

# Prognostic significance of N-terminal pro-atrial natriuretic factor (1-98) in acute myocardial infarction: comparison with atrial natriuretic factor (99-126) and clinical evaluation

Torbjørn Omland, Vernon V S Bonarjee, Dennis W T Nilsen, Johan A Sundsfjord, Rolv Terje Lie, Gaetan Thibault, Kenneth Dickstein

## Abstract

**Objective**—To evaluate the prognostic significance of plasma N-terminal pro-atrial natriuretic factor (1-98) concentrations measured in the subacute phase after acute myocardial infarction, and to compare the predictive value of measurement of N-terminal pro-atrial natriuretic factor (1-98) with the measurement of atrial natriuretic factor (99-126) and with clinical assessment of the degree of heart failure.

**Design**—Prospective observational.

**Setting**—Norwegian central hospital.

**Patients**—139 patients (mean (SD) age 66.9 (11.1) years, 71.2% males) with acute myocardial infarction. Patients in cardiogenic shock or with severe heart failure (New York Heart Association class IV) were excluded.

**Main outcome measure**—Cardiovascular death within 12 months.

**Results**—During the follow up period 15 patients died. In a univariate Cox proportional hazards model N-terminal pro-atrial natriuretic factor (1-98) was significantly related to mortality ( $p = 0.0003$ ). In a multivariate model the prognostic value of N-terminal pro-atrial natriuretic factor (1-98) was better than that of atrial natriuretic factor (99-126) and clinical assessment of heart failure (N-terminal pro-atrial natriuretic factor (1-98),  $p = 0.0003$ ; atrial natriuretic factor (99-126),  $p = 0.4513$ ; heart failure,  $p = 0.0719$ ). The odds ratio estimate of patients in whom plasma concentrations of N-terminal pro-atrial natriuretic factor (1-98) were greater than 2000 pmol/l was 25 (95% confidence interval 2.8-225.0) compared with patients with plasma concentrations less than 1000 pmol/l.

**Conclusions**—These results suggest that determination of plasma N-terminal pro-atrial natriuretic factor (1-98) in the subacute phase of myocardial infarction may provide clinically relevant prognostic information that is superior to that obtained from atrial natriuretic factor (99-126) measurements and clinical evaluation.

Atrial natriuretic factor is derived from a 126 amino acid prohormone synthesised and stored in secretory granules in the atrial cardiocytes.<sup>1</sup> Until now research has focused mainly on the physiological and pathophysiological role of the atrial natriuretic factor (99-126) in cardiovascular and renal homeostasis: only recently has the N-terminal fragment of the prohormone of atrial natriuretic factor been identified as a peptide in human plasma,<sup>2,3</sup> circulating predominantly as a single, high molecular weight form (pro-atrial natriuretic factor (1-98)).<sup>4</sup> Though the role of N-terminal pro-atrial natriuretic factor (1-98) in cardiovascular homeostasis has not yet been clearly defined, raised plasma concentrations have been detected during exercise<sup>5,6</sup> and in patients with essential hypertension,<sup>7</sup> myocardial infarction,<sup>8</sup> and cardiac and renal failure.<sup>2,6,7,9</sup>

Many data indicate that atrial natriuretic factor (99-126) is a marker of cardiac function. Significant correlations between plasma concentrations of the peptide and haemodynamic indices have been demonstrated in chronic heart failure<sup>10-13</sup> and in myocardial infarction.<sup>14,15</sup> Recently it has also become evident that measurement of atrial natriuretic factor (99-126) may provide valuable prognostic information in chronic heart failure<sup>16,17</sup> as well as in acute myocardial infarction.<sup>18</sup> The prognostic significance of N-terminal pro-atrial natriuretic factor (1-98), however, remains to be established.

Consequently, the main objectives of the present study were to evaluate the prognostic information obtained by the determination of N-terminal pro-atrial natriuretic factor (1-98) in acute myocardial infarction and to compare the predictive value of N-terminal pro-atrial natriuretic factor (1-98) with that of atrial natriuretic factor (99-126). Furthermore, we used a multivariate Cox proportional hazards model to establish whether plasma N-terminal pro-atrial natriuretic factor (1-98) and atrial natriuretic factor (99-126) concentrations measured soon after infarction provide additional prognostic information to that obtained by in-hospital clinical assessment of cardiac impairment. Blood samples for analysis of N-terminal pro-atrial natriuretic factor (1-98) were collected from 139 patients with acute myocardial infarction

Cardiology Division,  
Medical Department,  
Central Hospital in  
Rogaland, Stavanger,  
Norway  
T Omland  
V V S Bonarjee  
D Nilsen  
K Dickstein

Institute of Medical  
Biology, University of  
Tromsø, Tromsø,  
Norway  
J A Sundsfjord

Section for Medical  
Informatics and  
Statistics, University  
of Bergen, Bergen,  
Norway  
R Terje Lie

Clinical Research  
Institute of Montreal,  
Montreal, Canada  
G Thibault

Correspondence to:  
Dr Torbjørn Omland,  
Department of Cardiology,  
Bergen University Hospital,  
N-5021 Bergen, Norway.

