Plasma N-terminal B type natriuretic peptide as an Indicator of Long-term Survival After Acute Myocardial Infarction: Comparison With Plasma Mid-regional Pro-atrial Natriuretic Peptide - Leicester Acute Myocardial Infarction Peptide (LAMP) Study

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Abstract (word count 208)

Objectives

Our aim was to assess the prognostic value of MR-proANP in patients post acute myocardial infarction (AMI).

Background

Multimarker strategies may assist risk stratification post-AMI. Midregional Pro-Atrial Natriuretic Peptide (MR-proANP) is a newly described stable fragment of N-terminal Pro-Atrial Natriuretic Peptide. We compared the prognostic value of MR-proANP and an established marker N-terminal pro-B-type natriuretic peptide (NT-proBNP) post-AMI.

Methods

We recruited 983 consecutive post-AMI patients (720 men, median [range] age 65[24-95] years) in a prospective study with follow-up over 343[0-764] days.

Results

Plasma MR-proANP was raised in patients who died (n=101) compared to survivors (median [range] pmol/L, 310[48-1150] vs. 108[4.9-1210], P<0.0001). Using Cox modelling $\log_{10}$MR-proANP (HR 3.87) and $\log_{10}$NT-proBNP (HR 3.25) were significant independent predictors of death. In patients stratified by NT-proBNP in the highest quartile (> ~5900 pmol/L), MR-proANP in the top quartile (~330 pmol/L) was associated with poorer outcome (P<0.0001). Findings were similar for heart failure as an individual endpoint. However neither marker predicted recurrent AMI.

Conclusions

The A and B-type natriuretic systems are activated post AMI. MR-proANP is a powerful predictor of adverse outcome especially in those with an elevated NT-proBNP. MR-proANP may represent a clinically useful marker of prognosis after an AMI as part of a multimarker strategy targeting the natriuretic neurohormonal pathway.

Condensed Abstract

MR-proANP is a stable fragment of N-terminal Pro-Atrial Natriuretic Peptide. We compared the prognostic value of MR-proANP and NT-proBNP in 983 post-AMI patients.Plasma MR-proANP was raised in patients who died (n=101) compared to survivors. MR-proANP and NT-proBNP were found to be significant independent predictors of death. In patients stratified by NT-proBNP in the highest quartile (> ~5900 pmol/L), MR-proANP in the top quartile (~330 pmol/L) was associated with poorer outcome (P<0.0001). MR-proANP is a powerful predictor of death especially in those with an elevated NT-proBNP and may represent a clinically useful marker of prognosis targeting the natriuretic neurohormonal pathway.

Introduction

Acute myocardial infarction results as a consequence of plaque rupture and superimposed thrombus formation. This acute event leads to acute disruption of myocardial contractility and neurohormonal activation triggering the release of the natriuretic peptide hormones from the myocardial tissues [1]. The natriuretic peptide hormones atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) and the amino-terminal fragments of their prohormones (NTproANP and NT-proBNP, respectively) have now been well established as predictors of poor outcome in patients post-acute myocardial infarction (AMI) and in patients with heart failure [2, 3, 4,5,6,7]. The prognostic information provided by these hormones is over and above that of impaired left ventricular function, Killip class and renal impairment [6]. Both hormones promote natriuresis and diuresis and have vasodilator properties. The prohormones have a larger molecular weight, lack binding interactions with cells or other proteins and receptors, and have longer half-lives as there are no active excretion pathways and may therefore be easier to measure in plasma. B-type natriuretic peptide and its more stable counterpart N-terminal pro-B-type natriuretic peptide (NT-proBNP) have shown the greatest promise in this area[1] as their use covers a range of acute coronary syndromes.[8] A multimarker approach looking specifically at the natriuretic neurohormones however may be more beneficial. Previous studies have investigated combinations of BNP, NT-proBNP, ANP and N-ANP. Richards et al proposed NT-proBNP as the strongest independent marker for death or heart failure, with no additional contribution from N-ANP[5]. In Omland et al's study it was found that only BNP gave additional prognostic information on mortality over and above left ventricular systolic function [9]. Our group has previously directly compared N-ANP and NT-proBNP and found N-ANP may be of benefit in predicting late mortality and NT-proBNP at predicting early mortality [10]. Here we investigate midregional Pro-Atrial Natriuretic Peptide (MR-proANP) in a multimarker approach. Epitopes of the antibodies used in the assay for MR-proANP cover amino acids 53-90 of N-terminal Pro-

Atrial Natriuretic Peptide pro-ANP [11]. Midregional epitopes of prohormones may be more stable to degradation by exoproteases, unlike epitopes in the N- or C-terminals of proANP used in previous immunoassays. Previous studies may therefore have underestimated the utility of ProANP as a biomarker. The diagnostic use of MR-proANP has recently been described in the differential diagnosis of acute decompensated heart failure where it has been shown to be comparable to that of BNP and NT-proBNP [12]. Our aim was to investigate whether MR-proANP alone or in combination with NT-proBNP would be of benefit in determining the prognosis post-AMI, particularly for death and heart failure (HF). We were particularly interested to see if a multimarker approach using combined information from two natriuretic markers could give prognostic information over and above just NT-proBNP, a peptide of established prognostic value benefit in this group of patients.[5,10,13]

Methods

Study population

We studied 983 consecutive AMI patients admitted to the Coronary Care Unit of the Leicester Royal Infirmary. AMI was diagnosed if a patient had a plasma creatine kinase-MB elevation greater than twice normal or cardiac troponin I level >0.1 ng/mL with at least one of the following, chest pain lasting >20 minutes or diagnostic serial electrocardiographic (ECG) changes consisting of new pathological Q waves or ST-segment and T-wave changes. AMI was sub categorised into ST segment elevation myocardial infarction (STEMI) or non-ST segment myocardial infarction (NSTEMI). The study complied with the Declaration of Helsinki and was approved by the local ethics committee; written informed consent was obtained from all patients. Exclusion criteria were known malignancy, or surgery in the previous month. The estimated GFR (eGFR) was calculated from the simplified formula derived from the Modification of Diet in Renal Disease (MDRD) study, recently validated in patients with HF [14].

