# C-terminal Pro-Endothelin-1 Offers Additional Prognostic Information in Patients After Acute Myocardial Infarction. Leicester Acute Myocardial Infarction Peptide (LAMP) Study

**Sohail Q. Khan, MRCP, Onkar Dhillon, MRCP, Joachim Struck*, PhD, Paulene Quinn, MS, Nils G. Morgenthaler*, PhD, Ian B. Squire, MD, Joan E. Davies, PhD, Andreas Bergmann*, PhD, Leong L. Ng, MD**

University of Leicester

Department of Cardiovascular Sciences

Clinical Sciences Building

Leicester Royal Infirmary

Leicester LE2 7LX, UK

*Research Department

B·R·A·H·M·S Aktiengesellschaft

Neuendorfstr. 25

D-16761 Hennigsdorf

Germany

**Corresponding author:** Dr Sohail Q. Khan

Department of Cardiovascular Sciences

Clinical Sciences Building

Leicester Royal Infirmary

Leicester, LE2 7LX, UK

Phone:+1162523132 ; fax:+1162523108; e-mail:sqk1@le.ac.uk

Word count: 4765

**Short Title:** CT-proET-1 and prognosis in AMI

**Conflicts of Interest:**

A Bergmann holds ownership in BRAHMS AG, patent rights to the markers of the study and is a member of the board of directors of BRAHMS AG.

J Struck holds patent rights to the markers 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 CT-proET-1 assay, for which it owns patent rights.

This study was not financed by BRAHMS AG.

**Abstract**

**Background** Endothelin-1 is elevated in heart failure (HF) and after acute myocardial infarction (AMI) and gives prognostic information on mortality. Another part of its precursor, C-terminal Pro-Endothelin-1 (CT-proET-1), is more stable in circulation and ex-vivo. We investigated the cardiovascular prognostic value post-AMI of CT-proET-1 and compared it to N-terminal B-type natriuretic peptide (NTproBNP), a marker of death and HF. **Methods** We measured plasma CT-proET-1 and NTproBNP in 983 consecutive post-AMI patients (721 men, mean age 65.0±(SD)12.2 years), 3-5 days after chest pain onset. **Results** There were 101 deaths and 49 readmissions with HF during follow up (median 343, range 0-764 days). CT-proET-1 was raised in patients with death or HF compared to survivors (median [range]pmol/L, 119.0[14.0-671.0] vs. 73.0[4.6-431.0]; $p<0.0001$). Using a Cox proportional hazards logistic model, log CT-proET-1 (HR 6.82) and log NTproBNP (HR 2.62) were significant independent predictors of death or HF (along with age, gender, past history of AMI and therapy with beta blockers). The areas under the receiver-operating curve (AUC) for CT-proET-1, NTproBNP and the logistic model with both markers were 0.76, 0.76 and 0.81 respectively. Findings were similar for death and HF as individual endpoints. **Conclusion** The endothelin system is known to be activated post-AMI. CT-proET-1 is a powerful predictor of adverse outcome along with NTproBNP. CT-proET-1 may represent a clinically useful marker of prognosis after AMI.

**Keywords** Myocardial infarction; heart failure; peptides; Pro-Endothelin; N-terminal pro B type natriuretic peptide; prognosis

**Introduction**

Acute myocardial infarction (AMI) remains a challenging condition and prognosis of patients varies. Prognostic information and the identification of patients at high risk of adverse events can be gauged to some extent from clinical and biochemical data. Amongst the biochemical information that may offer useful prognostic information is natriuretic peptide levels such as N-terminal pro B type natriuretic peptide (NTproBNP) which provides information regarding the risk of death and heart failure following AMI.[1] The prognostic value of this biomarker compared to clinical features has been borne out in a range of acute coronary syndromes.[2] Complementary information can however be obtained in a multi-marker strategy particularly with NTproBNP. Here we investigate C-terminal Pro-Endothelin-1 (CT-proET-1) as a potential peptide in a multi-marker strategy. Endothelin-1 is a well known 21 amino acid potent vasoconstrictor peptide which was originally isolated from vascular endothelial cells.[3] Subsequently ET-1 has been found in smooth muscle, renal and pulmonary cells.[4]

ET-1 exerts its vascular effects by activation of the ET(A) and ET(B) receptors on smooth muscle cells which cause an increase in cellular calcium. [5] ET-1 is synthesized as part of a larger precursor molecule, termed preproendothelin-1. ET-1 is difficult to measure because of its instability and it's binding to receptors and plasma proteins. [6,7]

However an immunoluminometric assay for the measurement of the C-terminal endothelin-1 precursor fragment in human plasma has been reported which indirectly estimates activity of the endothelin system. [8] This breakdown fragment is more stable than the active molecule and is presumably inactive in the circulation. [9] The biological activity of ET-1 in the cardiovascular system is varied. It has been implicated in the development of hypertension, [10] chronic heart failure [11] and myocardial infarction, [12] including unstable angina and non q-wave myocardial infarction. [13]

Interestingly plasma ET-1 is increased in AMI, in proportion to the severity of the disease[12]

Plasma ET-1 has been investigated previously in a small study as a prognostic marker where it was found to be an independent predictor of long-term mortality independent of clinical variables.[14] The potential utility of the more stable prohormone fragment CT-proET-1 in prognostication after AMI is unknown.

