP in samples collected and stored in various ways.

Patients and methods

We obtained blood samples from 18 patients (nine women) admitted to our institution. The mean age was 76 (range 55–88) years. All patients underwent echocardiography. Nine patients had heart failure secondary to left ventricular systolic dysfunction and ischaemic heart disease. Four patients had no cardiovascular abnormality (unexplained falls in one, arthritis in one, and cancer in two), two had chronic obstructive airways disease, and one each had chronic renal failure, isolated atrial fibrillation, and hypertension with left ventricular hypertrophy. The study was approved by the local ethics committee and written informed consent obtained from patients.

COLLECTION AND STORAGE OF SAMPLES

Patients remained seated for 20 min before sampling. Blood (35 ml) was drawn from a forearm vein into polypropylene tubes containing EDTA and Trasylol in 12 cases and EDTA alone in six. Two 5 ml aliquots were taken into chilled tubes and centrifuged at 4°C. The supernatant plasma from one tube was stored at –70°C, and from the other at –20°C. Two 5 ml aliquots were taken into unchilled tubes, centrifuged at room temperature, and kept at room temperature for 24 or 72 h before freezing at –20°C. Three 5 ml aliquots were taken into unchilled tubes and kept as whole blood at room temperature for 6, 24, and 72 h before centrifuging. Supernatant plasma was stored at –20°C until assay within 4–6 weeks.

ASSAY

C-terminal ANP (ANP99–126) was measured by radioimmunoassay after extraction of acidified plasma using C18 Sep-pak cartridges.⁶ N-terminal ANP (ANP1–30) was measured by radioimmunoassay with an antibody supplied by Peninsula Laboratories (Belmont,

Plasma concentrations of C-terminal ANP, and N-terminal ANP before and after extraction

Peptide assay and storage conditions	Mean difference from control (pg/ml) (%)	95% confidence interval (pg/ml)	95% confidence interval as a percentage of control mean
C-terminal ANP storage			
Spun immediately and stored at -20°C	27.3 (24%)	-0.1 to 54.7	-0.1 to 48
Kept as blood for (h)			
6	-12.6 (-11%)	-40.0 to 14.9	-35 to 13
24	-48.8 (-43%)*	-76.2 to -21.4	-56 to -19
72	-86.6 (-76%)*	-114.0 to -59.2	-100 to -52
Kept as plasma for (h)			
24	-18.9 (-17%)	-46.3 to 8.5	-41 to 7
72	-39.1 (-34%)*	-66.5 to -11.7	-58 to -10
N-terminal ANP extracted			
Spun immediately and stored at -20°C	-610 (-9%)	-1338 to 118	-19 to 2
Kept as blood for (h)			
6	-464 (-7%)	-1190 to 266	-17 to 4
24	-152 (-2%)	-881 to 576	-13 to 8
72	-503 (-7%)	-1231 to 226	-18 to 3
Kept as plasma for (h)			
24	-53 (-1%)	-781 to 675	-11 to 10
72	-280 (-4%)	-1008 to 448	-14 to 6
N-terminal ANP unextracted			
Spun immediately and stored at -20°C	1544 (11)	-271 to 3358	-2 to 23
Kept as blood for (h)			
6	798 (6%)	-1017 to 2312	-7 to 18
24	794 (6%)	-1020 to 2609	-7 to 18
72	2426 (17%)*	611 to 4240	4 to 30
Kept as plasma for (h)			
24	1999 (14%)*	185 to 3814	1 to 17
72	2097 (15%)*	282 to 3911	2 to 27

*P < 0.05; **P < 0.01; ***P < 0.001 (ANOVA then paired t test as appropriate). ANP, atrial natriuretic peptide.

California, USA). The antibody cross reacted with ANP (1–30) (100%) but did not cross react with either human C-terminal ANP or brain natriuretic peptide. The radioimmunoassay has an IC₅₀ of 30 pg/tube. N-terminal ANP was measured before and after extraction in plasma. Acidified plasma was extracted using C18 Sep-pak cartridges. The recovery of N-terminal ANP added to plasma was 74% (n = 6) and the interassay coefficient of variation was 8%. Plasma in which N-terminal ANP was not extracted was assayed by substituting 5 ml of assay buffer with 5 ml of plasma. The coefficient of variation was 7%.

STATISTICAL ANALYSIS

Differences in plasma concentrations of ANP with each method of processing were tested with analysis of variance and paired t tests if appropriate.

Results

There was a good correlation between plasma C- and N-terminal ANP in the samples centrifuged immediately and stored at -70°C (r = 0.82 and 0.95 for N-terminal ANP before and after extraction, respectively, P < 0.0001).

Mean plasma concentrations of C-terminal ANP were not significantly different when centrifuged immediately and stored at -70°C or -20°C or if kept at room temperature as blood for 6 h or plasma for up to 24 h (table). In specimens kept at room temperature as plasma for 72 h or as blood for 24 and 72 h there was a substantial decline in concentrations of C-terminal ANP.

Mean plasma concentrations of N-terminal ANP after extraction were similar regardless of the conditions of collection or storage, whether or not Trasylol was present. The plasma concentration of N-terminal ANP measured before extraction increased slightly after 72 h in blood

or plasma.