Plasma samples

Blood samples were drawn on one occasion 3 to 5 days after the onset of chest pain for determination of plasma MR-proANP and NT-proBNP. After 15 minutes bed rest, 20mL blood was collected into tubes containing EDTA and aprotinin. All plasma was stored at -70°C until assayed in a blinded fashion in a single batch. In a subgroup of 132 patients from the original 983 patient cohort, blood sampling was performed daily for 5 days from admission to discharge.

Echocardiography

Transthoracic echocardiography was performed in patients using a Sonos 5500 instrument (Philips Medical Systems, Reigate, UK). Left ventricular ejection fraction (LVEF) was calculated using the biplane method of discs formula.[15]

NT-proBNP assay

Our NT-proBNP assay was based on a non-competitive assay as previously published.[8] Sheep antibodies were raised to the N-terminus of human NT-proBNP and monoclonal mouse antibodies were raised to the C-terminus. Samples or NT-proBNP standards were incubated in C-terminal IgG–coated wells with the biotinylated N-terminal antibody for 24 hours at 4°C. Detection was with methyl-acridinium ester (MAE)–labelled streptavidin on a MLX plate luminometer (Dynex Technologies Ltd., Worthing, UK). The lower limit of detection was 0.3 pmol/L. There was no cross reactivity with atrial natriuretic peptide, BNP, or C-type natriuretic peptide.

MR-proANP assay

MR-proANP was detected using a novel commercial sandwich immunoassay in the chemiluminescence/-coated tube-format (BRAHMS AG) as described [11] . Briefly, patient

samples (1:40 dilution of 5 µl plasma in incubation buffer) or standards were added in duplicate to antibody-coated tubes (affinity purified sheep polyclonal antibodies directed against proANP peptide 73–90) and incubated for 30 min at room temperature. After five washes with 1 ml washing buffer, 200 µl tracer was added, containing acridinium ester-labelled anti-proANP antibody (affinity purified sheep polyclonal antibodies directed against proANP peptide 53–72), followed by 30 min incubation at room temperature. Tubes were washed three times with 1 ml washing buffer, and detection was performed in a LB952T luminometer (Berthold, Germany; 1 s detection time per sample). Relative light units of the chemiluminescence assay were expressed in pmol/L MR-proANP, as calculated from a calibration curve (4–1800 pmol/l) that was included in every analytical run. The lower detection limit of the assay is 4.3 pmol/l and the functional sensitivity of the assay is 11 pmol/L MR-proANP. The interassay coefficient of variation (CV) within the range of plasma measurements was under 10% (8,0% CV at 100 pmol/L; 6.5% CV at 400 pmol/L).

End points

Our primary endpoint was death. We also investigated, hospitalization for HF and recurrent AMI as individual secondary endpoints. Hospitalization for HF was defined as a hospital readmission for which HF was the primary reason. Myocardial infarction (MI) was diagnosed on established criteria as described above [16]. Endpoints were obtained by reviewing the Office of National Statistics Registry and by contacting each patient. There was a minimum 60-day follow-up of all surviving patients.

Statistical analysis

Statistical analyses were performed on SPSS Version 14 (SPSS Inc, Chicago, Illinois). Comparisons of continuous variables were made using the Mann Whitney U test. Comparisons in the daily sampling study were performed using the general linear model (GLM) with repeated

measures, with correction for multiple comparisons using the Bonferroni method. The GLM repeated measures procedure in SPSS provides analysis of variance when the same measurement is made several times on each subject or case. Spearman's correlations were performed. The relationship of baseline variables with death and HF was assessed using Cox proportional hazards analysis by univariate and multivariate analysis. Factors with univariate significance of $P < 0.1$ were included in multivariate analyses. Echocardiographic data was analysed in a substudy (see below). The Kaplan-Meier cumulative survival curves were constructed and compared by the log-rank test and the log-rank test for trend. Normality of continuous variables was assessed by the Kolmogorov-Smirnov test. Levels of NT-proBNP and MR-proANP were normalised by $\log_{10}$ transformation. Thus, odds ratios and hazard ratios refer to a tenfold rise in the levels of these markers. Hazard ratio (HR) and 95% confidence intervals for risk factors and significance level for chi-square (likelihood ratio test) are given.

To compare the accuracy of NT-proBNP and MR-proANP, receiver-operating characteristic (ROC) curves were generated and the area under the curves (AUC) was calculated. A two-tailed P value of less than 0.05 was deemed to be statistically significant. The study size of 980 patients had a 90% power at $P<0.01$ to detect a difference of 0.63 in the hazard ratio between the top quartile of the biomarker compared to the other quartiles. All authors had full access to the data and take responsibility for its integrity and accuracy of the analysis. All authors have read and agree to the manuscript as written.

Results

Patient characteristics

Patient details are recorded in Table 1. Median length of follow-up was 343 [range 0-764] days. The minimum length of follow-up for survivors was 60 days. During follow-up, 101 (10.3%) patients died, 49 (5.0%) were readmitted with heart failure and there were 79(8.0%) recurrent AMI. 1 year mortality was 18.1%. There were 783 STEMI patients. No patient was lost to follow-up.

Plasma profile of MR-proANP and NT-proBNP

In a subgroup of 132 patients (102 male, median age [range] 64 [32-90] years) daily blood samples were obtained for 5 days post-admission. 7 patients in the subgroup experienced the primary endpoint of death. The time course of plasma NT-proBNP is shown in Figure 1. This shows significant changes with day of sampling ($P<0.001$, Bonferroni corrected for 4 comparisons in 132 patients), with peak levels on day 2 ($P<0.001$ and 0.02 compared to day 1 and day 3 respectively using the Bonferroni correction for 4 comparisons in 132 patients). For plasma MR-proANP, the peak was most evident on day 1 (significantly elevated compared to day 2, $P=0.001$ using the Bonferroni correction for 4 comparisons in 132 patients), falling on day 2 before significantly rising again on day 3 ($P<0.001$, Bonferroni corrected for 4 comparisons in 132 patients).