Accepted for publication  
4 June 1993

who were followed for one year for survival analysis.

### Patients and methods

#### STUDY DESIGN

The randomised placebo controlled double blind Cooperative New Enalapril Scandinavian Survival Study (CONSENSUS II) studied the effect on mortality of enalapril administered within 24 hours after the onset of symptoms in patients with acute myocardial infarction.<sup>19</sup> Our substudy was designed to evaluate the prognostic value of N-terminal pro-atrial natriuretic factor (1-98) concentration in acute myocardial infarction.

The diagnosis of myocardial infarction was based on the combination of chest pain lasting >20 minutes and standard electrocardiographic criteria compatible with infarction or enzymatic evidence of myocardial necrosis. Exclusion criteria included supine systolic blood pressure <100 mm Hg or diastolic blood pressure <60 mm Hg, cardiogenic shock or the need for pressor support, haemodynamically significant valvar stenosis, or severe congestive heart failure (New York Heart Association class IV).

Throughout their hospital stay patients were regularly examined and their clinical status recorded. Clinical heart failure was defined as pulmonary congestion warranting treatment with diuretics.

Venous blood for atrial natriuretic factor determination was collected at 8 am on day 3 after the onset of symptoms. The follow up period was 360 days.

#### PATIENTS

One hundred and thirty nine patients with acute myocardial infarction included in the CONSENSUS II trial (99 men and 40 women) participated in this substudy. The average (SD) age was 66.9 (11.1) years. Prior medical history included 33 patients with previous myocardial infarction (24%), 51 with angina pectoris (37%), six with congestive heart failure (4%), 25 with hypertension (18%), and 13 with diabetes mellitus (9%).

In-hospital treatment included thrombolytic therapy with streptokinase in 64 patients (46%), 68 patients received  $\beta$  adrenoceptor blocking drugs (49%) while 67 patients required diuretics (48%), 108 morphine (78%), and 116 intravenous glyceryl trinitrate (83%).

New pathological Q waves developed in 69 patients (50%). Electrocardiographic criteria indicated an anterior (including lateral) infarction in 65 patients (47%). There were no significant differences in demographic or clinical data between the enalapril group and the placebo group.

#### BLOOD SAMPLING PROCEDURES AND RADIOIMMUNOASSAY

Blood samples were drawn by direct venepuncture after at least 30 minutes' supine rest. For atrial natriuretic factor analyses samples were collected into chilled

silicone coated tubes containing ethylenediaminetetraacetic acid and aprotinin (500 kallikrein inactivator units/ml blood). Test tubes were immediately placed on ice and centrifuged within 30 minutes for 10 minutes at 3000 revolutions per minute at 4°C. Plasma samples were stored at -70°C until analysis. N-terminal pro-atrial natriuretic factor (1-98) was measured by a specific radioimmunoassay without prior extraction as described previously.<sup>2</sup> We measured plasma atrial natriuretic factor (99-126) by radioimmunoassay after extraction on a C 18 octadecyl silica microcolumn with a kit from Amersham International, England, as described in a previous report.<sup>20</sup>

#### STATISTICAL ANALYSIS

Results are presented as mean (SEM). Because the plasma concentrations of the hormone were not normally distributed these values were logarithmically transformed. The prognostic value of our variables was tested in a Cox proportional hazards regression analysis using the program 2L in BMDP.<sup>21</sup> Kaplan-Meier estimates of the survival function were plotted using a cutpoint of 1100 pmol/l.<sup>22</sup> We obtained estimates of odds ratios with confidence intervals from a logistic model with the program LR in BMDP.<sup>23</sup> To illustrate the predictive value of a discriminant function for one year survival based on plasma N-terminal pro-atrial natriuretic factor (1-98) we also performed discriminant analysis with the program 7M in BMDP.<sup>24</sup> Probabilities for death within one year based on the estimated discriminant function were plotted for different values of the plasma variable. Backward and forward multivariate regression analyses were performed to assess the effect of other variables on plasma N-terminal pro-atrial natriuretic factor (1-98) concentrations. *t* Tests and  $\chi$  tests with Yates' correction were used to compare means and proportions between groups.