We postulated that plasma CT-proET-1 and NTproBNP would be useful in determining the prognosis following AMI, particularly for predicting death and heart failure, and that CT-proET-1 would have incremental value for risk stratification to NTproBNP, a biomarker with established prognostic value in post AMI patients. [1,15,16]

## Methods

### Study population

We studied 983 consecutive acute myocardial infarction patients admitted to the Coronary Care Unit of Leicester Royal Infirmary. The study complied with the Declaration of Helsinki and was approved by the local ethics committee; written informed consent was obtained from patients. 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. .[17] AMI was sub-categorised into ST segment elevation myocardial infarction (STEMI) or non-ST segment myocardial infarction (NSTEMI). The estimated GFR (eGFR) of these subjects was calculated from the simplified formula derived from the Modification of Diet in Renal Disease (MDRD) study, recently validated in patients with HF. [18] Exclusion criteria were known malignancy, or surgery in the previous month.

**Plasma samples**

Blood samples were drawn on one occasion 3 to 5 days after the onset of chest pain (patients who did not have chest pain were included time zero was set as admission time) for determination of plasma CT-proET-1 and NTproBNP. 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, blood sampling was performed daily for 5 days from admission to discharge.

**NTproBNP assay**

Our NTproBNP assay was based on a non-competitive assay as previously published.[2] Sheep antibodies were raised to the N-terminal of human NTproBNP and monoclonal mouse antibodies were raised to the C- terminal. The N-terminal IgG was affinity-purified and biotinylated. Samples or NTproBNP 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.

**CT-proET-1 assay**

CT-proET-1 was detected using a novel commercial assay in the chemiluminescence/ coated tube-format (BRAHMS AG), as described.[8] Briefly, tubes were coated with a purified sheep polyclonal antibody raised against a peptide representing amino acids 168–181 of pre-proET-1 (see figure 1). A purified sheep polyclonal antibody raised against a peptide representing amino acids 200–212 of pre-proET-1 was labelled with MACN-Acridinium-NHS-Ester (InVent GmbH, Germany) and used as tracer. Dilutions of a peptide representing amino acids 169–212 of pre-proET-1 in normal horse serum served as standards. The immunoassay was performed by

incubating 50 µl of samples/standards and 200µl tracer in coated tubes for 2 h at room temperature. Tubes were washed 4 times with 1 ml of LIA wash solution (B.R.A.H.M.S AG), and bound chemiluminescence was measured using a LB952T luminometer (Berthold, Germany).

**End points**

We assessed the value of both CT-proET-1 and NTproBNP for the prediction of the combined primary endpoint of death and hospitalization for heart failure and for death or hospitalization for heart failure as individual secondary endpoints. Hospitalization for heart failure was defined as a hospital admission for which heart failure was the primary reason. 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). The 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 with repeated measures, with correction for multiple comparisons using the Bonferroni method. To test the independent predictive power for death or heart failure of peptide levels Cox proportional hazards analyses were conducted. We included as variables baseline patient characteristics (age, gender, eGFR, Killip class, territory of AMI, past history of myocardial infarction or heart failure, therapy with ACE inhibitors, angiotensin receptor blockers, beta-blockers, and peptide markers CT-proET-1 and NTproBNP). Cox models were always constructed with the same variables entered simultaneously (which included variables statistically significant in univariate analyses and those variables that may have an effect on the end point on the basis of previous studies).

Levels of NTproBNP and CT-proET-1 were normalised by log transformation. Thus hazard ratios refer to a tenfold rise in the levels of these markers. Spearman’s correlations were

performed for peptide values and continuous variables. To compare the accuracy of NTproBNP and CT-proET-1 receiver-operating characteristic (ROC) curves were generated and the area under the curves (AUC) was calculated. Kaplan Meier survival curves were generated to visualise the relationship between the peptides NTproBNP and CT-proET-1 and the primary and secondary endpoints. The Mantel-Cox log rank test was used to assess the significance of the stratification using medians of CT-proET-1 (and log rank tests for linear trend of factor levels for stratification using ordered quartiles of CT-proET-1) dichotomised according to NTproBNP median levels. A two-tailed p value of less than 0.05 was deemed to be statistically significant. 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

The demographic features of the patient population are shown in Table 1. Median range of follow-up was 343 days ranging from 0–764 days. No patient was lost to follow-up and the minimum length of follow-up for survivors was 60 days, enabling a censored primary end-point of death or HF to be determined at this time point for ROC analysis. During follow-up, 101 (10.3%) patients died and 49 (5.0%) were readmitted with heart failure. In 783 patients, the AMI was a STEMI event.

### Plasma profile of CT-proET-1 and NTproBNP

Daily blood samples were obtained for 5 days post-admission in a subgroup of 132 patients (102 male, median age range] 64 [32-90] years), 16 of whom subsequently experienced the primary endpoint of death or heart failure. Figure 2a illustrates the time course of plasma NTproBNP showing significant changes with day of sampling ($p<0.001$), with peak levels on day 2 ($p<0.001$ and 0.02 compared to day 1 and day 3 respectively using the Bonferroni correction). In contrast,

the plasma CT-proET-1 rose to peak on day 2 (significantly elevated compared to day 1, p<0.001 using the Bonferroni correction), before a plateau was reached between days 3-5 (figure 2b).

**CT-proET-1 levels in patients**

Plasma levels of CT-proET-1 in patients with AMI ranged from 4.63- 671.0 pmol/L with a median of 71.0 pmol/L. CT-proET-1 was higher in patients who died (p<0.0001) or were readmitted with heart failure (p<0.0001) compared to event free survivors. Levels were higher in females compared with males (p<0.0001), in patients with history of prior AMI (p<0.0001), hypertension (p<0.0001), those with a Killip class above 1 (p<0.0001) and in patients with prior history of heart failure (p<0.0001). CT-proET-1 levels were not significantly different between STEMI and NSTEMI patients. CT-proET-1 was lower in patients who received thrombolytic therapy (p=0.021) (see table 2).

CT-proET-1 correlated with age ($r_s$= 0.390, p< 0.0001), eGFR ($r_s$ = -0.481, p< 0.0001) and NTproBNP ($r_s$ =0.548, p< 0.0001).