MR-proANP levels

MR-proANP was raised in patients with death compared to event-free survivors There was no significant difference in MR-proANP levels between anterior or other site of AMI, STEMI vs. NSTEMI. However there was a significantly higher level in females vs. males, patients with a prior history of AMI, patients with a prior history of hypertension, patients who had a prior history of HF or diabetes and higher levels in patients who were not thrombolysed. MR-proANP levels were higher in patients with Killip class above 1. Plasma MR-proANP correlated with age

(r_s=0.59, P<0.0005), eGFR (r_s=-0.54, P<0.0005), Killip class (r_s=0.25, P<0.0005) and NT-proBNP (r_s=0.63, P<0.0005, Figure 2). There were some patients with markedly raised MR-proANP but low NT-proBNP levels. These patients had the same demographic characteristics as the main cohort of patients described but a significantly better eGFR. No difference in outcomes was noted.

NT-proBNP levels

Significant differences in NT-proBNP levels were noted between males and females, those with a Killip class above 1 and in patients with a prior history of HF, hypertension, AMI or diabetes. Plasma NT-proBNP levels were also higher in STEMI vs. NSTEMI patients, and those with anterior site of AMI. Plasma NT-proBNP was correlated with age, eGFR and Killip class. There were some patients with markedly raised NT-proBNP but low MR-proANP levels. These patients had the same demographic characteristics as the main cohort of patients described but a significantly worse eGFR. No difference in outcomes was noted.

Primary Endpoints: MR-proANP and NT-proBNP as predictors of death

When clinical characteristics were entered into a Cox proportional hazards model (Table 2), MR-proANP (HR 3.87) and NT-proBNP (HR 3.25) together with use of beta-blockers and ACE/Angiotensin receptor blockers and age independently predicted the primary endpoint. Past history of hypertension or diabetes, Killip class and eGFR were not predictors.

The area under the receiver-operating-characteristic curve (AUC ROC) for MR-proANP (0.83[95% CI: 0.78-0.87]) and NT-proBNP (0.83[95% CI: 0.78-0.87]) were similar. The AUC for troponin was 0.42 (95% CI: 0.32-0.51, P=NS) and for peak CK 0.40 (95% CI: 0.29-0.50, P=NS).

For prediction of mortality, stratification by NT-proBNP (< or >median) correctly identified 83 endpoints, with an additional 10 identified using stratification by MR-proANP (< or >median). Using MR-proANP levels for risk stratification, 87 endpoints were correctly identified, with an additional 8 identified using stratification by NT-proBNP. Thus, only 6 endpoints were incorrectly identified using both markers.

The Kaplan-Meier survival curves plotting quartiles of MR-proANP or NT-proBNP (figure 3) show that both MR-proANP and NT-proBNP are useful predictors of death post-AMI. Patients in the top quartile for MR-proANP (above 331 pmol/L) had a significantly higher mortality than those in quartiles 1 to 3 ($P<0.0001$ for all). In patients stratified by NT-proBNP in the highest quartile (median 5934 pmol/L), MR-proANP in the highest quartile gave additional information on death in those patients who had NT-proBNP level above the highest quartile (log rank test χ^2 for linear trend of factor levels, pooled over NT-proBNP strata, 14.47, $P=0.0001$, figure 4). MR-proANP in the top quartile had predictive value in those patients in the lower three quartiles of NT-proBNP (log rank test χ^2 39.28, $P<0.0001$). For NT-proBNP in the lower three quartiles, those patients in the top quartile of MR-proANP had higher event rates than those in quartile 1 ($P<0.0001$), 2 ($P<0.0001$) and 3 ($P=0.0004$). Patients can therefore be classified into low (both markers<lowest quartile), intermediate (either marker> highest quartile) or high-risk (both markers>highest quartile) groups (log rank for trend, $P<0.0005$).

Echocardiographic substudy

Echocardiographic parameters were available for 584 subjects (59.4%) for the index admission. Plasma MR-proANP and NT-proBNP were elevated in patients with impaired LV systolic function. In this subgroup, there were 54 deaths. Cox modelling analysis of clinical and biomarker variables with echocardiographic presence of impaired LV systolic function (biplanar ejection fraction as a continuous variable) revealed both biomarkers MR-proANP and

NTproBNP along with use of beta-blockers, age and ejection fraction, as significant independent predictors of death (Table 2).

Secondary Endpoints: MR-proANP and NT-proBNP as predictors of heart failure or recurrent myocardial infarction as individual endpoints

MR-proANP and NT-proBNP levels were significantly higher in patients who were readmitted with HF compared to event-free survivors. Cox modelling revealed the following independent significant predictors; MR-proANP, past history of diabetes, and Killip class. Kaplan-Meier analysis revealed a lower readmission rate for HF in those in the lower three quartiles of MR-proANP ($P<0.0001$) and the highest HF readmission rates in those with both biomarkers elevated in the highest quartile ($P<0.0005$).

Compared to survivors with no endpoints, patients who had recurrent AMI, had similar NT-proBNP and MR-proANP levels.

Discussion

Our data indicated that, NT-proBNP and MR-proANP are powerful predictors of death after MI. The combination of markers from the A and B type natriuretic peptide systems gives added prognostic information above existing clinical characteristics thus enabling patients to be stratified into low, intermediate or high-risk groups.

Risk stratification at an early stage after AMI remains important and may be useful in helping to select treatment regimes in the future. ROC curve analysis indicated that NT-proBNP and MR-proANP were of similar accuracy in prediction of death, more so than markers of structural myocardial damage such as troponin or peak CK. Even though both markers are targeting the natriuretic hormone pathway they are clearly giving additive and complementary information. Kaplan-Meier analysis revealed MR-proANP was useful irrespective of whether NT-proBNP was high or low. A raised MR-proANP and NT-proBNP in the highest quartile was particularly

useful in defining a high-risk group of patients. Multimarker strategies for outcome post-AMI using biomarkers that integrate different pathways have been utilised before.[17] However here we show that complementary information can be gained by targeting different biomarkers of the 2 natriuretic peptide neurohormonal systems.

