TITLE PAGE

Pro-substance P for evaluation of risk in acute myocardial infarction (PROSPER-AMI)

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STRUCTURED ABSTRACT

Objectives

We assessed whether pro-substance P (ProSP)was associated with poor prognosis after acute myocardial infarction(AMI), to identify novel pathophysiological mechanisms.

Background

ProSP is a stable surrogate marker for labile substance P (SP). Substance P (SP) has pro-inflammatory effects, increases platelet aggregation and clot strength, and reduces fibrinolysis.

Methods

ProSP was measured in 1148 (825 male, mean age 66.2 ± 12.8years) AMI patients. Endpoints were major adverse events (composite of death, re-AMI, heart failure(HF) hospitalization), death/re-AMI and death/HF. GRACE scores were compared with ProSP for the death and/or re-AMI endpoint at 6 months.

Results

During 2 years follow-up, endpoints were 140 deaths, 112 HF hospitalisations and 149 re-AMIs. ProSP levels were highest on the first 2 days following admission and related to eGFR, age, history of diabetes, IHD or hypertension, Killip class, LV wall motion index and sex. Multivariate Cox regression models showed ProSP level was a predictor of major adverse events (hazard ratio HR 1.30(95%CI 1.10-1.54,p<0.002)), death and/or AMI (HR 1.42(1.20-1.68,p<0.0005)), death and/or HF (HR 1.38(1.14-1.67,p<0.001)). ProSP levels were

independent predictors of 6 month death and/or re-AMI together with GRACE scores(p<0.0005 for both). ProSP-adjusted GRACE scores reclassified patients significantly (overall category-free net reclassification improvement NRI (>0) of 31.6(95%CI 14.3-49.0, P<0.0005)) mainly by downclassifying those without endpoints.

Conclusions

ProSP levels post-AMI are prognostic for death, recurrent AMI or HF and improve risk prediction of GRACE scores predominantly by downclassifying risk in those without events. The tachykinin system may be important in determining outcomes in post-AMI patients.

ABBREVIATIONS AND ACRONYMS

AMI = Acute Myocardial Infarction

eGFR = estimated glomerular filtration rate

MACE = Major Adverse Cardiac Events

Re-AMI = Recurrent Myocardial Infarction

HF = Heart Failure

NSTEMI = Non–ST-segment Elevation Myocardial Infarction

NTproBNP = N-terminal Pro-B-type Natriuretic Peptide

ProSP = Pro-substance P

SP = Substance P

STEMI = ST-segment Elevation Myocardial Infarction

IHD = Ischemic heart disease

NRI = Net reclassification improvement

Introduction

Over the last few decades, the treatment of acute myocardial infarction has improved the prognosis of patients substantially, with introduction of thrombolysis, percutaneous coronary intervention, beta-blockers, ACE inhibitors and angiotensin receptor antagonists, statins, anti-platelet agents (such as aspirin and ADP receptor antagonists) and aldosterone antagonists. There is a need to identify new pathways that may affect outcomes post infarction.

Subtance P (SP) and the neurokinins belong to the tachykinin family and are widely distributed in the central and peripheral nervous system (1). Low levels are present in myocardium, and mainly in nerve fibres (1). SP plays a role in nociception, inflammation, plasma extravasation, platelet and leukocyte aggregation in post-capillary venules, and leukocyte chemotactic migration through vessel walls (1). Neurokinin (NK) receptors are mainly present in coronary vessels and intracardiac ganglia, and not on ventricular or atrial myocardium (2). A direct action on the NK1 receptor in coronary arteries may cause NO-mediated vasodilatation (1), although this effect may be impaired in patients with coronary artery disease (3), leading to a dominant NK2 mediated vascoconstriction. SP and neurokinin A are negatively inotropic and chronotropic, acting via cholinergic neurons (2). In contrast, NK1 antagonists improve inotropy and lusitropy in rat myocardial infarction (AMI) models whilst SP attenuates the positive inotropic effect of norepinephrine (4). SP (via the NK1 receptor) has been implicated in myocardial stunning post-AMI in guinea pigs (5).
SP is also expressed in platelets, has a pro-aggregatory effect on platelets (6). Furthermore, NK1 receptor inhibition reduces thrombus formation. Administration of a NK1 receptor antagonist reduced fibrinous adhesion formation and increased tissue plasminogen

activator mRNA and activity, suggesting a role of SP in fibrinolysis(7). SP also strengthens the clot formed in blood, an effect that may be mediated via leucocytes and the magnitude of which is dependent on full length or truncated NK1 receptor isoform expression(8).

Myocardial (9) and pulmonary (10) SP has been observed to be increased in animal models of AMI suggesting a role in AMI. Investigations in man have been hampered by the very short half life of SP (12 min)(11) and there are no large studies examining the role of SP in AMI. The recent development of an assay for stable protachykinin A (ProSP) which is a surrogate for labile SP(12), has enabled studies on the role of this tachykinin system in human disease. We therefore investigated the potential role of SP in AMI, by measuring ProSP and studying its association with major adverse cardiac events (MACE) such as death, heart failure (HF) or readmission with AMI (re-AMI).

Methods

Study Population. We studied 1148 STEMI and NSTEMI patients admitted to University Hospitals of Leicester NHS trust between August 2004 and April 2007. This observational cohort 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 cardiac troponin I level above the 99th centile with at least one of the following:- chest pain lasting >20 minutes or diagnostic serial electrocardiographic changes consisting of new pathological Q waves or ST-segment and T-wave changes (13). Patients with known malignancy, renal replacement therapy or surgery in the previous month were excluded. Estimated glomerular filtration rate (eGFR) was calculated from the simplified Modification of Diet in Renal Disease formula (14). All patients received standard medical treatment and revascularisation at the discretion of the attending physician.