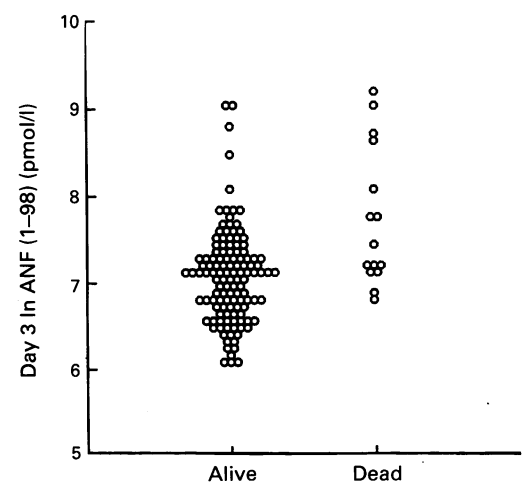
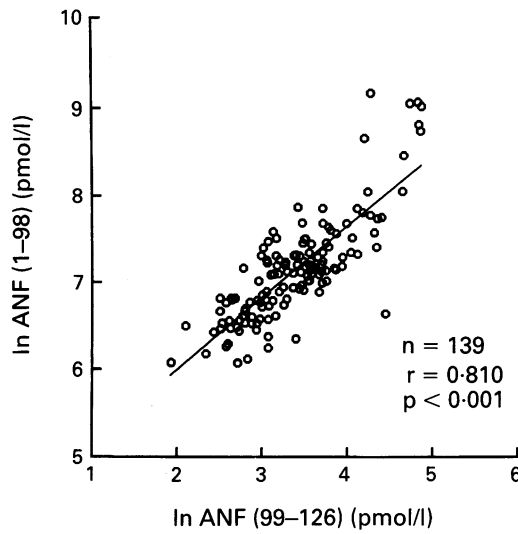


Figure 1 Individual plasma concentrations of N-terminal pro-atrial natriuretic factor (1-98) (ANF (1-98)) in patients with acute myocardial infarction by 1 year survival (alive, n = 123; dead, n = 15; data from one patient were censored).

Figure 2 Relation between the plasma concentration of N-terminal pro-atrial natriuretic factor (1-98) (ANF (1-98)) and atrial natriuretic factor (99-126) (ANF (99-126)) after logarithmic transformation (ln, natural logarithm) in 139 patients with acute myocardial infarction.



**Results**

During the one year follow up period 16 patients died. One of these patients died of a non-cardiovascular cause on day 94 after myocardial infarction and was subsequently censored from the analysis. Figure 1 shows the distribution of individual plasma concentrations of N-terminal pro-atrial natriuretic factor (1-98) in those who were alive at 1 year and in those who were dead. There were six deaths in the group of patients originally randomised to enalapril and nine in the original placebo group.

The concentration of N-terminal pro-atrial natriuretic factor (1-98) on day 3 was 1603 (133) pmol/l (median 1241 pmol/l), while the mean concentration of plasma atrial natri-

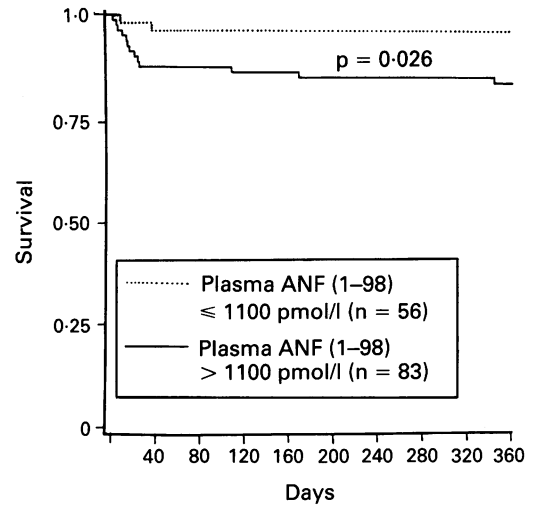


Figure 3 Kaplan-Meier survival plots for patients with acute myocardial infarction divided into two groups according to the concentration of N-terminal pro-atrial natriuretic factor (1-98) (ANF (1-98)) in plasma.

uretic factor (99-126) on day 3 was 36.1 (2.2) pmol/l. The correlation between plasma concentrations of N-terminal pro-atrial natriuretic factor (1-98) and atrial natriuretic factor (99-126) ( $r = 0.81, p < 0.001$ ) was good (fig 2). Serum creatinine concentrations at admission correlated significantly with day 3 plasma concentrations of N-terminal pro-atrial natriuretic factor (1-98) ( $n = 123, r = 0.53; p < 0.001$ ). There was no significant difference in day 3 plasma N-terminal pro-atrial natriuretic factor concentrations between the enalapril and the placebo group (1439 (146) v 1774 (224) pmol/l,  $p = 0.233$ ). In a multivariate model, treatment with diuretics and lack of thrombolytic therapy during the in-hospital phase were associated with higher plasma concentrations of N-terminal pro-atrial natriuretic factor (1-98) (diuretics,  $p = 0.005$ ; thrombolytic therapy,  $p = 0.043; r^2 = 0.09$ ). In contrast, treatment with converting enzyme inhibitors or  $\beta$  adrenoceptor blockers had no evident effect on concentrations.