**NTproBNP levels in patients**

NTproBNP obtained in the plateau phase was higher in patients who died (p<0.0001) or were readmitted with heart failure (p<0.0001). Significant differences in NTproBNP levels were noted between males and females (p<0.0001) and those with Killip class above 1 (p<0.0001) and in patients with a prior history of heart failure (p<0.0001) or AMI (p=0.001) (see table 2).

**Primary Endpoints: CT-proET-1 and NTproBNP as predictors of death and heart failure**

CT-proET-1 was raised in patients with death or heart failure compared to survivors (median [range] pmol/L, 119; [14.0-671.0] vs. 73; [4.63-431]; p<0.0001).

When clinical and demographic characteristics (age, gender, eGFR, Killip class, territory of AMI, past history of myocardial infarction or heart failure therapy with ACE inhibitors, angiotensin

receptor blockers, beta-blockers, and peptide markers CT-proET-1 and NTproBNP) were entered into a Cox proportional hazards model CT-proET-1 (HR 6.82, 95% CI: 3.01-15.42, p<0.0001) and NTproBNP (HR 2.62, 95% CI: 1.80-3.83, p<0.0001) independently predicted the primary endpoint along with age (HR 1.04), gender (HR for male vs. female 0.74), prior history of AMI (HR 1.74) and use of beta blocker (HR 0.69) (Table 3). Killip class and eGFR were no longer independent predictors of death and heart failure. The area under the curves (AUC) of the receiver-operating-characteristic curve for CT-proET-1 of 0.76 (95% CI: 0.71-0.82, p<0.0001) and NTproBNP AUC 0.76 (95% CI: 0.70-0.82, p<0.0001) were similar. The predicted probability from the binary logistic model combining the 2 markers yielded an AUC of 0.81 (95% CI: 0.75-0.86, p<0.0001), which exceeded that of either peptide alone.

The bootstrapped C-statistic with 95% confidence intervals (computed from a bootstrap sample of 3000 repeated 50 times) was 0.75 (95% CI: 0.73-0.76) for NTproBNP and 0.75 (95% CI: 0.74-0.77) for CT-proET-1.

Kaplan-Meier survival analysis confirmed the findings obtained in the Cox regression model and revealed a significantly better clinical outcome in patients with CT-proET-1 below the median (71 pmol/L) compared with those with CT-proET-1 above the median (log rank 54.02, p<0.0001, figure 3). This was also true for NTproBNP (log rank 68.98, p<0.0001, figure 4). In addition there was a grading to the primary end point, which increased as the levels of CT-proET-1 or NTproBNP increased. A positive CT-proET-1 and NTproBNP (ie, both above their respective median values) was associated with a significantly higher rate of the primary end point than having either peptide level above their medians, or both peptides below their medians (log rank 78.26, p<0.0001, figure 5).

**Secondary Endpoints: CT-proET-1 and NTproBNP as predictors of death or heart failure as individual endpoints.**

On Cox proportional hazards modelling the strongest independent predictors of death were NTproBNP (HR 3.59, p<0.0001), age (HR 1.06, p<0.0001), therapy with beta-blockers (HR 0.54, p=0.003) and CT-proET-1 (HR 4.00, p=0.005), the other independent predictors were prior history of AMI (HR 1.48, p=0.069) and therapy with ACE inhibitors or angiotensin receptor blockers (HR 0.76, p=0.059). Such modelling on heart failure readmissions yielded the following independent predictors: CT-proET-1 (HR 5.71, p=0.002), NTproBNP (HR 1.66, p=0.044), Killip class above 1 (HR 2.00, p=0.018), and PMH of HF (HR 3.11, p=0.001). Kaplan-Meier analysis on death or heart failure as individual endpoints revealed a significantly better clinical outcome in patients with CT-proET-1 below the median compared with those with CT-proET-1 above the median (log rank 31.02 and 27.68 respectively, p<0.0001).

**Discussion**

We have shown using survival analysis that CT-proET-1 is a powerful independent predictor of death and heart failure, with combined levels of CT-proET-1 and NTproBNP performing better at identifying patients with the highest risk of an adverse event than either marker alone. The prognostic ability of CT-proET-1 at predicting death or heart failure is independent of well established clinical and biochemical factors including the well established NTproBNP. This has been borne out in the multivariate analyses with Cox regression.

When the combination of CT-proET-1 and NTproBNP was investigated in a multi-marker risk stratification approach this actually generated an increased area under the ROC curve than for either marker individually and greater predictive accuracy. This suggests that the two markers may be giving complementary information after an AMI. Kaplan-Meier analysis revealed a positive CT-proET-1 and NTproBNP was associated with a significantly higher rate of the primary end point than having one raised peptide level or two low levels of peptides.

There are some associations between CT-proET-1 and NTproBNP; both levels increase with age and both show higher levels in females. There is also a strong correlation between the two peptides raising the possibility of release of this peptide from the myocardium itself. Another alternative suggestion may be that the impaired LV systolic function increases left atrial pressures and this increase in pressure is then the stimulus for endothelin release.

A multimarker strategy for post-AMI prognosis using independent biomarkers has been utilised and proposed previously as it provides complementary information through different mechanistic pathways.[19] Our data indicate that individually CT-proET-1 and NTproBNP have similar prognostic utility, however the two markers considered together provide complementary information.

The plasma profile of endothelin has been described previously showing a peak at around 6 hours post AMI before attaining normal levels by 24 hours after chest pain onset. [20] Interestingly those patients who then developed complications continue to get a sustained secretion of endothelin whereas those with an uncomplicated AMI show declining levels. This has also been borne out for unstable angina and non Q-wave myocardial infarction. [13] In contrast, the plasma profile for CT-proET-1 rises to a peak on day 2 before reaching a steady state. This is different to the secretion pattern of both endothelin itself and to NTproBNP which has a biphasic type response.