Plasma samples. Blood samples (anticoagulated with EDTA and aprotinin) were drawn after 15 minutes bed rest, immediately after diagnosis and within 36 h of symptom onset. Plasma was stored at -80°C until assayed in a single batch for blinded determination of plasma ProSP and NTproBNP.

Echocardiography. Transthoracic echocardiography was performed in 895(77.9%) patients during the index admission, using either a Sonos 5500 or IE 33 instrument (Philips Medical Systems, Reigate, UK). A 16-segment left ventricular wall motion index (LVWMI) score was performed based on the American Society of Echocardiography method(15). In suitable patients left ventricular ejection fraction (LVEF) was calculated using the biplane method of

discs formula. LV systolic dysfunction (LVSD) was defined as either an LVEF<40% or a LVWMI >1.8.

Global Registry of Acute Coronary Events (GRACE) Scoring. Based on an international observational database of acute coronary syndrome patients, GRACE scores can be calculated on initial presentation to predict in-hospital mortality(16) or for 6 month major adverse cardiac events (MACE), defined as death and/or re-AMI(17) . We used GRACE scores on discharge for comparison with 6 month death and/or re-AMI.

Biomarker assays. The NTproBNP assay was based on a non-competitive assay as previously published (18). Troponin I was measured using the Centaur cTnI Ultra immunoassay (Siemens Healthcare Diagnostics), which has a CV (coefficient of variation) of 10% at 0.03 µg/L with a 99th percentile of 0.04 µg/L. An assay for stable ProSP has been previously reported in detail(12) and was modified as follows: in brief, a mouse monoclonal anti-ProSP antibody (against amino acid sequence 21-36 of ProSP) was used to coat polystyrene tubes. Polyclonal antibodies against amino acids 3-22 of the ProSP sequence were labelled with methyl-acridinium ester and served as the detector antibody. Standards (ProSP peptide; amino acids 1-37 of ProSP) and samples (50 µL) were incubated in tubes with the detector antibody (200 µl). After equilibration, tubes were washed and bound chemiluminescence was detected with a luminometer LB952T/16 (Berthold, Wildbad, Germany).The lower detection limit of the immunoassay was 4.4 pmol/L.

End points. The primary composite endpoint was major adverse cardiac events (MACE) which included all-cause mortality, heart failure (HF)hospitalization or recurrent AMI (re-AMI), within 2 years of the index event. Hospitalization for HF was defined as a hospital readmission for which HF was the primary reason requiring treatment with high dose diuretics, inotropes or intravenous nitrate. Recurrent AMI was diagnosed using the universal definition (13). Other secondary composite endpoints were death and/or re-AMI and death and/or HF readmission. The endpoint of death and/or re-AMI at 6 months was used in analyses involving the GRACE score as this time-point was used in development of the risk score. Endpoints were obtained by reviewing the local hospital databases and patients' records, the Office of National Statistics Registry and phone calls to patients. We achieved 100% follow-up.

Statistical analysis. Statistical analyses were performed on SPSS Version 20 (SPSS Inc, Chicago, Illinois) and Stata 12.1 (Texas,USA). Biomarker levels were $\log_{10}$ transformed and hazard ratios for these refer to 1 SD increment of the $\log_{10}$ transformed biomarker. GRACE scores were used as the original scores. Non-parametric tests were employed for data analysis (Chi-squared test, Kruskal Wallis test and Spearman (r_s) correlations). Independent predictors of ProSP levels were assessed using general linear models, with coefficients and P values reported for 2000 bootstrap samples. To assess prognostic value of biomarkers, a 'base' model was generated using Cox survival analysis, which included variables that were significantly ($p<0.10$) associated with any of the study end points on univariate analysis (age, sex, previous history of ischemic heart disease (IHD), hypertension or diabetes, Killip Class, eGFR, and biomarkers (log troponin I and log NTproBNP)). ProSP was added to this base

model to evaluate its added prognostic value. A second 'comparative' Cox model, was used to assess the relative prognostic power of NTproBNP and ProSP and the GRACE score. To demonstrate independence from clinical variables and NTproBNP or the GRACE score with and without NTproBNP, the added value of ProSP was evaluated based on the likelihood ratio χ^2 test for nested regression models. The additional prognostic value of ProSP in the base and comparative Cox models was further evaluated by reclassification analysis with calculation of category-free net reclassification improvement (NRI) as described by Pencina *et al* (19). We constructed classification trees using Chi-squared Automatic Interaction Detection (CHAID, analysis performed using SPSS), which detects which biomarker has the strongest interaction with the dependent variable in step-wise analysis.

Results

Patient Characteristics

The characteristics of the study population are shown in Table 1, according to ProSP quartiles. Patients with higher ProSP levels were older, female, with more having past histories of hypertension, IHD, diabetes, HF. They had higher GRACE scores, NTproBNP and glucose levels and lower ejection fractions and eGFR.

Correlation analysis

Spearman correlation analysis (r_s) showed ProSP was significantly correlated to age (0.521), eGFR (-0.555), diastolic BP (-0.178), NTproBNP (0.428), wall motion score index (0.173) and heart rate (0.172) (all P<0.0005). ProSP was not correlated to troponin or peak creatine kinase levels.

A general linear model with 2000 bootstrap samples showed eGFR, age and Killip class above 1 as independent predictors of ProSP level (Table 2).