The plasma concentration of N-terminal pro-atrial natriuretic factor (1-98) was significantly related to mortality in a univariate Cox proportional hazards regression model ( $p = 0.0003$ ). Atrial natriuretic factor (99-126) and clinical heart failure were also significantly related to survival in univariate analysis (atrial natriuretic factor (99-126),  $p = 0.0004$ ; heart failure,  $p = 0.0010$ ). There was no significant relation between age ( $p = 0.1536$ ), heart rate ( $p = 0.4039$ ), or systolic blood pressure ( $p = 0.4544$ ) and mortality (table 1). In a multivariate model only N-terminal pro-atrial natriuretic factor (1-98) provided independent prognostic information (table 1).

Furthermore, division of patients into two groups according to the upper normal limit of the plasma concentration of pro-atrial natriuretic factor (1-98) (1100 pmol/l) showed that mortality was significantly higher in the group with raised concentrations of N-terminal pro-atrial natriuretic factor (1-98) (13

Table 1 Effects of N-terminal pro-atrial natriuretic factor (1-98), atrial natriuretic factor (ANF) (99-126), and clinical heart failure on 1 year mortality according to a Cox proportional hazards model

	Univariate		Final model	
	$\chi^2$	p Value	$\chi^2$	p Value
In ANF (99-126) (pmol/l)	12.35	0.0004	0.57	0.4513
In pro-ANF (1-98) (pmol/l)	13.08	0.0003	13.08	0.0003*
Heart failure	10.83	0.0010	3.24	0.0719
Age (yr)	2.04	0.1536	0.07	0.7888
Heart rate day 3 (beats/min)	0.70	0.4039	0.18	0.6700
SBP day 3 (mm Hg)	0.56	0.4544	0.70	0.4300

\*Factor in the model.  
SBP, systolic blood pressure.

Table 2 Comparison of demographic, clinical, and biochemical variables\* grouped according to a plasma N-terminal pro-atrial natriuretic factor (1-98) cut-off value of 1100 pmol/l

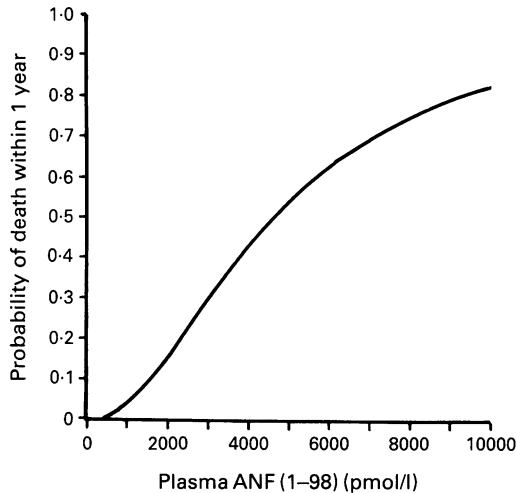
	Pro ANF (1-98) <1100 pmol/l	Pro ANF (1-98) >1100 pmol/l	p Value
Total number	56	83	
Deaths	2	13	0.048
Age	60.7 (1.6)	71.2 (0.9)	<0.001
Male sex	40 (71%)	59 (71%)	NS
Q-wave myocardial infarction	28 (50%)	41 (49%)	NS
Anterior myocardial infarction	27 (48%)	38 (46%)	NS
Treatment during primary admission to hospital:			
$\beta$ Adrenoceptor blockers	25 (45%)	43 (51%)	NS
Converting enzyme inhibitors	29 (52%)	41 (49%)	NS
Diuretics	21 (38%)	46 (55%)	NS
Thrombolytics	31 (55%)	33 (40%)	NS
Biochemical variables:			
Serum sodium (mmol/l)	139 (2.5)	139 (1.7)	NS
Serum potassium (mmol/l)	4.1 (0.1)	4.1 (1.1)	NS
Peak creatine kinase-MB (IU/l)	122 (16)	145 (14)	NS

\*Continuous variables presented as mean (SEM).