In a previous study investigating endothelin as a prognostic marker after an AMI it was found to be predictive of 1-year mortality in a small cohort of 142 patients. [14] This study unlike ours however did not investigate the predictive power of endothelin for heart failure.

Endothelin is most likely to have a detrimental effect after an AMI possibly causing extension of the infarct and has been shown to be grossly elevated in patients following cardiogenic shock.[21] Endothelin is known to significantly reduce coronary blood flow [22] and endothelin antagonists reduce the extent of experimental AMI. [23]

We have measured activation of the endothelin system indirectly by measurement of the prohormone over the active peptide endothelin, which is elevated transiently post-AMI. CT-proET-1 is not involved in receptor binding or protein interactions and the longer half-life results in higher easily measurable plasma levels which may act as an integral of activity of the endothelin system.

**Limitations of the study**

This was a single centre study and the results need to be replicated in larger multicentre studies. There was a preponderance of ST elevation AMI, as cut-points for non-ST elevation AMI may need to be independently established. Our study employed blood samples in the recovery phase of AMI, and the utility of initial triage blood samples should be investigated for even earlier stratification of risk post-AMI.

In conclusion this report confirms activation of the ET system post AMI and CT-proET-1 to be a powerful new prognostic marker of death or heart failure in patients with AMI, independent of established conventional risk factors and newer plasma biomarkers such as NTproBNP. A multimarker approach with CT-proET-1 and NTproBNP is more informative than either marker alone and may be useful for risk stratification in AMI patients.

**References**

1) Richards AM, Nicholls MG, Yandle TG, et al. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: new neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. Circulation 1998;97:1921-9.

2) Omland T, Persson A, Ng L, et al. N-terminal pro-B-type natriuretic peptide and long-term mortality in acute coronary syndromes. Circulation 2002;106:2913-18.

3) Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411-15.

4) Inoue A, Yanagisawa M, Kimura S, et al. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci USA 1989;86:2863-67.

5) Haynes WG, Strachan FE, Webb DJ. Endothelin ET(A) and ET(B) receptors cause vasoconstriction of human resistance and capacitance vessels in vivo. Circulation 1995;92:357-63.

6) Rossi GP, Seccia TM, Albertin G, et al. Measurement of endothelin: clinical and research use. Ann Clin Biochem 2000;37:608-26.

7) Brunner F, Stessel H, Watzinger N, et al. Binding of endothelin to plasma proteins and tissue receptors: effects on endothelin determination, vasoactivity, and tissue kinetics. FEBS Lett 1995;373:97-101.

8) Papassotiriou J, Morgenthaler NG, Struck J, et al. Immunoluminometric assay for measurement of the C-terminal endothelin-1 precursor fragment in human plasma. Clin Chem 2006;52:1144-51.

9) Struck J, Morgenthaler NG, Bergmann A. Proteolytic processing pattern of the endothelin-1 precursor in vivo. Peptides 2005;26:2482-86.

10) Kiely DG, Cargill RI, Struthers AD, et al. Cardiopulmonary effects of endothelin-1 in man. Cardiovasc Res 1997;33:378-86.

11) Wei CM, Lerman A, Rodeheffer RJ, et al. Endothelin in human congestive heart failure. Circulation 1994;89:1580-86.

12) Tomoda H. Plasma endothelin-1 in acute myocardial infarction with heart failure. Am Heart J 1993;125:667-72.

13) Wieczorek I, Haynes WG, Webb DJ, et al. Raised plasma endothelin in unstable angina and non-Q wave myocardial infarction: relation to cardiovascular outcome
British Heart Journal 1994;72:436-41.

14) Omland T, Lie RT, Aakvaag A, et al . Plasma endothelin determination as a prognostic indicator of one year after acute myocardial infarction. Circulation 1994;89:1573-9.

15) Omland T, de Lemos JA, Morrow DA, et al. Prognostic value of N-terminal pro-atrial and pro-brain natriuretic peptide in patients with acute coronary syndromes. Am J Cardiol 2002;89:463-5.

16) Squire IB, O'Brien RJ, Demme B, et al. N-terminal pro-atrial natriuretic peptide (N-ANP) and N-terminal pro-B-type natriuretic peptide (N-BNP) in the prediction of death and heart failure in unselected patients following acute myocardial infarction. Clin Sci (Lond) 2004;107:309-16.

17) Alpert JS, Thygesen K, Antman E, et al. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36:959-69.

18) Smilde TD, van Veldhuisen DJ, Navis G, et al. Drawbacks and prognostic value of formulas estimating renal function in patients with chronic heart failure and systolic dysfunction. Circulation 2006;114:572-80.

19) Sabatine MS, Morrow DA, de Lemos JA, et al. Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: simultaneous assessment of troponin I, C-reactive protein, and B-type natriuretic peptide. Circulation 2002;105:1760-63.

20) Stewart DJ, Kubac G, Costello KB, et al. Increased plasma endothelin-1 in the early hours of acute myocardial infarction. J Am Coll Cardiol 1991;18:38-43.

21) Cernacek P, Stewart DJ. Immunoreactive endothelin in human plasma: marked elevations in patients in cardiogenic shock. Biochem Biophys Res Commun 1989;161:562-67.

22) Kurihara H, Yamaoki K, Nagai R, et al. Endothelin: a potent vasoconstrictor associated with coronary vasospasm. Life Sci 1989;44:1937-43.

23) Grover GJ, Dzwonczyk S, Parham CS. The endothelin-1 receptor antagonist BQ-123 reduced infarct size in a canine model of coronary occlusion and reperfusion. Cardiovasc Res 1993;27:1613-18.