Day curves for ProSP

Sequential plasma sampling over 5 days was available for 110 patients, of which 29 had a MACE within 2 years. Figure 1 demonstrates the plasma profile along with a general linear model with repeated measures that shows significant changes in ProSP over time (p<0.001), and higher levels in those with MACE p<0.03). In post-hoc testing, ProSP levels on day 1 was higher than days 3, 4 or 5 (p<0.001, 0.004 and 0.002 respectively, Bonferroni corrected for multiple comparisons). ProSP levels on days 1 and 2 were similar. There was no statistically significant interaction of the time profile of ProSP with MACE.

Survival analysis

During follow-up over 2 years, patients with elevated ProSP levels (log_{10} transformed and standardised by 1 SD) had more MACE, deaths, and rehospitalizations with HF or re-AMI (Table 1). Table 3 reports the univariate hazard ratios of various factors that affected the outcome of MACE at 2 years. In multivariate analysis for predicting MACE at 2 years, significant independent predictors included age, Killip class above 1, eGFR, and NTproBNP. Addition of ProSP to the model (model 2 in Table 3) showed ProSP had independent predictive value with a hazard ratio (HR(95% confidence interval) of 1.30(1.10 -1.54), $p<0.002$, and the added value of ProSP as evaluated by the likelihood ratio χ^2 test for nested regression models was $p<0.0001$. Kaplan-Meier survival analysis visualises the MACE rates in ProSP quartiles (Figure 2), showing quartile 4 was significantly different from all other quartiles ($p<0.0005$, log rank test (Mantel-Cox)), and quartile 3 was significantly different from 4($p<0.0005$), 2($p<0.022$) and 1($p<0.001$).

Inclusion of glucose in Cox survival models for MACE showed the hazard ratio HR (95% confidence interval) of glucose was 1.11 (0.97-1.26) (p non-significant) with the HR of ProSP being 1.24 (1.03-1.49), p=0.02. Inclusion of white cell count in survival models for MACE showed white cell count had a HR of 0.98 (0.95-1.01) (p non-significant) whilst that of ProSP was 1.27 (1.06-1.53), p=0.01.

In other models for prediction of the secondary composite endpoints of death and/or re-AMI (Table 4) and death and/or HF readmission (Table 5), ProSP remained an independent predictor ($p<0.0005$ and $p<0.001$ respectively) of these endpoints, and in both models,

ProSP showed added value to the clinical variables and NTproBNP (log likelihood χ^2 test p value <0.0001 for both composite endpoints).

Comparison with GRACE scores

The GRACE risk score (17) was originally derived for prediction of death and/or re-AMI at 6 months. We investigated the utility of the biomarkers NTproBNP and ProSP for prediction of death and/or re-AMI as well as other composite endpoints (MACE, death and/or HF). GRACE scores and the biomarkers NTproBNP and ProSP were predictors of all composite endpoints in univariate analysis (Table 6). In multivariate analysis for MACE, death and/or re-AMI and death and/or HF at 6 months, both NTproBNP and ProSP demonstrated added value to the GRACE score, and in addition, ProSP showed added value to models with GRACE and NTproBNP for all composite endpoints analysed (p<0.0001 for all, Table 6).

Using receiver operating characteristic curve (ROC) analysis for death and/or re-AMI at 6 months, the area under the curve (AUC) increased from 0.69(95% CI 0.65-0.74) for GRACE scoring only to 0.72 (0.68-0.77) with the addition of ProSP (P=0.01). Addition of NTproBNP to GRACE score yielded a higher area (0.70 (0.65-0.75) (p not significant)). Comparison of the areas for GRACE score and NTproBNP (0.70 (0.65-0.75)) and that of GRACE score, NTproBNP and ProSP (0.72 (0.68-0.77)) was not significant (p=0.06).

Reclassification Analysis

Category-free reclassification analysis (with no arbitrary cut-off probabilities) was employed as described by Pencina *et al* (19) to calculate the NRI (>0), for the effect of addition of

NTproBNP or ProSP to the probabilities derived from the GRACE score in predicting the endpoints of death and/or re-AMI, MACE and death and/or HF (Table 7). NTproBNP upclassified risk in all those with events for all these composite endpoints. However, it wrongly (and significantly) upclassified risk in those without events for the death and/or re-AMI and MACE endpoints, although correctly downclassifying risk in those without events for the death and/or HF endpoint. However, ProSP correctly downclassified risk in those without events for all composite endpoints. When ProSP was added to a composite risk score comprised of GRACE and NTproBNP, ProSP downclassified risk in those without events for the endpoints of MACE, and death and/or MI, and had no significant reclassification of those with the death and/or HF endpoint (Table 7).

Decision tree analysis

In order to determine optimal cut-points for biomarkers, we constructed decision trees (using ProSP and NTproBNP levels and GRACE scores) to classify patients into survivors or those with the endpoint of death and/or re-AMI at 6 months. Using ProSP as an initial classifier (figure 3) a ProSP level under 72.08pmol/L and GRACE score under 137 defines a low risk group of patients (n=512, 44.6% of the total) in whom the event rate was 3.0%. Of these, only 3 patients (0.26%) had died within 6 months, and 1 (0.09%) had died within 30 days. ProSP levels above 121.6 pmol/L defined a high risk group of patients with a death/re-AMI rate of 37.7% and a death rate of 30.7% (figure 3).

Discussion

In addition to the known effects of SP on pain and inflammatory pathways, SP may also have important effects on platelet aggregation (6),fibrinolysis (7) and strengthening of clot

(8),which may suggest a potential role in affecting outcomes after AMI. SP itself is very labile and difficult to measure in plasma. A novel ProSP assay (12) has therefore enabled us to examine the hypothesis that SP may play a role following AMI.