Table 3 One year mortality by the concentration of N-terminal pro-atrial natriuretic factor (1-98) in plasma

Plasma ANF (1-98) (pmol/l)	Deaths	Total	Odds ratio estimate	95% Confidence interval
0-1000	1	51	1.0	
1001-2000	7	67	5.8	(0.7 to 50.0)
>2000	7	21	25.0	(2.8 to 225.0)

Figure 4 Plot of the probability of death within 1 year as a function of plasma concentrations of N-terminal pro-atrial natriuretic factor (1-98) (ANF (1-98)).



patients) than in the patients with values within the normal range (two patients) ( $p = 0.026$ ) (fig 3). Table 2 compares the demographic, clinical, and biochemical variables in patients with high concentrations of N-terminal pro-atrial natriuretic factor (1-98) and patients with low concentrations.

Logistic regression analysis showed that the odds ratio of patients with plasma concentrations of N-terminal pro-atrial natriuretic factor (1-98) > 2000 pmol/l was 25.0 (95% confidence interval 2.8 to 225.0) compared with an odds ratio of 1.0 in patients with plasma concentrations < 1000 pmol/l (table 3).

Figure 4 shows the estimated probability of cardiac death within 1 year as a function of plasma N-terminal pro-atrial natriuretic factor (1-98) according to a discriminant analysis model. The discriminant model showed that the optimal cut-off value for pro-atrial natriuretic factor (1-98) to divide patients into a high risk group and a low risk group was 1648 pmol/l. This cut-off value gave a sensitivity of 53.3% (false negative rate 47.7%) and a specificity of 79.8% (false positive rate 20.2%). The corresponding positive and negative predictive values were 24.2% and 93.4%, respectively. The arbitrary cut-off value of 1100 pmol/l gave a sensitivity of 86.7% (false negative rate 13.3%) and a specificity of 43.5% (false positive rate 56.5%) for prediction of one year mortality (positive predictive value 15.7%, negative predictive value 96.4%).

### Discussion

Our data confirm and extend current knowledge of the prognostic significance of atrial natriuretic factor in cardiac disease. Though

both the presence of clinical heart failure and the plasma concentration of atrial natriuretic factor (99-126) were related to mortality in the univariate analysis, the addition of these two factors to the multivariate Cox model did not improve the prognostic information compared with the information provided by N-terminal pro-atrial natriuretic factor (1-98) determination alone.

Atrial natriuretic factor (99-126) and mortality were related in patients with chronic cardiac failure<sup>16,17</sup> and in patients with acute myocardial infarction.<sup>18</sup> However, the instability of atrial natriuretic factor (99-126) (plasma half life < 3 minutes)<sup>25</sup> and the time-consuming and cumbersome method of detection tend to limit the clinical usefulness of such measurements. In contrast, the plasma half-life of the N-terminal pro-atrial natriuretic factor (1-98) fragment is approximately one hour,<sup>26</sup> and incubation of samples at room temperature for up to 24 hours did not significantly reduce the plasma concentration.<sup>2</sup> Further, direct assay of the N-terminal pro-atrial natriuretic factor (1-98) fragment without prior extraction is feasible and simplifies the analysis.<sup>2</sup> The significant relation that we found between subacute plasma concentrations of N-terminal pro-atrial natriuretic factor (1-98) and 1-year mortality after acute myocardial infarction may thus be clinically important.

The relation between the plasma concentration of N-terminal pro-atrial natriuretic factor and mortality after acute myocardial infarction is not likely to be causal. Infusion studies in healthy individuals,<sup>27</sup> in patients with chronic heart failure<sup>28</sup> and myocardial infarction<sup>29</sup> all indicate that atrial natriuretic factor (99-126) has beneficial, albeit weak, physiological actions. Furthermore, intravenous administration of the endopeptidase inhibitor candoxatrilat, which inhibits the degradation of atrial natriuretic factor (99-126), had beneficial haemodynamic and natriuretic effects in patients with severe chronic heart failure.<sup>30</sup> Fragments of N-terminal pro-atrial natriuretic factor (1-98) can also have vasodilatory and natriuretic properties.<sup>31,32</sup> Significant correlations between plasma concentrations of atrial natriuretic factor (99-126) and various indices of cardiac function have been reported in several studies.<sup>10-15</sup> Because atrial distension is the main stimulus for the release of atrial natriuretic factor<sup>33</sup> it is not surprising that in patients with predominant left ventricular failure there is a close relation between left ventricular end diastolic pressure and plasma concentrations of atrial natriuretic factor (99-126) ( $r = 0.88$ ).<sup>13</sup> Furthermore, because left ventricular function is a major determinant of survival in patients with previous myocardial infarction,<sup>34</sup> the association between concentrations of N-terminal pro-atrial natriuretic factor (1-98) and mortality can probably be explained by the ability of the factor to reflect increases in cardiac filling pressures secondary to left ventricular dysfunction. Interestingly, concentrations of

atrial natriuretic factor in the ventricles increase during the progression of heart failure.<sup>35</sup> Recent data also suggest that acute myocardial infarction can trigger the synthesis of atrial natriuretic factor in ventricular tissue.<sup>36</sup> Consequently, left ventricular distension may be a stimulus for the production of atrial natriuretic factor in the ventricular myocyte after myocardial infarction.