**Acknowledgments**

Dr Sohail Q Khan is supported by a British Heart Foundation Junior Research Fellowship (FS/03/028/15486).

**Legends**

Figure 1 Principle of the CT-proET-1 assay. A sketch of the Endothelin-1 precursor is shown, and amino acid positions in prepro-ET-1 are denoted, at which the precursor is proteolytically processed. Tracer- and solid phase-antibodies used in the sandwich immunoassay for the detection of CT-proET-1 are indicated. Signal: signal peptide; ET-1: Endothelin-1; CT-proET-1: C-terminal pro-Endothelin-1.

Figure 2 Time dependent changes (mean ± SE) 5 days post-AMI of plasma NTproBNP (a) and CT-proET-1 (b) (n=132)

Figure 3 Kaplan-Meier Curve: Time to death or heart failure related to plasma CT-proET-1

Figure 4 Kaplan-Meier Curve: Time to death or heart failure related to plasma NTproBNP

Figure 5 Kaplan–Meier curve: Time to death or heart failure related to low or high plasma CT-proET-1 and NTproBNP levels. (1) Low CT-proET-1 and NTproBNP, (2) Low CT-proET-1 and high NTproBNP, (3) High CT-proET-1 and low NTproBNP and (4) High CT-proET-1 and NTproBNP

**Table 1** Characteristics of the 983 patients in the study separated by CT-proET-1 quartiles.

Values are means (SD) or numbers (%)

| | 1st quartile | 2nd quartile | 3rd quartile | 4th quartile | p value |
|---|---|---|---|---|---|
| Age (in years) | 58.9 ± 11.7 | 63.5 ± 11.7 | 65.9 ± 11.3 | 71.7 ± 10.7 | <0.0001 |
| Male Sex | 202 (84.2) | 184 (75.7) | 168 (70.0) | 158 (64.8) | <0.0001 |
| Previous Medical History | | | | | |
| AMI | 26 (10.8) | 40 (16.5) | 38 (15.8) | 60 (24.6) | 0.001 |
| Angina Pectoris | 56 (23.3) | 62 (25.5) | 52 (21.7) | 79 (32.4) | 0.038 |
| Hypertension | 82 (24.2) | 106 (43.6) | 101 (42.1) | 131(53.7) | <0.0001 |
| Diabetes mellitus | 34 (14.2) | 47 (19.3) | 51 (21.3) | 79 (32.4) | <0.0001 |
| High cholesterol | 47 (19.6) | 52 (21.4) | 66 (27.5) | 60 (24.6) | 0.176 |
| Current/Ex-Smokers | 149 (62.1) | 159 (65.4) | 162 (67.5) | 135 (55.3) | 0.030 |
| ST-elevation AMI | 196 (81.7) | 179 (72.8) | 209 (83.8) | 208 (80.3) | 0.284 |
| Thrombolytic | 143 (59.6) | 126 (51.9) | 143 (59.6) | 119 (48.8) | 0.034 |
| Territory of Infarct | | | | | 0.077 |
| Anterior | 105 (43.8) | 102 (42.0) | 102 (42.5) | 102 (41.8) | |
| Inferior | 103 (42.9) | 89 (36.6) | 98 (40.8) | 83 (34.0) | |
| Other | 32 (13.3) | 52 (21.4) | 40 (16.7) | 58 (23.8) | |
| Killip Class on Admission | | | | | <0.0001 |
| I | 162 (67.5) | 132 (54.3) | 114 (46.3) | 84 (34.4) | |
| II | 68 (28.3) | 93 (38.3) | 104 (43.3) | 109 (44.7) | |
| III | 9 (3.8) | 14 (5.8) | 24 (10.0) | 41 (16.8) | |
| IV | 1 (0.4) | 0 (0) | 0 (0) | 9 (3.7) | |

| | | | | | |
|---|---|---|---|---|---|
| Peak CK (IU/L) | 838.5± 794.9 | 895.4 ± 1091.8 | 1261.5 ± 1243.1 | 1273.0 ± 1590.7 | <0.0001 |
| eGFR (ml/min/1.73m$^2$) | 76.9 ± 16.0 | 74.5 ± 16.1 | 67.7 ± 17.5 | 52.7 ± 18.5 | <0.0001 |
| NTproBNP (pmol/L) | 1031.6 ± 2267.7 | 1224.9 ± 1783.0 | 2141.5 ± 2400.5 | 4350.6 ± 3547.1 | <0.0001 |

**Table 2** Table comparing CT-proET-1 and NTproBNP levels in different patient sub-groups

| | Median CT-proET-1 (pmol/L) | p value | Median NTproBNP (pmol/L) | p value |
|---|---|---|---|---|
| Death vs. Survivors | 126.0 vs. 74.0 | p<0.0001 | 5929.3 vs.802.4 | p<0.0001 |
| Admission with HF vs. No HF | 112 vs. 74.9 | p<0.0001 | 3932.9 vs. 839.0 | p<0.0001 |
| Males vs. Females | 73.0vs. 89.0 | p<0.0001 | 788.7 vs. 1632.6 | p<0.0001 |
| Previous AMI vs. No AMI | 85.0 vs. 75.0 | p<0.0001 | 1332.3 vs. 844.4 | P<0.001 |
| Hypertension vs. Normotensives | 84.0 vs. 73.2 | p<0.0001 | 1105.6 vs. 802.4 | p<0.0001 |
| Previous HF vs. No HF | 108.0 vs. 75.9 | P<0.0001 | 4160.1 vs. 856.6 | P<0.0001 |
| STEMI vs. NSTEMI | 78.1 vs. 72.9 | p=NS | 1017.9 vs. 624.6 | P<0.002 |
| Killip class above 1 vs. Killip class 1 | 88.9 vs. 70.0 | p<0.0001 | 1595.1 vs. 631.5 | p<0.0001 |