In our cohort of AMI patients, ProSP was most strongly correlated with renal function, and was also influenced by age, past history of diabetes and IHD, Killip class, wall motion index, sex and blood pressure. There was no relation to infarct size. ProSP may therefore closely integrate a patient's previous history with renal function at AMI presentation. ProSP levels peaked at days 1 and 2 following chest pain onset, permitting an early assessment of risk.

During follow-up, ProSP was associated with cardiovascular outcomes such as death, recurrent AMI and HF rehospitalization. Existing biomarkers such as NTproBNP were mainly predictive of mortality and HF, with poorer detection of death and/or recurrent AMI. In contrast, ProSP provided independent prognostic information for the composite of MACE, death and/or re-AMI, and also death and/or HF. These analyses suggest that SP may potentially have a role in the pathophysiology of outcomes post-AMI.

Analysis of the increment in ROC AUC showed that addition of ProSP had small effects on this area. However, it is recognised that such analyses are relatively insensitive to the addition of novel biomarkers (20) and reclassification analyses should also be performed. For example, NTproBNP demonstrated a small non-significant increase in ROC AUC, whereas it showed added value in reclassification analyses for both up and down classifying risk in those with MACE, death and/or MI and death and/or HF. In reclassification analysis, ProSP

demonstrated additional utility to the GRACE score, used as a standard risk classification tool in AMI, mainly by down-classifying risk in those without endpoints. Such a biomarker would be especially useful in detecting patients with low risk, and this was confirmed on decision tree analysis. ProSP levels under 72.1pmol/L may also define a low-risk group of patients, who potentially could be discharged from hospital earlier. The event rates in this group were very low during follow-up with only 3 deaths (0.26%) by 6 months.

Reclassification analyses also suggested that NTproBNP could up or downclassify risk in those assessed with the GRACE score, and that ProSP only showed added value for the MACE and death and/or MI endpoints and not the death and/or HF endpoint. However, since the GRACE score was derived mainly for prediction of death and/or MI, this could be a limitation when including HF as an endpoint.

Association of ProSP with poor outcomes may reflect some of the known effects of SP on physiology and pathophysiology. The association with heart failure rehospitalisation could be due to the known negative inotropic and lusitropic effects of SP which have been demonstrated in animal models of AMI (4,5). SP is expressed in monocytes and macrophages (21), may play a role in inflammation (22) and leukocyte chemotaxis and egress from vessels (1,2), which may also affect myocardial function. Recent emerging evidence suggests SP is a mast cell secretagogue via the NK1 receptor, and mast cells may play a role in adverse remodelling (23,24). In animal models of remodelling from volume overload, NK1 antagonists prevented an increase in mast cell density and myocardial TNF-α (23) and remodelling was also reduced in TAC1 gene knockout mice. Mast cells are

colocalised with cardiac nerves (24) and secrete proteases to activate collagenases and gelatinases, the putative mediators of remodelling. Furthermore, mast cells secrete stored renin, resulting in local activation of the renin-angiotensin system (9).

The association of SP with readmission with AMI may be due to effects of SP on platelet aggregation (6,25). Tachykinin family peptides (SP, endokinins A and B) are found in platelets (25), and receptors on platelet membranes (NK1 and NK3) may mediate a modulatory positive paracrine feedback on platelet activation. NK1 inhibition reduces thrombus formation. SP also strengthens a clot after formation (8), an effect which is dependent on NK1 receptors on leucocytes. This effect is more marked in those patients with a full length NK1 receptor (8). In addition, SP may reduce tissue plasminogen activator activity and expression (7), hence potentially promoting thrombosis.

On the other hand, SP may also have some potentially beneficial roles in ischemia post-conditioning (26) and in mobilisation of progenitor cells which may play a role in angiogenesis within ischemic tissue (27). Our findings are hypothesis generating for investigating the role of SP on outcomes post-AMI, as it is uncertain whether beneficial effects of SP could be outweighed by the deleterious effects.

Limitations

Our findings are based on a population from a single centre, with 2 admitting hospitals, and should be verified in other larger populations. The rate of early revascularisation in our

NSTEMI population was low and may not reflect the more contemporary invasive approach of revascularisation within 72 h of presentation. One advantage is that the relation of ProSP with poor outcomes would not have been confounded by higher early revascularisation rates.

Conclusions

Following AMI, circulating ProSP levels provide added value to the prognostic information determined by the GRACE score and the prognostically important biomarker NTproBNP. The ability of ProSP to predict recurrent AMI in addition to mortality may confer clinical utility on the tachykinin system in risk stratification after AMI.

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Figure Legends

Figure 1: Profile of plasma ProSP over 5 days following AMI, in those with (in red) or without (in green) MACE at 2 years.

Figure 2: Kaplan-Meier Plots for the endpoint of MACE, according to ProSP quartiles.

Figure 3: Classification tree for the endpoint of death and/or re-AMI at 6 months.