The relative prognostic merits of measurements of N-terminal pro-atrial natriuretic factor (1–98) and commonly employed indices of ventricular performance derived from the assessment of central haemodynamics or of cardiac chamber volumes are unknown. Do neurohumoral variables provide prognostic information that is additional to the estimation of ventricular volumes based on echocardiographic or radionuclide techniques (perhaps the clinically most versatile diagnostic aids at present)? Certainly the sensitivity and specificity of N-terminal pro-atrial natriuretic factor assessment seem to be comparable to those obtained from the determination of left ventricular ejection fraction and heart rate variability, which are both established prognostic indices in acute myocardial infarction.<sup>37</sup>

Previous studies of the prognostic value of neurohumoral measurements in acute myocardial infarction have been limited by small patient samples and selection of patients. In contrast to the study of Svanegaard *et al.*<sup>18</sup> who included less than 30% of eligible patients, we studied more than 60% of patients admitted to our coronary care unit during the six months recruitment period. Furthermore, our sample may be more representative of the general myocardial infarction population because patients were included irrespective of concurrent therapy, whereas patients receiving cardiovascular drugs before blood sampling were excluded from the Danish study.<sup>18</sup> Though we excluded patients with severe heart failure and patients in cardiogenic shock, we believe that this tends to underestimate rather than overestimate the relation between atrial natriuretic factor and prognosis, because both groups have markedly increased plasma concentrations of atrial natriuretic factor and a poor prognosis.<sup>38</sup> The choice of day 3 after myocardial infarction as the time for blood sampling was arbitrary, but it coincides with peak plasma concentrations of atrial natriuretic factor in most previous reports<sup>39,40</sup> and has the advantage of being performed during daytime and after haemodynamic stabilisation of the patient.

The patients with plasma concentrations N-terminal pro-atrial natriuretic factor (1–98) greater than 1100 pmol/l were significantly older than those with a value less than 1100 pmol/l. This observation might contribute to the association between N-terminal pro-atrial natriuretic factor and mortality. However, though there was a positive relation between the plasma concentration of N-terminal pro-atrial natriuretic factor and age ( $r = 0.34$ ,  $p < 0.001$ ), adjustment for age in the Cox

proportional hazards model did not alter the relation between N-terminal pro-atrial natriuretic factor (1–98) and survival.

In conclusion, these results suggest that the measurement of plasma concentrations of N-terminal pro-atrial natriuretic factor (1–98) may provide a readily obtainable prognostic index in acute myocardial infarction that is superior to that obtained from atrial natriuretic factor (99–126) measurements and risk stratification according to the presence of symptomatic ventricular impairment. The prognostic value of N-terminal pro-atrial natriuretic factor (1–98) is emphasised by the fact that its significance is evident despite the presence of various factors that can affect plasma concentrations of the peptide—for example, concurrent medical treatment, renal function, and sodium intake. Further, because the greater stability and subsequent increase in plasma concentrations simplifies analysis, assessment of the N-terminal pro-atrial natriuretic factor (1–98) may prove to be more versatile than measuring the atrial natriuretic factor (99–126) fragment. Whether measurement of plasma N-terminal pro-atrial natriuretic factor (1–98) is a better indicator of outcome than echocardiographic and haemodynamic indices of cardiac performance remains to be determined.

We thank Dr Harald Vik-Mo, for critical evaluation of the paper and Dr Asbjørn Aakvaag for performing the atrial natriuretic factor (99–126) analyses. Torbjørn Aarsland RN provided valuable assistance in the implementation of the study. This study was supported by a grant from Merck Research Laboratories (Norwegian subsidiary).