The complementary information provided by MR-proANP and NT-proBNP may partly be due to the different secretion patterns of both markers. There is a clear difference in secretion profile post-AMI of both markers, with MR-proANP peaking on day 1 as compared to the NT-proBNP peak by day 2. Also NT-proBNP levels appear to be more dependent on renal function than MR-proBNP levels. Patients with markedly raised MR-proBNP but low NT-proBNP levels had significantly greater eGFR values and vice versa; this however did not have an impact on outcomes. These are the only identifiable differences between the two markers, similarities between the two being elevated levels in females compared to males and a strong correlation with eGFR (a surrogate marker of renal function) and left ventricular systolic dysfunction. In the subset with echocardiography data, both biomarkers remained independent predictors of poor outcome. Our data is consistent with previous analyses (eg with ejection fraction and a single marker NT-proBNP [6]) which have retained imaging parameters in predicting similar outcomes. MR-proBNP has been investigated in patients after an acute myocardial infarction where it has also been shown to be comparable to NT-proBNP for the detection of impaired left ventricular function [18]. This study unlike ours is a small investigation in post AMI patients at a remote timepoint after the acute event (mean follow-up 687 days).

The benefit of measuring both prohormones over their bioactive peptides include the lack of receptor binding or protein interactions and the longer half-lives resulting in higher plasma levels. The prohormones are also more stable in blood ex-vivo, and this makes them generally more applicable in clinical practice[11]. Previous studies with ANP or NTproANP have not revealed independent predictive value of the A-type over the B-type natriuretic peptide

systems[5,9]. This is in contrast to what we have found and this could be attributed to performance of different assays. In particular, assays directed to the N- and C-terminals of proANP may be more susceptible to endogenous proteases, providing the rationale for measuring mid-regional epitopes in the current MR-proANP assay[11]. There is evidence of much heterogeneity in molecular forms of both NT-proBNP and NT-proANP, with evidence that midregional epitopes may be relatively preserved in plasma samples[19].

Natriuretic peptides in the post-AMI period probably have a beneficial effect causing vasodilatation and increasing diuresis at a time when the ventricle has sustained a significant insult. The current findings confirm previous studies about the benefits of measuring the natriuretic peptides after an AMI and suggest that a combination marker approach may be more specific at identifying higher risk group of patients associated with poor outcome after AMI.

There are some limitations which should be mentioned. This was a single centre study with a preponderance of relatively higher risk ST elevation AMI patients, so that cut-points for non-ST elevation AMI may need to be independently established. Although we used very minimal exclusion criteria there was a disproportionately higher number of STEMI than NSTEMI. This may in part be due to the admission policies which naturally exist where patients with STEMI are more likely to be admitted to a coronary care unit than NSTEMI. Our study employed blood samples in the recovery phase of AMI, and the utility of initial triage blood samples should be investigated. Finally, although both markers predicted 95 of the 101 events, 6 remaining events eluded prediction.

Conclusion

This is the first report showing MR-proANP to be a new prognostic marker of death in patients with AMI, independent of established conventional risk factors. A multimarker approach with

MR-proANP and NT-proBNP targeting both the A and B-type natriuretic neurohormonal pathways is more informative than either marker alone and may be useful for risk stratification in AMI patients.

Conflicts of Interest Disclosures

S.Q. Khan, O. Dhillon, D. Kelly, I. Squire, P.A. Quinn, J.E. Davies have no disclosures. L.L.Ng has submitted patents on behalf of the University of Leicester on biomarkers of cardiac disease. A Bergmann holds ownership in BRAHMS AG, patent rights to the MR-proANP assay and is a member of the board of directors of BRAHMS AG. J Struck holds patent rights to the MR-proANP assay and is an employee of BRAHMS AG. N Morgenthaler is an employee of BRAHMS AG.

BRAHMS is a mid-sized company, based in Hennigsdorf, Germany, commercializes immunoassays and has developed the MR-proANP assay, for which it owns patent rights.

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Legends

Figure 1a. Patient plot for 60 patients

Changes in plasma MR-proANP and NT-proBNP in 60 individual patients within the first 5 days post-AMI.

Figure 1b. MR-proANP and NT-proBNP box plots

Median and interquartile range of plasma peptide levels in the 132 patients with serial sampling data.

Figure 2. Scatter diagram

Spearman correlation between MR-proANP and NT-proBNP, r_s=0.63.

Figure 3. Kaplan-Meier Survival Curves

Event rate (death) in patients grouped according to quartiles of plasma MR-proANP or NT-proBNP (1=lowest quartile, 4=highest quartile).

Figure 4. Combined Kaplan-Meier Survival Curve

MR-proANP levels (highest quartile vs. lower three quartiles) predicting the primary endpoint of death, in patients stratified by NT-proBNP (highest quartile vs. lower three quartiles).

Table 1: Characteristics of Patients in the Study. Values are medians [range] or numbers (percentage).

(eGFR, estimated glomerular filtration rate, STEMI, ST-segment elevation myocardial infarction, PCI, percutaneous coronary intervention, MR-proANP , midregional Pro-atrial natriuretic peptide, NT-proBNP, N-terminal pro-B-type natriuretic peptide)

	AMI Patients
Number	983
Age (in years)	66 [24-95]
Male Gender	720
eGFR (ml/min/1.73m^2 surface area)	68.4 [14.9-166.1]
NT-proBNP (pmol/L)	907.4 [0.3-28886.8]
MR-proANP (pmol/L)	117.0 [4.9-1210]
Previous Medical History	
Angina Pectoris (%)	251 (25.5)
Myocardial infarction (%)	165 (16.8)
Hypertension (%)	430 (43.7)
Diabetes Mellitus (%)	213 (21.7)
Heart Failure (%)	57 (5.8)
Hypercholesterolaemia (%)	227 (23.1)
STEMI (%)	783 (79.7)
Revascularization (fibrinolysis)	580/783 (79.1)
Revascularization (PCI)	122 (12.4)
Cardiogenic shock	10 (1.0)
Current smokers/Ex-smokers	617 (62.8)