**Table 3** Multivariate Cox proportional hazards regression model of significant predictors of death or heart failure. Variables entered into the regression model included age, gender, territory of AMI, past history myocardial infarction (PMH AMI), past history of heart failure (PMH HF), use of thrombolysis, Killip class>1, eGFR, use of beta blockers or ACE inhibitors/Angiotensin receptor blockers, log NTproBNP, log CT-proET-1

| Variable | Hazard Ratio | 95% CI | p value |
|---|---|---|---|
| Log CT-proET-1 | 6.82 | 3.01-15.42 | <0.0001 |
| Log NTproBNP | 2.62 | 1.80-3.83 | <0.0001 |
| Age | 1.04 | 1.02-1.06 | <0.0001 |
| PMH of AMI | 1.74 | 1.22-2.47 | 0.002 |
| Use of beta blockers | 0.69 | 0.49-0.98 | 0.049 |
| Gender | 0.74 | 0.52-1.04 | 0.081 |

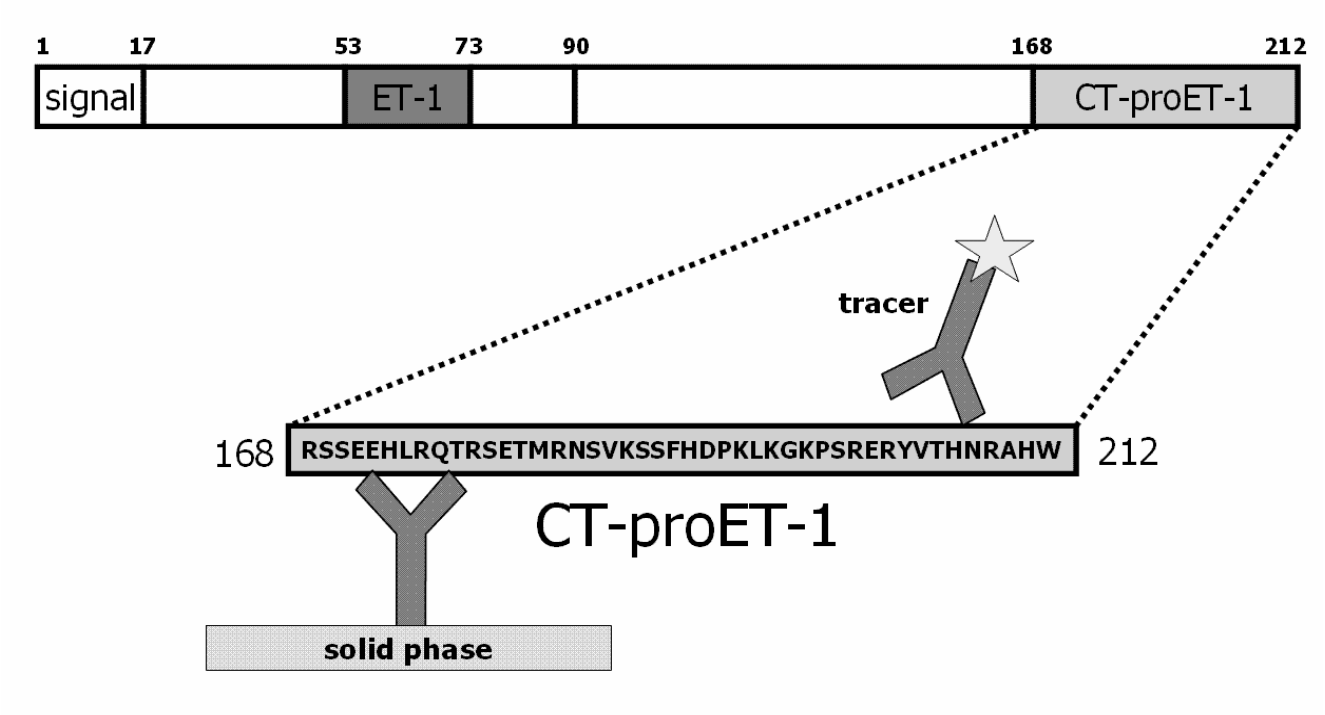
1
17
53
73
90
168
212
signal
ET-1
CT-proET-1
tracer
168
RSSEEHLRQTRSETMRNSVKSSFHDPKLKGKPSRERYVTHNRAHW
212
CT-proET-1
solid phase