- 1 Thibault G, Garcia R, Gutkowska J, Bilodeau J, Lazure C, Seidah NG, *et al.* The propeptide Asn 1-Tyr 126 is the storage form of rat atrial natriuretic factor. *Biochem J* 1987;241:265–72.
- 2 Sundsfjord JA, Thibault G, Larochelle P, Cantin M. Identification and plasma concentrations of the N-terminal fragment of proatrial natriuretic factor in man. *J Clin Endocrinol Metab* 1988;66:605–10.
- 3 Itoh H, Nakao K, Sugawara A, Saito Y, Mukoyama M, Morii N, *et al.*  $\gamma$ -Atrial natriuretic polypeptide ( $\gamma$ ANP)-derived peptides in human plasma: Cosecretion of N-terminal ANP fragment and  $\alpha$ ANP. *J Clin Endocrinol Metab* 1988;67:429–37.
- 4 Buckley MG, Sagnella GA, Markandu ND, Singer DRJ, MacGregor GA. Concentrations of N-terminal ProANP in human plasma: evidence for ProANP (1–98) as the circulating form. *Clin Chim Acta* 1990;191:111–4.
- 5 Baker BJ, Wu WCL, Winters CJ, Dinh H, Wyeth R, Sallman AL, Vesely DL. Exercise increases the circulating concentration of the N-terminus of the atrial natriuretic factor prohormone in normal individuals. *Am Heart J* 1991;122:1395–1402.
- 6 Winters CJ, Baker BJ, Dinh H, Sallman AL, Rico DM, Vesely DL. Prohormone atrial natriuretic peptides 1–98 and 31–67 increase with exercise in congestive heart failure patients. *Am J Med Sci* 1989;298:377–82.
- 7 Buckley MG, Sagnella GA, Markandu ND, Singer DRJ, MacGregor GA. Immunoreactive N-terminal pro-atrial natriuretic peptide in human plasma: plasma levels and comparisons with  $\alpha$ -human atrial natriuretic peptide in normal subjects, patients with essential hypertension, cardiac transplant and chronic renal failure. *Chin Sci* 1989;77:573–9.
- 8 Ngo L, Vesely DL, Bissett JK, Murphy ML, Dinh H, Sallman AL, *et al.* Acute and sustained release of the atrial natriuretic factor prohormone N-terminus with acute myocardial infarction. *Am J Med Sci* 1991;301:157–64.
- 9 Winters CJ, Sallman AL, Baker BJ, Meadows J, Rico DM, Vesely DL. The N-terminus and a 4,000-MW peptide from the midportion of the N-terminus of the atrial natriuretic factor prohormone each circulate in humans and increase in congestive heart failure. *Circulation* 1989;80:438–49.
- 10 Raine AEG, Erne P, Bürgisser E, Müller FB, Bolli P, Burkhardt F, *et al.* Atrial natriuretic peptide and atrial