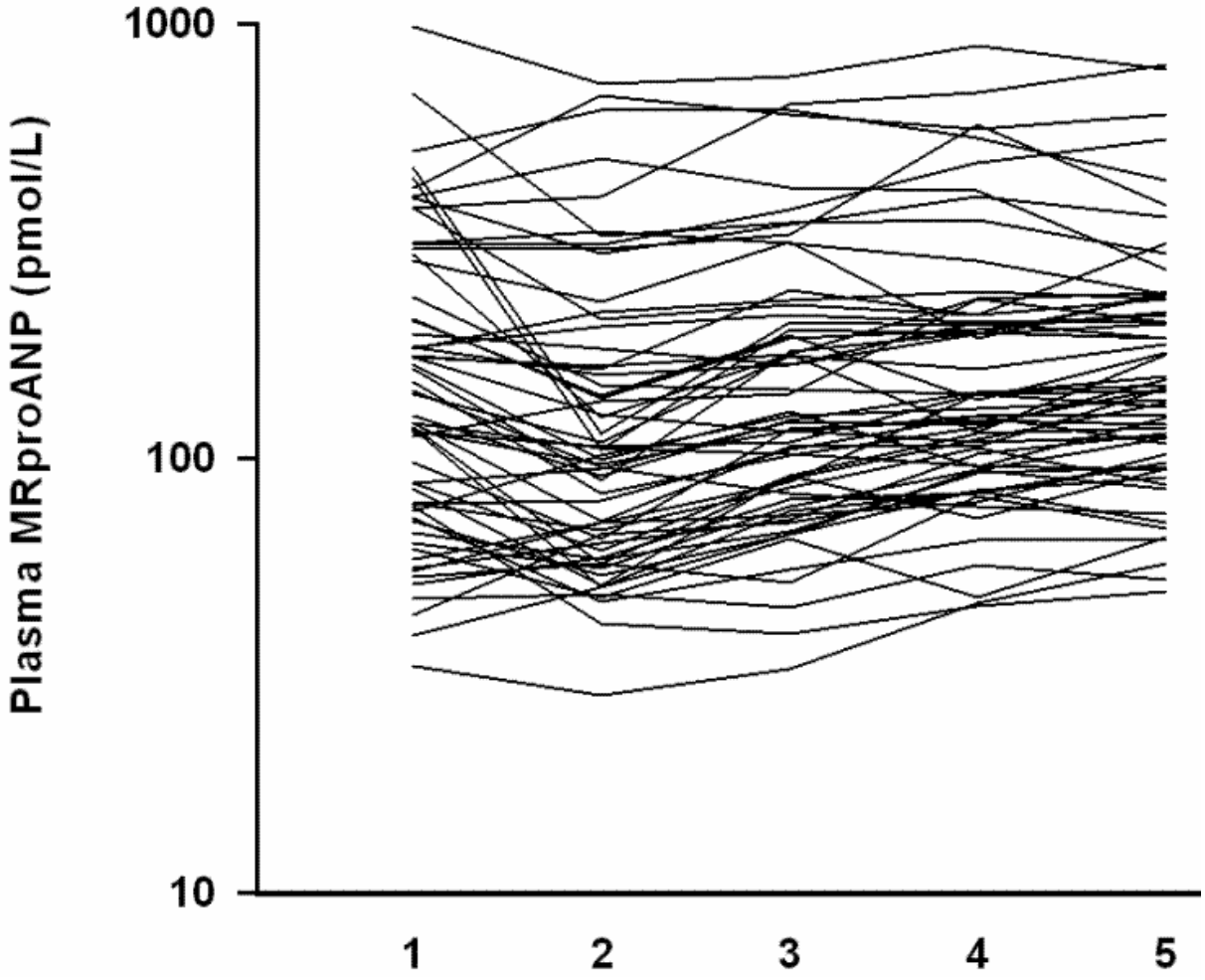
Table 2. Cox regression analysis for death post-AMI. Multivariate analysis results are reported for the whole group and for the subgroup with echocardiography data (N=584)

(eGFR, estimated glomerular filtration rate, MR-proANP , midregional Pro-atrial natriuretic peptide, NT-proBNP, N-terminal pro-B-type natriuretic peptide, CK, creatine kinase, NA, not applicable)

	Univariate Analysis		Multivariate Analysis (Whole group)		Multivariate Analysis (Echocardiography subgroup)	
	Hazard Ratio [95% CI]	P value	Hazard Ratio [95% CI]	P value	Hazard Ratio [95% CI]	P value
Age	1.10 [1.08-1.12]	<0.0005	1.04 [1.02-1.07]	0.002	1.05 [1.01-1.09]	0.009
Male Gender	0.47 [0.32-0.70]	<0.0005	0.69 [0.44-1.08]	0.11	0.75 [0.41-1.38]	0.35
Previous History of :-						
AMI	2.52 [1.66-3.80]	<0.0005	1.44 [0.93-2.44]	0.10	1.02 [0.56-1.87]	0.94
Heart Failure	1.64 [0.85-3.15]	0.14	NA	NA	NA	NA
Hypertension	1.57 [1.06-2.32]	0.024	1.17 [0.77-1.77]	0.46	1.54 [0.87-2.72]	0.13
Diabetes mellitus	1.74 [0.95-2.26]	0.082	1.16 [0.73-1.85]	0.52	0.95 [0.50-1.82]	0.87
Hypercholesterolaemia	0.91 [0.57-1.48]	0.716	NA	NA	NA	NA
Smoking	0.69 [0.46-1.02]	0.063	1.19 [0.75-1.88]	0.46	1.01 [0.54-1.87]	0.99
Anterior AMI	1.01 [0.78-1.31]	0.93	NA	NA	NA	NA
ST-elevation AMI	1.06 [0.66-1.70]	0.82	NA	NA	NA	NA
Thrombolytic use	0.58 [0.39-0.86]	0.007	1.11 [0.72-1.71]	0.63	1.04 [0.58-1.86]	0.91
Killip class > 1	2.50 [1.63-3.83]	<0.0005	0.93 [0.58-1.48]	0.75	1.44 [0.74-2.93]	0.32
Use of beta blockers	0.29 [0.20-0.43]	<0.0005	0.44 [0.49-0.67]	0.001	0.46 [0.20-0.80]	0.006
Use of ACE/Angiotensin receptor blockers	0.62 [0.41-0.92]	0.016	0.63 [0.41-0.96]	0.034	0.77 [0.42-1.42]	0.40
Log NT-proBNP	8.45 [5.35-13.35]	<0.0005	3.25 [1.89-5.89]	<0.0005	3.28 [1.49-7.25]	0.003
Log MR-proANP	22.54 [12.82-69.81]	<0.0005	3.87 [1.51-9.93]	0.005	3.34 [1.03-10.84]	0.044
eGFR	0.95 [0.94-0.96]	<0.0005	0.99 [0.98-1.01]	0.65	1.00 [0.98-1.02]	0.95