Patients with heart or renal failure had higher levels of C- and N-terminal ANP compared with those in other diagnostic groups (fig).

Discussion

These data suggest that ANP, in particular the N-terminal form, is stable for long periods at room temperature, even in whole blood, making it practical to explore its use as a routine diagnostic test for heart failure. Confusion between diagnoses of renal and heart failure as causes of raised ANP can be resolved readily by measuring serum creatinine. The higher N-terminal ANP values found by direct assay without an extraction phase compared with the values after extraction can be explained by an approximate 30% loss of the peptide during the extraction procedure. It is also possible that the direct assay measures other N-terminal fragments not recovered by extraction.

N-terminal ANP may prove to be a useful tool to define the epidemiology of heart failure and give appropriate priority to its management. Lack of good epidemiological data on heart failure, often not mentioned when cardiovascular statistics are being reported,⁷ has led, in turn, to relative neglect of heart failure as a problem by health services. For example heart failure is not mentioned in the British Government's recent document entitled *The health of the nation* despite the fact that heart failure is one of the most common reasons for admission to hospital.^{8,9}

N-terminal ANP also has a potential role for monitoring the progression of heart failure and predicting or following the response to treatment.¹⁰ N-terminal ANP may also help select patients in whom it is safe to start an angiotensin converting enzyme inhibitor in the community. Initiation of angiotensin converting enzyme inhibitors for heart failure in the

Plasma concentrations of C-terminal ANP, and N-terminal ANP before and after extraction collected under optimal conditions (spun immediately and stored at -70°C) (x axis) and after 72 h kept at room temperature as plasma or blood (y axis) ($1\text{ pg/ml} \times 0.285 = 1\text{ pmol/l} = (1\text{ ng/ml} \times 285)$). Line of identity is shown on each graph. ●, patients with heart failure; ○, those without cardiovascular disease. ANP, atrial natriuretic peptide; 1, hypertension with left ventricular hypertrophy; 2, atrial fibrillation; 3, chronic obstructive airways disease; 4, renal failure.

community is less expensive than hospital initiation but controversy over the safety of the former approach persists. Although the incidence of first dose hypotension is only 0.5–3%, the patients were highly selected in large clinical trials.⁸ The risks of hypotension and renal dysfunction may be greater in the wider heart failure population⁸ and this requires formal study. Low levels of ANP in a patient considered to have heart failure and receiving a diuretic may be due to a wrong diagnosis or overaggressive treatment. Such patients may be at especial risk of hypotension when starting an angiotensin converting enzyme inhibitor.

ANP will not make echocardiography redundant but ANP may prove to be a superior tool for confirming the presence of heart failure.^{11 12} Although echocardiographic assessment of left ventricular systolic function seems to be fairly accurate, its practical utility in identifying patients with diastolic heart failure has been found wanting.^{13 14} Echocardiography will still be required to help determine the aetiology of heart failure. However, N-terminal ANP may

allow the physician to select patients who require further investigation more accurately, thereby reducing the workload and increasing the yield from echocardiography.

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SHORT CASES IN CARDIOLOGY

Persistent left sided and absent right sided superior vena cava complicating permanent pacemaker insertion

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The radiographic image (figure) shows a venogram after insertion of an antitachycardia pacemaker in a patient with atrioventricular (AV) nodal re-entrant tachycardia. Two previous attempts at radiofrequency ablation of the slow AV nodal pathway had failed but a persistent left sided superior vena cava (SVC) was identified at electrophysiology study. The pacemaker was therefore implanted on the right. The cephalic vein was sought but was not large enough so a right subclavian approach was used. The active fixation, non-preshaped electrode passed along the right subclavian vein, across the midline into the persistent left sided SVC, down the coronary sinus into the right atrium. The right SVC was absent. A satisfactory threshold and stable position were achieved in the superior part of the right atrium.

Venogram showing the path taken by the pacemaker electrode. There is a persistent left superior vena cava and an absent right superior vena cava.

A persistent left sided SVC is the commonest anomaly of the major veins. It occurs in 0.1 to 0.2% of the general population and between 3 and 8% in those with congenital cardiac malformations.¹ An associated absent right SVC is much less common and fewer than 150 cases in patients with situs solitus have been reported. It is most likely to result from flow reversal between the left and right anterior cardinal and innominate veins during early development but the cause is not known.² Associations with conduction system abnormalities also remain unclear. Fragmentation and stretching of the atrioventricular node over a large coronary sinus have been reported.³ The slow atrioventricular nodal pathway is usually situated close to the coronary sinus os. Presumably the very large os in this case reduced the distance between the fast and slow pathways and this made selective ablation of one pathway difficult. Variation in the venous drainage system is not usually physiologically important and is probably often unrecognised during life. However, recognition is important if the patient requires, for example, cardiopulmonary bypass, surgery involving the systemic veins, radiofrequency ablation, or permanent pacemaker insertion. Primary elective epicardial electrode implantation has been recommended for those with a persistent left sided SVC in association with an absent right SVC requiring pacemaker insertion.⁴ The circuitous path taken by the pacemaker lead here explains the difficulty of transvenously obtaining a stable electrode position and sustained capture. The problems were circumvented in this case by the use of an active fixation, screw-in electrode.

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