- pressure in patients with congestive heart failure. *N Engl J Med* 1986;315:533-7.
- 11 Bates ER, Shenker Y, Grekin RJ. The relationship between plasma levels of immunoreactive atrial natriuretic hormone and hemodynamic function in man. *Circulation* 1986;73:1155-61.
  - 12 Richards AM, Cleland JFG, Tonolo G, McIntyre GD, Leckie BJ, Dargie HJ, et al. Plasma  $\alpha$ -atrial natriuretic peptide in cardiac impairment. *Br Med J* 1986;293:409-12.
  - 13 Tsutamato T, Bito K, Kinoshita M. Plasma atrial natriuretic polypeptide as an index of left ventricular end-diastolic pressure in patients with chronic left-sided heart failure. *Am Heart J* 1989;117:599-606.
  - 14 Tomoda H. Atrial natriuretic peptide in acute myocardial infarction. *Am J Cardiol* 1988;62:1122-3.
  - 15 Matsubara H, Nishikawa M, Umeda Y, Taniguchi T, Iwasaka T, Kurimoto T, et al. The role of atrial pressures in secreting atrial natriuretic polypeptides. *Am Heart J* 1987;113:1457-63.
  - 16 Gottlieb SS, Kukin ML, Ahern D, Packer M. Prognostic importance of atrial natriuretic peptide in patients with chronic heart failure. *J Am Coll Cardiol* 1989;13:1534-9.
  - 17 Swedberg K, Eneroth P, Kjekshus J, Wilhelmssen L. Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. *Circulation* 1990;82:1730-6.
  - 18 Svanegaard J, Angelo-Nielsen K, Pindborg T. Plasma concentrations of atrial natriuretic peptide at admission and risk of cardiac death in patients with acute myocardial infarction. *Br Heart J* 1992;68:38-42.
  - 19 Swedberg K, Held P, Kjekshus J, Rasmussen K, Ryden L, Wedel H on behalf of the CONSENSUS II study group. Effects of the early administration of enalapril on mortality in patients with acute myocardial infarction. *N Engl J Med* 1992;327:678-84.
  - 20 Omland T, Barvik S, Aakvaag A, Aarsland T, Dickstein K. Plasma atrial natriuretic factor concentration during maximal cardiopulmonary exercise in men with mild heart failure. *Int J Cardiol* 1990;29:179-84.
  - 21 Hopkins A. Survival analysis with covariates. In: Dixon WJ, ed. *BMDP statistical software manual*. Berkeley: University of California Press, 1990;769-804.
  - 22 Benedetti J, Yuen K, Young L. Life tables and survivor functions. In: Dixon WJ, ed. *BMDP statistical software manual*. Berkeley: University of California Press, 1990;739-68.
  - 23 Engelmann L. Stepwise logistic regression. In: Dixon WJ, ed. *BMDP statistical software manual*. Berkeley: University of California Press, 1990;1013-46.
  - 24 Jennrich R, Sampson P. Stepwise discriminant analysis, in Dixon WJ, ed. *BMDP statistical software manual*. Berkeley: University of California Press, 1990;339-58.
  - 25 Yandle TG, Richards AM, Nicholls MG, Cuneo R, Espiner EA, Livesey JH. Metabolic clearance rate and plasma half-life of  $\alpha$ -human atrial natriuretic peptide in man. *Life Sci* 1986;38:1827-33.
  - 26 Thibault G, Murthy KK, Gutkowska J, Seidah NG, Lazure C, Chretien M, Cantin M. NH<sub>2</sub>-terminal fragment of rat pro-atrial natriuretic factor in the circulation: identification, radioimmunoassay and half-life. *Peptides* 1988;9:47-53.
  - 27 Richards AM, Nicholls MG, Ikram H, Webster MWI, Yandle TG, Espiner EA. Renal, haemodynamic, and hormonal effects of human  $\alpha$ -atrial natriuretic peptide in healthy volunteers. *Lancet* 1985;i:545-9.
  - 28 Crozier IG, Nicholls MG, Ikram H, Espiner EA, Gomez HJ, Warner NJ. Haemodynamic effects of atrial peptide infusion in heart failure. *Lancet* 1986;ii:1242-5.
  - 29 Nakamura M, Arakawa N, Kato M. Renal, hormonal, and hemodynamic effects of low-dose infusion of atrial natriuretic factor in acute myocardial infarction. *Am Heart J* 1990;120:1078-85.
  - 30 Münzel T, Kurz S, Holtz J, Busse R, Steinhauser H, Just H, Drexler H. Neurohormonal inhibition and hemodynamic unloading during prolonged inhibition of ANF degradation in patients with severe chronic heart failure. *Circulation* 1992;86:1089-98.
  - 31 Vesely DL, Norris JS, Walters JM, Jespersen RR, Baeyens DA. Atrial natriuretic prohormone peptides 1-30, 31-67 and 79-98 vasodilate the aorta. *Biochem Biophys Res Commun* 1987;148:1540-8.
  - 32 Martin DR, Pevahouse JB, Trigg DJ, Vesely DL, Buerkert JE. Three peptides from the pro ANF prohormone NH<sub>2</sub>-terminus are natriuretic and/or kaliuretic. *Am J Physiol* 1990;258:F1401-8.
  - 33 Edwards B, Zimmerman R, Schwab T, Heublein D, Burnett J. Atrial stretch, not pressure, is the principal determinant controlling the acute release of atrial natriuretic factor. *Circ Res* 1988;62:191-5.
  - 34 White HD, Norris RM, Brown MA, Brandt PWT, Whitlock RM, Wild CJ. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation* 1987;76:44-51.
  - 35 Takemura G, Fujiwara H, Mukoyama M, Saito Y, Nakao K, Kawamura A, et al. Expression and distribution of atrial natriuretic peptide in human hypertrophic ventricles of hypertensive hearts and hearts with hypertrophic cardiomyopathy. *Circulation* 1991;83:181-90.
  - 36 Jougasaki M, Yasue H, Mukoyama M, Nakao K, Takahashi K. Appearance of atrial natriuretic peptide in the ventricles in patients with myocardial infarction. *Am Heart J* 1990;119:92-6.
  - 37 Odemuyiwa O, Malik M, Farrell T, Bashir Y, Poloniecki J, Camm J. Comparison of the predictive characteristics of heart rate variability index and left ventricular ejection fraction for all-cause mortality, arrhythmic events and sudden death after acute myocardial infarction. *Am J Cardiol* 1991;68:434-9.
  - 38 Fontana F, Bernardi P, Spagnolo N, Capelli M. Plasma atrial natriuretic factor in low output heart failure syndromes. *Klin Wochenschr* 1990;68:313-9.
  - 39 Tan ACITL, van Loenhout TT, Lamfers EJP, Hooghoudt TEH, Kloppenborg PWC, Benraad TJ. Atrial natriuretic factor after myocardial infarction. *Am Heart J* 1989;118:490-3.
  - 40 Fontana F, Bernardi P, Spagnolo N, Capelli M. Plasma atrial natriuretic factor in patients with acute myocardial infarction. *Eur Heart J* 1990;11:779-87.