Figure 1

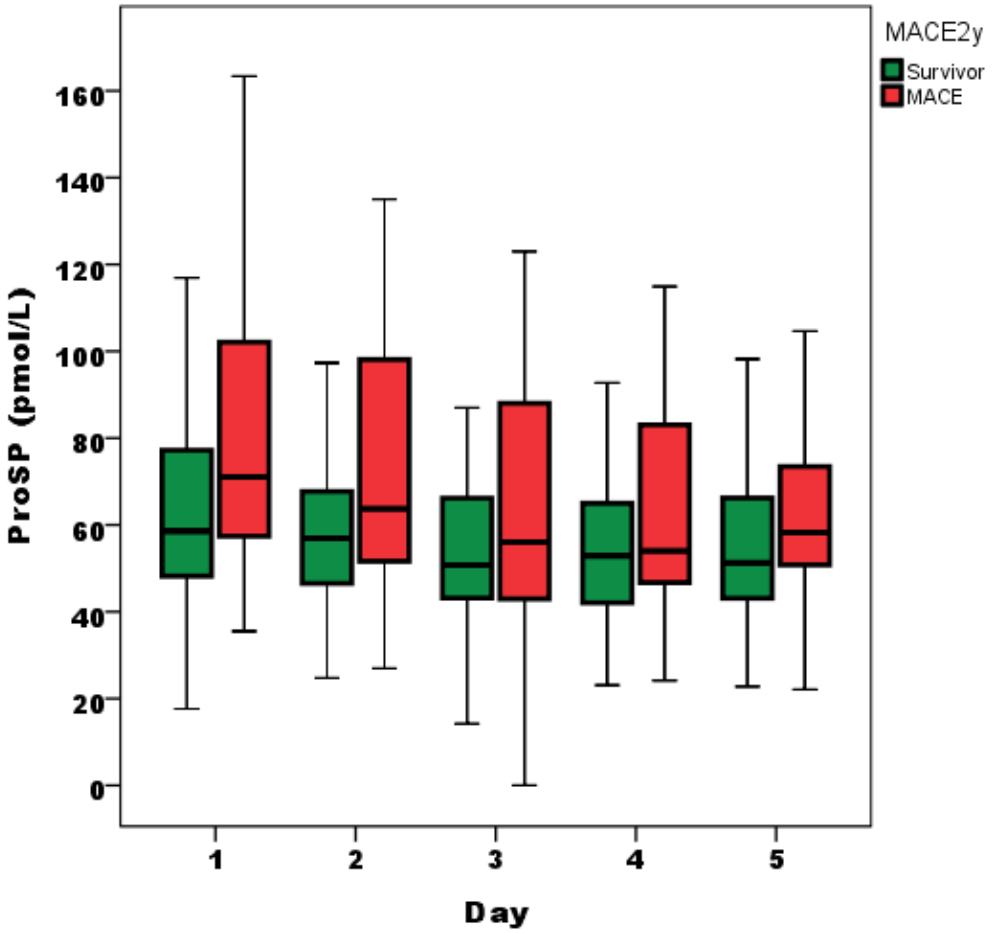
MACE2y
Survivor
MACE
ProSP (pmol/L)
160
140
120
100
80
60
40
20
0
1
2
3
4
5
Day

Figure 2

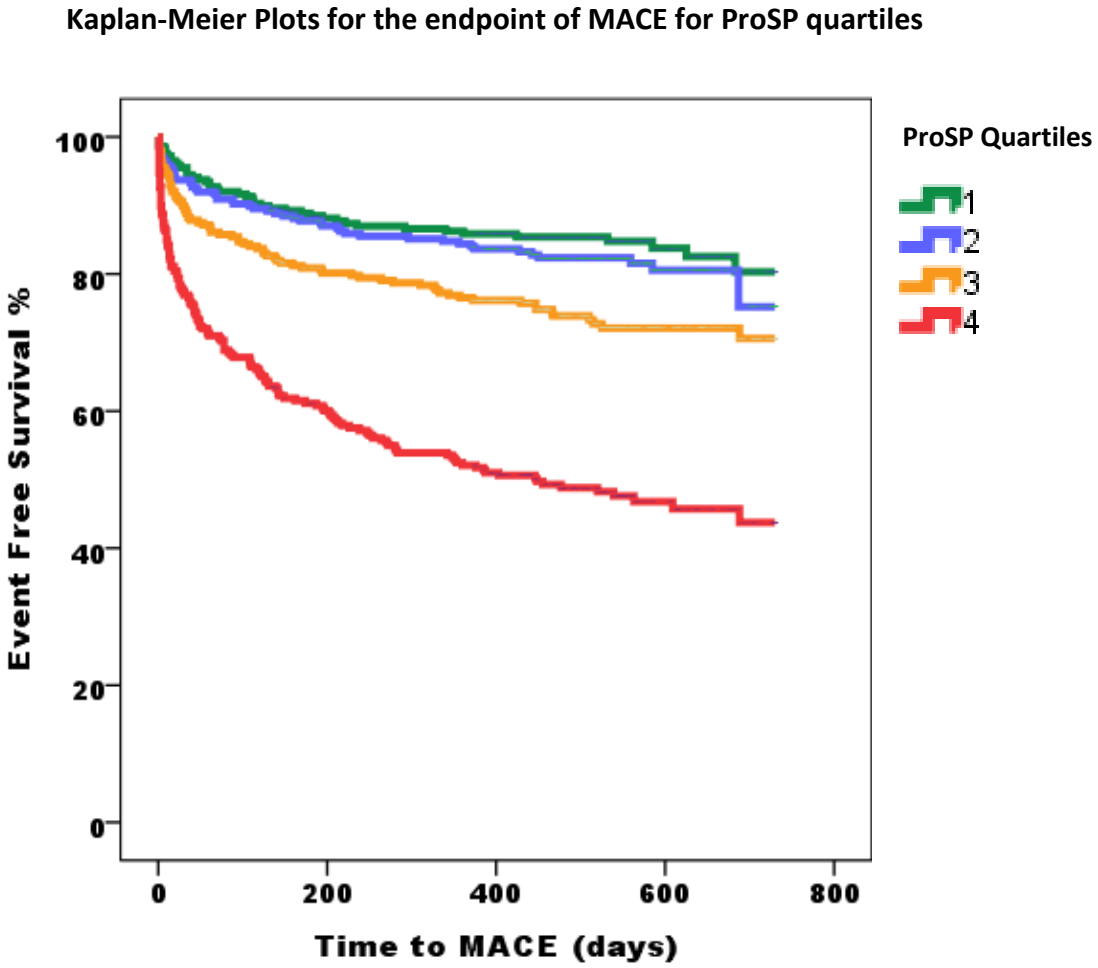
Kaplan-Meier Plots for the endpoint of MACE for ProSP quartiles
Event Free Survival %
100
80
60
40
20
0
0
200
400
600
800
Time to MACE (days)
ProSP Quartiles
1
2
3
4