Peak CK	1.01 [0.97-1.03]	0.87	NA	NA	NA	NA
Ejection Fraction	2.83 [1.89-4.22]	<0.0005			1.99 [1.10-3.59]	0.023

Figure 1a



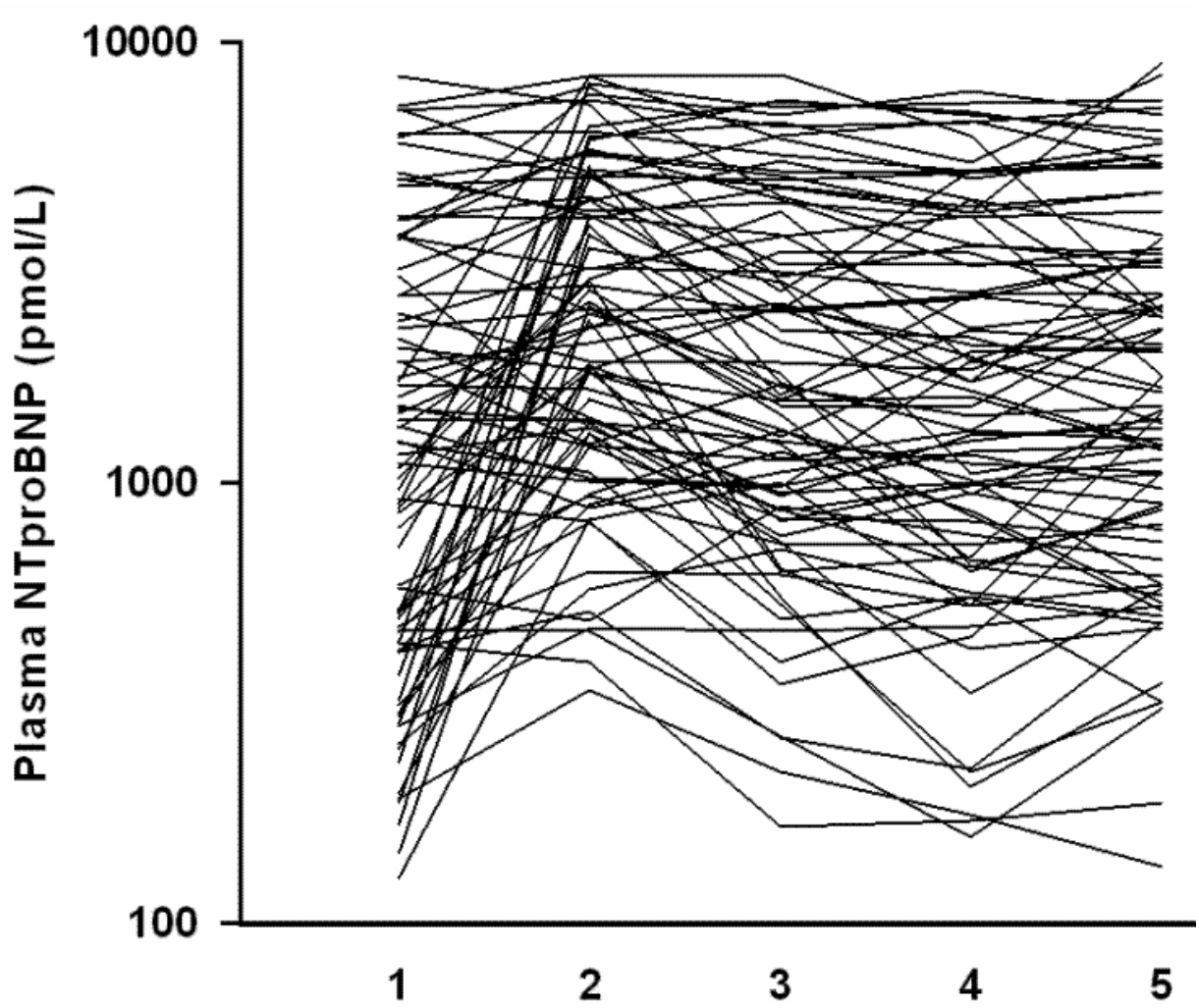

10000
1000
100
Plasma NTproBNP (pmol/L)
1
2
3
4
5

Figure 1b

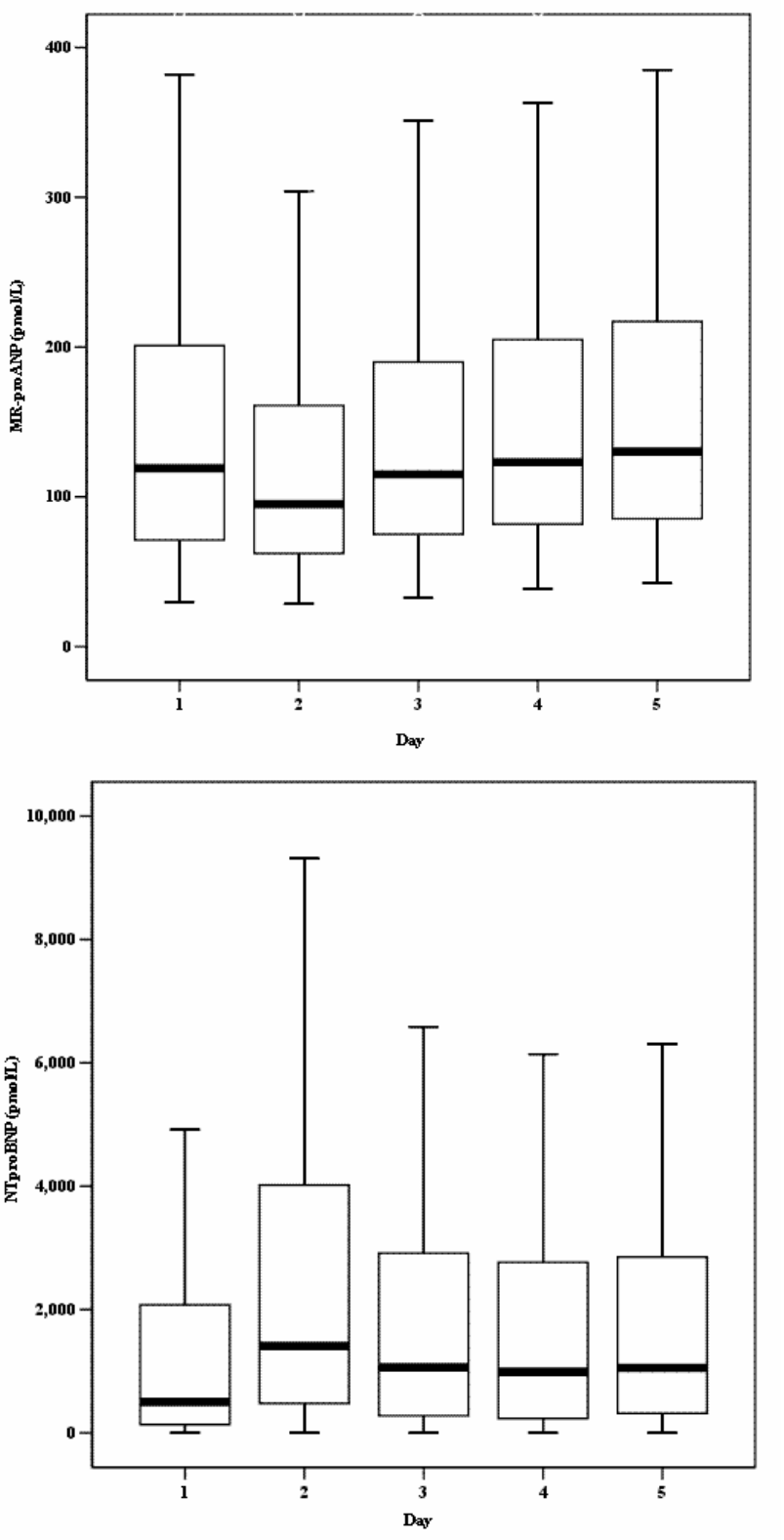