**Figure 1**

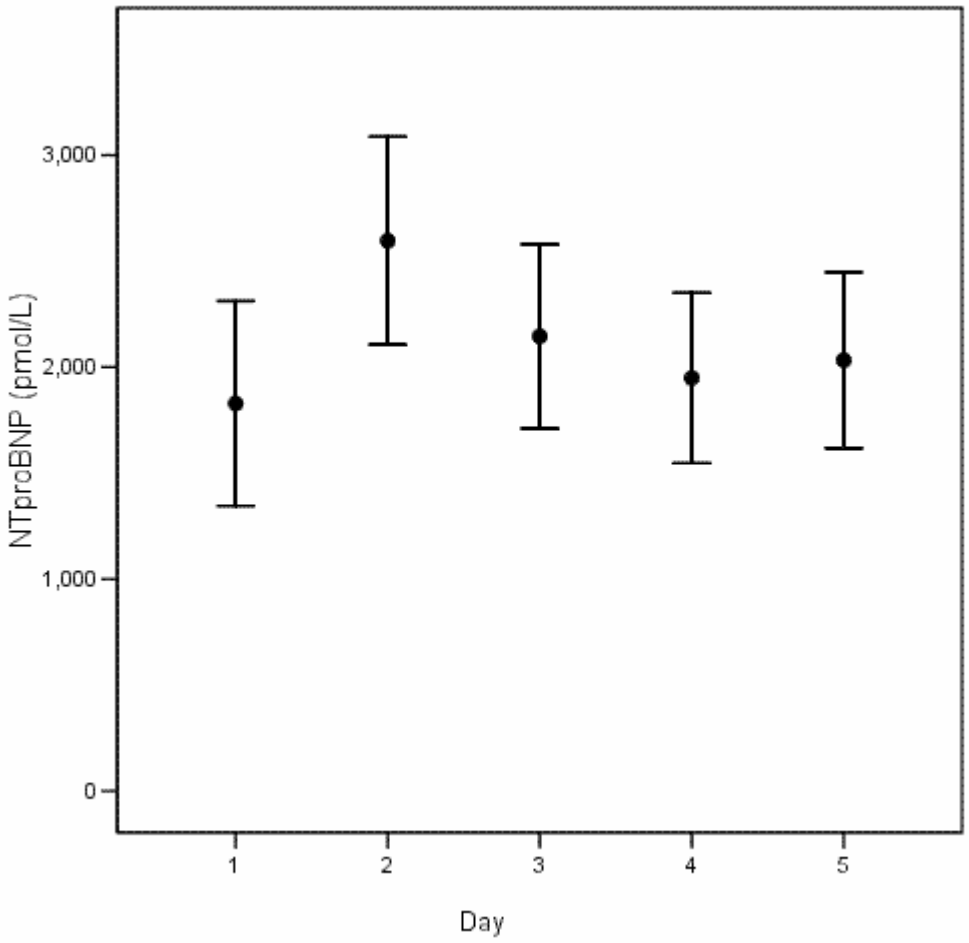


**Figure 2a**

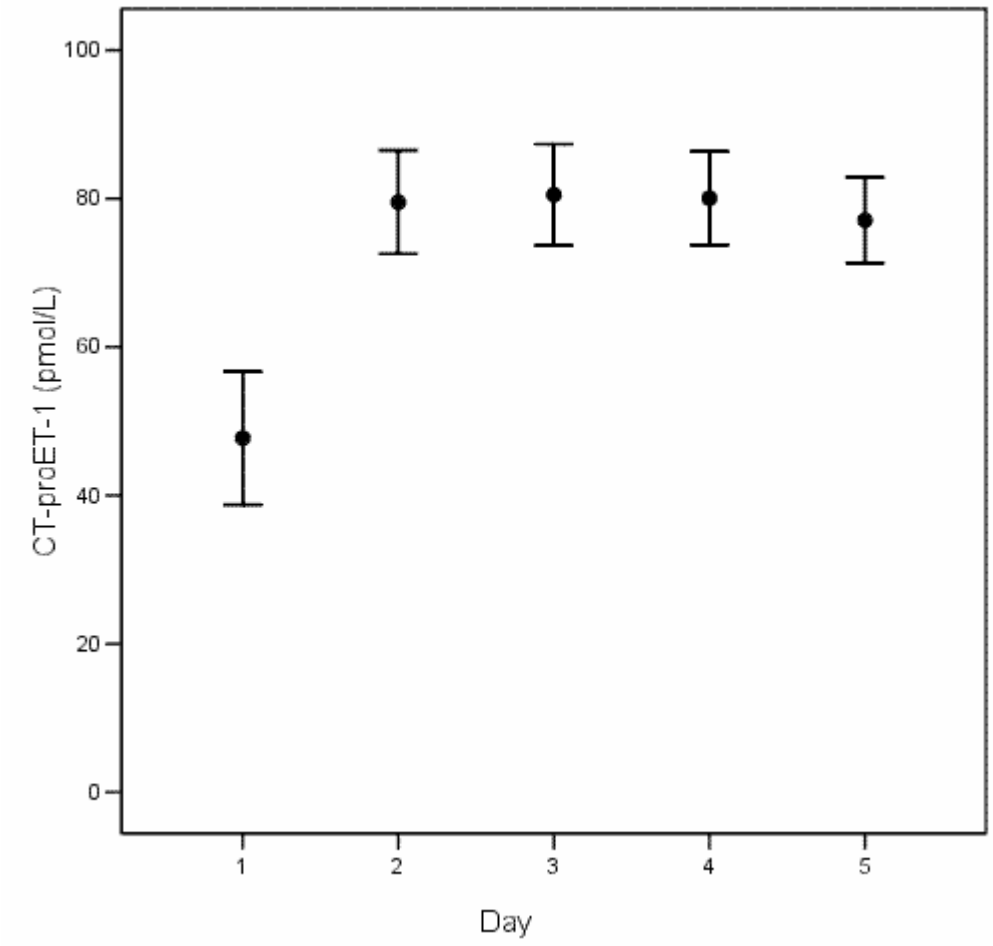


**Figure 2b**

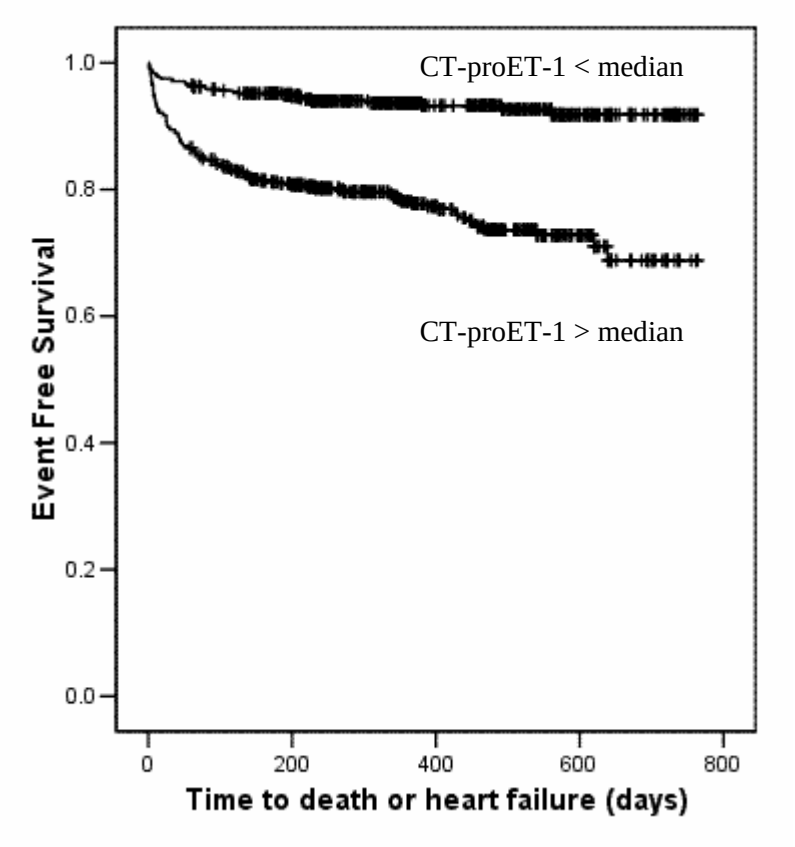