Figure 3

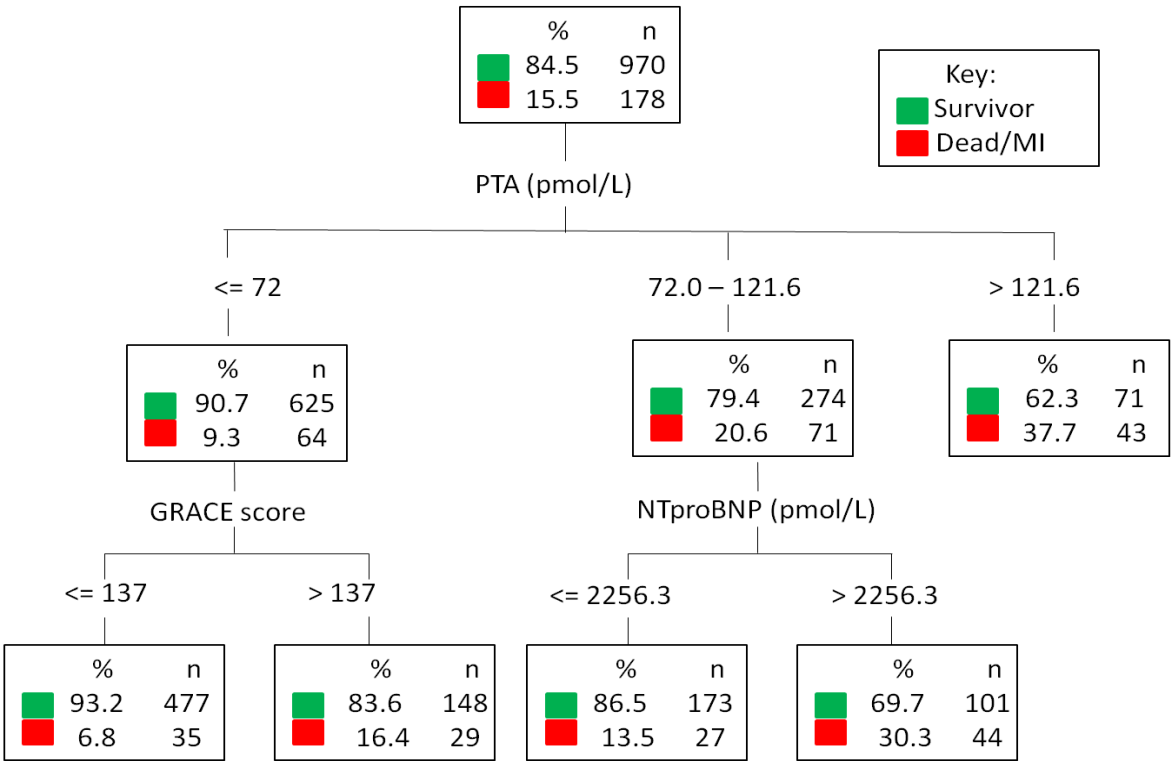
% n
84.5 970
15.5 178
Key:
Survivor
Dead/MI
PTA (pmol/L)
<= 72
72.0 – 121.6
> 121.6
% n
90.7 625
9.3 64
% n
79.4 274
20.6 71
% n
62.3 71
37.7 43
GRACE score
NTproBNP (pmol/L)
<= 137
> 137
<= 2256.3
> 2256.3
% n
93.2 477
6.8 35
% n
83.6 148
16.4 29
% n
86.5 173
13.5 27
% n
69.7 101
30.3 44

Table 1: Characteristics of the 1148 AMI patients according to ProSP quartiles on admission.Numerical data are presented as n (%). P values are quoted for the Kruskal Wallis or Chi squared tests for continuous or categorical variables respectively. Numbers (%) or Mean± SD are reported.

		ProSP quartiles				
	All n=1148	1 <52.0 pmol/L n=288	2 52.0-65.19pmol/L n=286	3 65.19-89.1pmol/L n=288	4 >89.1 pmol/L n=286	P Value
ProSPpmol/L	77.2 ± 55.7	42.2 ± 7.43	58.4 ± 4.0	75.6 ± 7.1	132.9 ± 87.4	<0.0005
NTproBNP(pmol/L)	1849 ± 2108	891.3 ± 1062	1339 ± 1641	1874 ±2030	3300 ± 2569	<0.0005
Demographics						
Age (years)	66.2 ± 12.8	58.3 ± 11.2	63.1 ± 11.0	68.1 ± 11.9	75.4 ± 10.3	<0.0005
Male (%)	825 (72)	235 (82)	214 (75)	208 (72)	168 (59)	<0.0005
ST elevation AMI	545 (47)	144 (50)	132 (46)	149 (52)	120 (42)	NS
Previous History						
IHD	379 (33)	67 (23)	80 (28)	91 (32)	141 (49)	<0.0005
Heart Failure	46 (4)	3 (1)	8 (3)	10 (3)	19 (7)	<0.003
Hypertension	596 (52)	125 (44)	134 (47)	152 (53)	185 (65)	<0.0005
Diabetes Mellitus	266 (23)	53 (18)	71 (25)	61 (21)	81 (28)	0.032
Killip Class>1	426 (40)	61 (24)	92 (35)	121 (45)	152 (56)	<0.0005
Glucose (mmol/L)	8.9 ± 4.2	8.5 ± 3.9	8.7 ± 3.9	8.4 ± 3.5	9.9 ± 5.4	<0.007
Troponin I (ng/mL)	13.1 ± 25.8	13.2 ± 26.7	12.0 ± 24.4	15.0 ± 27.9	12.1 ± 24.2	NS
eGFR (ml/min/1.73m^2)	65.6 ± 20.1	77.9 ± 17.7	71.4 ± 15.5	64.4 ± 16.6	48.9 ± 17.9	<0.0005