MR-proANP (pmol/L)
400
300
200
100
0
1
2
3
4
5
Day
NTproBNP (pmol/L)
10,000
8,000
6,000
4,000
2,000
0
1
2
3
4
5
Day

Figure 2

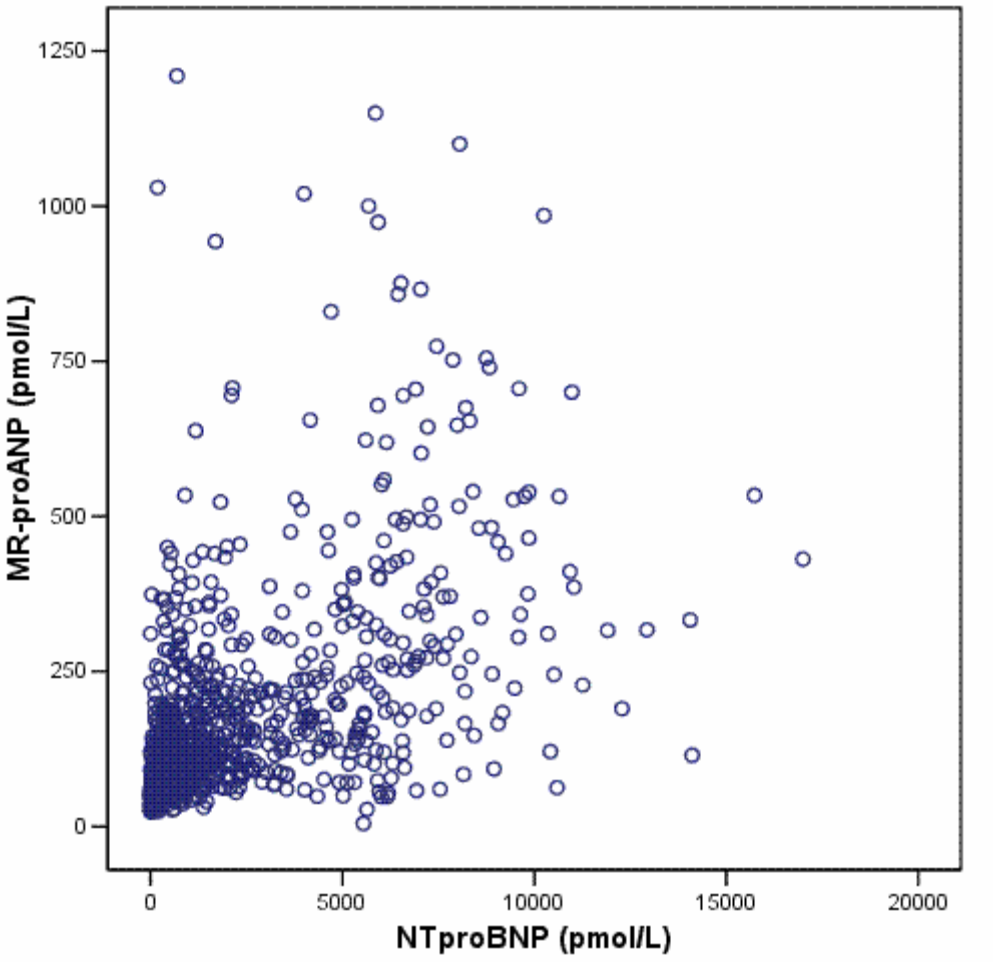

MR-proANP (pmol/L)
1250
1000
750
500
250
0
0
5000
10000
15000
20000
NTproBNP (pmol/L)