**Figure 3**

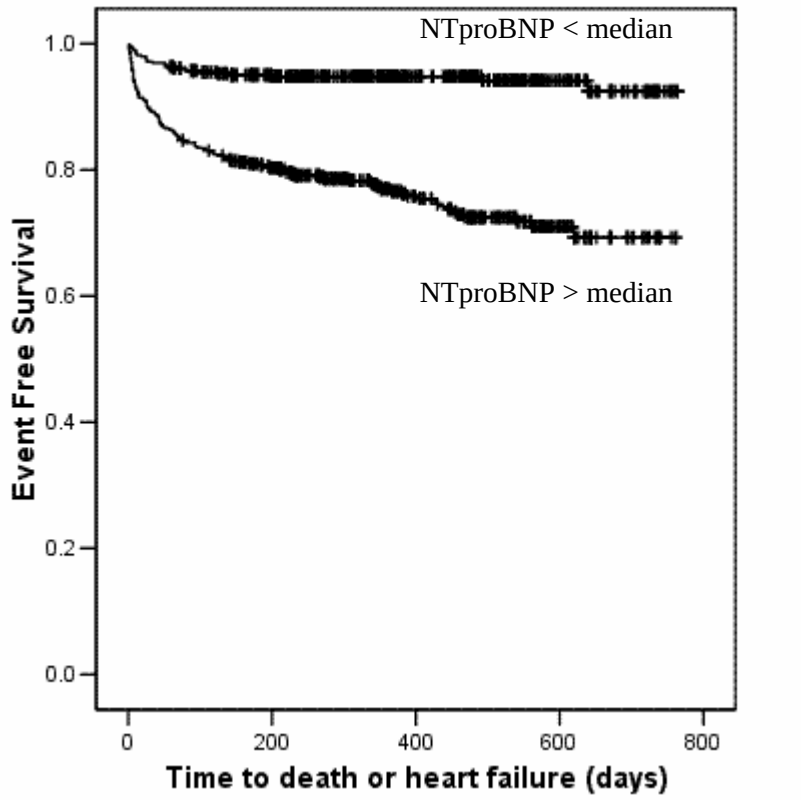


Figure 4

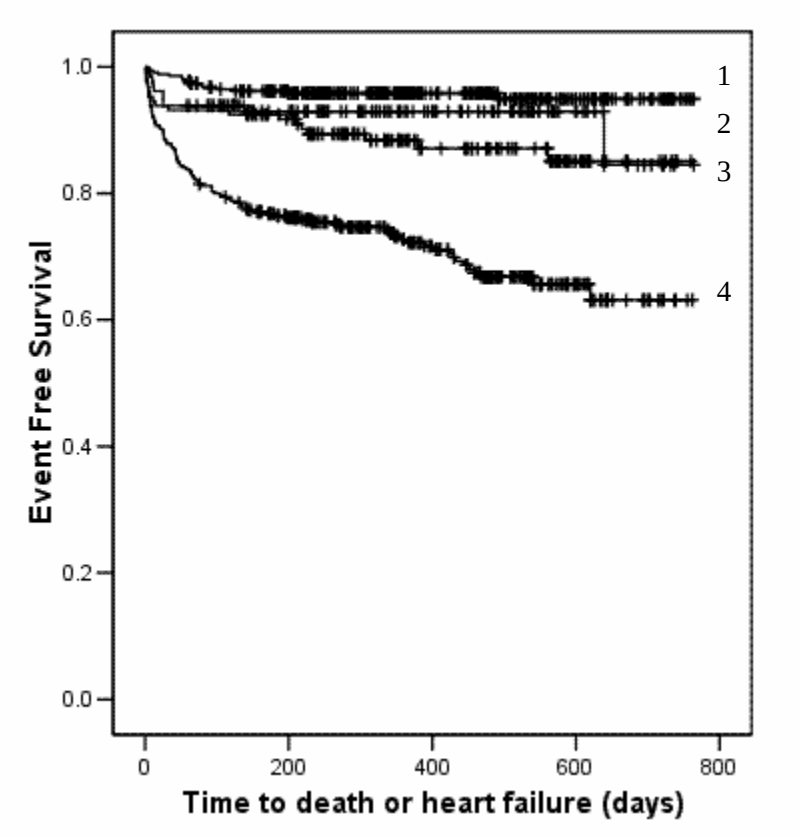
1.0
0.8
0.6
0.4
0.2
0.0
Event Free Survival
0
200
400
600
800
Time to death or heart failure (days)
1
2
3
4

Figure 5