Risk Markers on Discharge						
Echocardiographic LVSD [n=893]						
LV wall motion index	1.47 ± 0.42	1.38 ± 0.37	1.46 ± 0.42	1.46 ± 0.41	1.60 ± 0.43	<0.0005
LV ejection fraction	42.1 ± 14.5	44.8 ± 13.8	43.8 ± 14.3	41.4 ± 13.8	38.3 ± 15.2	<0.0005
GRACE score	120.0 ± 32.7	99.7 ± 26.6	109.6 ± 26.9	125.6 ± 28.4	144.5 ± 29.9	<0.0005
Treatment						
Aspirin	963 (84)	255 (89)	255(89)	238 (83)	215 (75)	<0.0005
Beta-blocker	920 (80)	256 (89)	238 (83)	230 (80)	196 (69)	<0.0005
ACE inhibitor or ARB*	940 (82)	249 (87)	234 (82)	245(85)	212 (74)	<0.0005
Statin	1002 (87)	270 (94)	258 (90)	260(90)	214(75)	<0.0005
Loop Diuretic	289 (25)	39 (14)	59 (21)	69(24)	122(43)	<0.0005
Revascularisation	343 (30)	95 (33)	99 (35)	79(27)	70 (24)	0.027
End Points (2 years)						
Major Adverse Cardiac Events	324 (28)	45 (16)	53 (19)	77 (27)	149 (52)	<0.0005
Death	140 (12)	11 (4)	11 (4)	31 (11)	87 (30)	<0.0005
Non-fatal major Adverse Cardiac Events	230 (20)	41 (14)	46 (16)	56 (19)	87 (30)	<0.0005
Heart Failure	112 (10)	13 (5)	19 (7)	28 (10)	52 (18)	<0.0005
Re-AMI	149 (13)	29 (10)	35 (12)	33 (11)	52 (18)	0.021

*ARB = Angiotensin 2 receptor blocker

Table 2: General linear model showing independent predictors of ProSP levels, with coefficients reported for 2000 bootstrapped samples. Lower and upper CI refer to 95% confidence intervals.

Variable	Coefficient	Standard error	Lower CI	Upper CI	P value
eGFR	-0.00345	0.00045	-0.004	-0.003	<.001
age	0.003272	0.000548	0.002	0.004	<.001
Killip Class >1	0.045352	0.012334	0.021	0.070	<.001
Male sex	0.011859	0.013149	-0.014	0.038	NS
Past history IHD	0.015876	0.012434	-0.008	0.040	NS
Past history hypertension	-0.00233	0.011583	-0.025	0.020	NS
Past history diabetes	0.024257	0.014706	-0.005	0.053	NS
Diastolic BP	-0.00036	0.000359	-0.001	0.000	NS
Heart Rate	0.00021	0.000231	0.000	0.001	NS
LV Systolic Dysfunction	0.004572	0.012434	-0.020	0.029	NS

Table 3.Cox regression analysis for MACE at 2 years post-AMI. Multivariable analysis results in model 1 included variables and biomarkers (except ProSP) which were significant on univariable analysis. Multivariable Model 2 used the variables in model 1 with the addition of ProSP as a continuous variable. * P value for the increment in log likelihood χ^2 for models.

	Univariable HR (95% CI)	**P**	**Multivariable Model 1 HR (95% CI)**	**P**	**Multivariable Model 2 HR (95% CI)**	**P**
Age (years)	1.05 (1.04-1.06)	0.001	1.03 (1.01-1.04)	0.001	1.02 (1.01-1.04)	0.002
Male Sex	0.62 (0.50-0.78)	0.001	1.09 (0.83-1.45)	NS	1.07 (0.81-1.42)	NS
ST elevation	1.09 (0.88-1.36)	NS	1.35 (0.98-1.85)	NS	1.28 (0.93-1.76)	0.035
Killip class>1	2.65 (2.10-3.34)	0.001	1.60 (1.22-2.11)	0.001	1.56 (1.18-2.06)	0.002
eGFR (ml min^{-1} /1.73m^2)	0.97 (0.96-0.98)	0.001	0.99 (0.98-0.99)	0.002	0.99 (0.98-1.00)	NS
Heart rate (beats min^{-1})	1.01 (1.01-1.01)	0.001	1.01 (1.00-1.01)	NS	1.00 (0.99-1.00)	NS
Systolic BP (mm Hg)	0.99 (0.99-1.00)	0.043	0.99 (0.99-1.00)	NS	0.99 (0.99-1.00)	NS
Past history						
Ischemic heart disease	1.54 (1.23-1.91)	0.001	1.01 (0.76-1.34)	NS	0.97 (0.73-1.29)	NS
Hypertension	1.64 (1.31-2.06)	0.001	1.16 (0.87-1.55)	NS	1.17 (0.87-1.55)	NS
Diabetes	1.55 (1.22-1.96)	0.001	1.32 (0.99-1.74)	NS	1.31 (0.99-1.74)	NS
Biomarkers						
Log Troponin (µg/L)	1.12 (0.99-1.26)	0.07	1.08 (0.93-1.25)	NS	1.08 (0.93-1.25)	NS
Log NTproBNP (pmol/L)	1.93 (1.65-2.25)	0.001	1.28 (1.04-1.57)	0.018	1.21 (0.98-1.48)	NS
Log ProSP (pmol/L)	1.81(1.65-1.99)	0.001	Excluded		1.30 (1.10 -1.54)	0.002
Log Likelihood χ^2			152.39		171.30	0.0001*

Table 4.Cox regression analysis for Death and/or re-AMI at 2 years post-AMI. Multivariable analysis results are reported for model 1 which included variables and biomarkers (except ProSP) which were significant on univariable analysis. Multivariable Model 2 used the variables in model 1 with the addition of ProSP. * P value for the increment in log likelihood χ^2 for models.