Figure 3a

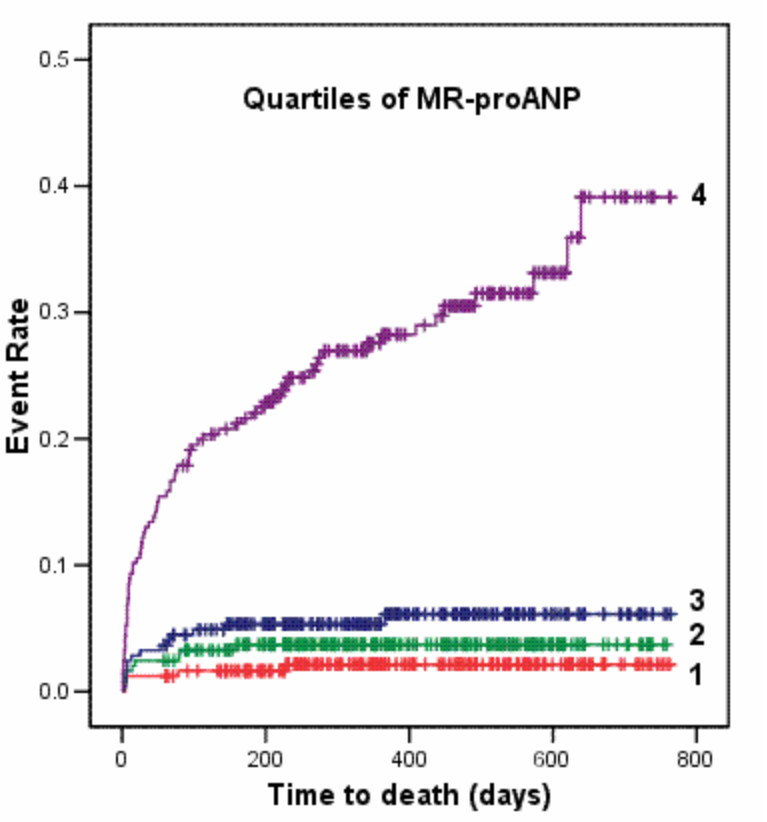

Quartiles of MR-proANP
Event Rate
0.5
0.4
0.3
0.2
0.1
0.0
0
200
400
600
800
Time to death (days)
4
3
2
1

Figure 3b

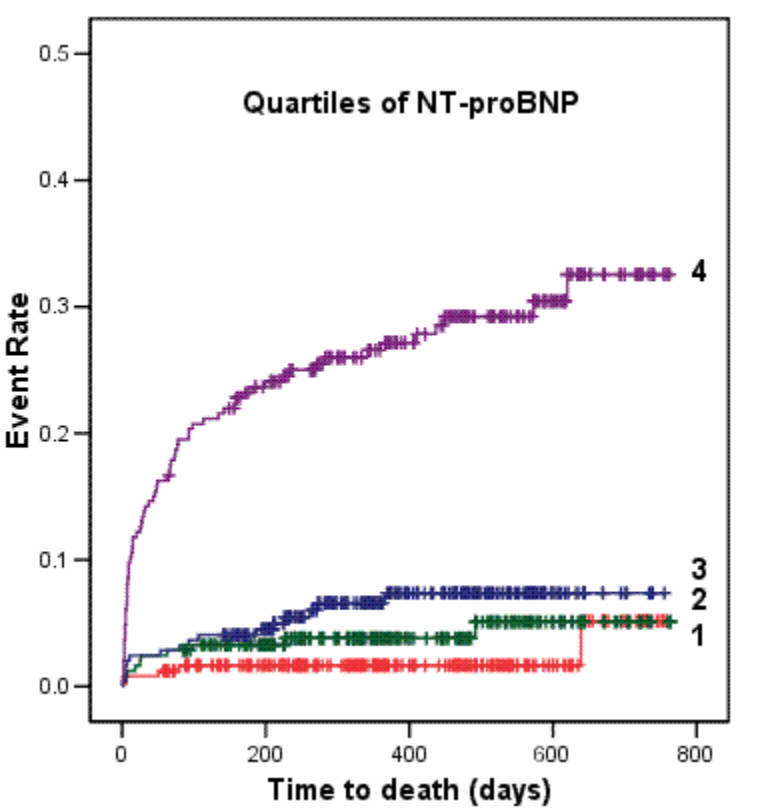
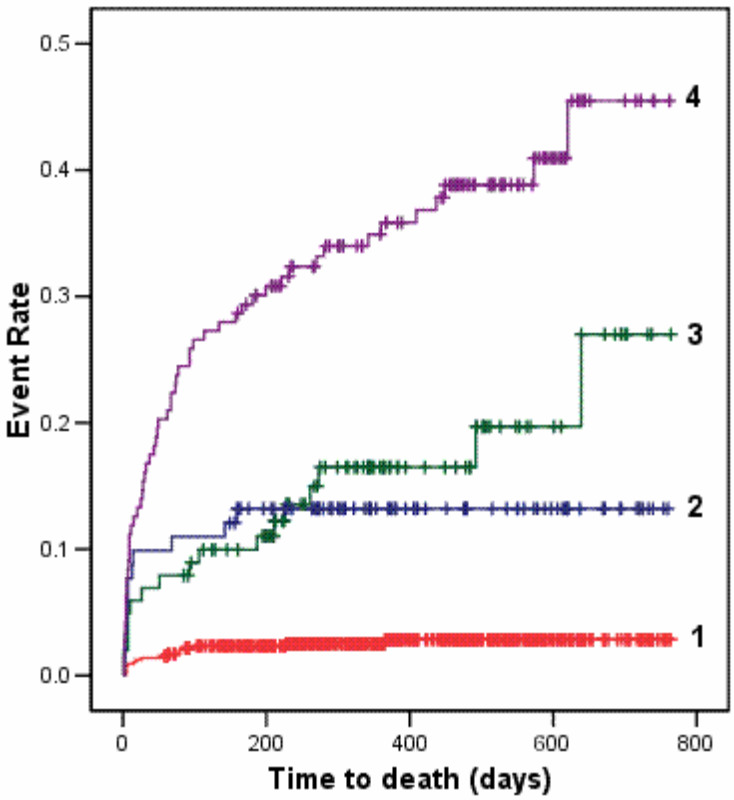

Quartiles of NT-proBNP
Event Rate
0.5
0.4
0.3
0.2
0.1
0.0
0
200
400
600
800
Time to death (days)
4
3
2
1

Figure 4



NT-proBNP	MR-proANP	Group
<lower three quartiles	<lower three quartiles	1
<lower three quartiles	>highest quartile	2
>highest quartile	<lower three quartiles	3
>highest quartile	>highest quartile	4