	Univariable HR (95% CI)	**P**	**Multivariable Model 1 HR (95% CI)**	**P**	**Multivariable Model 2 HR (95% CI)**	**P**
Age (years)	1.05 (1.04-1.06)	0.001	1.03 (1.01-1.04)	0.003	1.02 (1.01-1.04)	0.01
Male Sex	0.66 (0.51-0.85)	0.001	1.15 (0.83-1.58)	NS	1.11 (0.81-1.52)	NS
ST elevation	1.03 (0.81-1.32)	NS	1.21 (0.84-1.75)	NS	1.14 (0.79-1.64)	NS
Killip class>1	2.07 (1.61-2.67)	0.001	1.19 (0.87-1.62)	NS	1.14 (0.83-1.56)	NS
eGFR (ml min^{-1} /1.73m^2)	0.97 (0.96-0.98)	0.001	0.99 (0.98-0.99)	0.006	1.00 (0.99-1.01)	NS
Heart rate (beats min^{-1})	1.01 (1.00-1.01)	0.001	1.00 (0.99-1.00)	NS	1.00 (0.99-1.00)	NS
Systolic BP (mm Hg)	1.00 (0.99-1.00)	NS	1.00 (0.99-1.00)	NS	1.00 (0.99-1.00)	NS
Past history						
Ischemic heart disease	1.62 (1.27-2.07)	0.001	1.20 (0.87-1.65)	NS	1.15 (0.83-1.59)	NS
Hypertension	1.56 (1.21-1.99)	0.001	1.05 (0.77-1.45)	NS	1.06 (0.77-1.47)	NS
Diabetes	1.54 (1.18-2.00)	0.001	1.28 (0.93-1.75)	NS	1.25 (0.91-1.71)	NS
Biomarkers						
Log Troponin (μg/L)	1.06 (0.92-1.21)	NS	1.06 (0.89-1.26)	NS	1.06 (0.90-1.25)	NS
Log NTproBNP (pmol/L)	1.83 (1.54-2.17)	0.001	1.29 (1.02-1.63)	0.032	1.19 (0.95-1.50)	NS
Log ProSP (pmol/L)	1.76 (1.60-1.94)	0.001	Excluded		1.42 (1.20-1.68)	0.0005
Log Likelihood χ^2			93.45		119.72	0.0001*

Table 5.Cox regression analysis for Death and/or HF at 2 years post-AMI. Multivariable analysis results are reported for model 1 which included variables and biomarkers (except ProSP) which were significant on univariable analysis. Multivariable Model 2 used the variables in model 1 with the addition of ProSP. * P value for the increment in log likelihood χ^2 for models.

	Univariable HR (95% CI)	**P**	**Multivariable Model 1 HR (95% CI)**	**P**	**Multivariable Model 2 HR (95% CI)**	**P**
Age (years)	1.07 (1.06-1.09)	0.001	1.04 (1.02-1.06)	0.001	1.04 (1.02-1.06)	0.001
Male Sex	0.51 (0.39-0.66)	0.001	1.01 (0.72-1.41)	NS	0.98 (0.70-1.37)	NS
ST elevation	0.99 (0.77-1.29)	NS	1.13 (0.76-1.67)	NS	1.06 (0.72-1.57)	NS
Killip class>1	3.71 (2.76-4.99)	0.001	2.02 (1.42-2.86)	0.001	1.95 (1.37-2.77)	0.001
eGFR (ml $min^{-1}/1.73m^2$)	0.96 (0.95-0.97)	0.001	0.98 (0.97-0.99)	0.001	0.99 (0.98-1.00)	NS
Heart rate (beats min^{-1})	1.01 (1.01-1.02)	0.001	1.00 (0.99-1.00)	NS	1.00 (0.99-1.00)	NS
Systolic BP (mm Hg)	0.99 (0.98-0.99)	0.004	0.99 (0.98-0.99)	0.005	0.99 (0.98-0.99)	0.005
Past history						
Ischemic heart disease	1.59 (1.22-2.06)	0.001	0.87 (0.62-1.22)	NS	0.82 (0.58-1.16)	NS
Hypertension	1.70 (1.30-2.23)	0.001	1.02 (0.72-1.45)	NS	1.03 (0.72-1.46)	NS
Diabetes	1.58 (1.19-2.09)	0.001	1.42 (1.01-1.98)	0.043	1.41 (1.01-1.98)	0.047
Biomarkers						
Log Troponin (μg/L)	1.16 (1.00-1.33)	0.044	1.09 (0.91-1.32)	NS	1.09 (0.91-1.31)	NS
Log NTproBNP (pmol/L)	3.21 (2.57-4.02)	0.001	1.65 (1.23-2.21)	0.001	1.50 (1.12-2.01)	0.007
Log ProSP(pmol/L)	2.07 (1.87-2.29)	0.001	Excluded		1.38 (1.14-1.67)	0.001
Log Likelihood χ^2			201.25		227.63	0.0001*

Table 6. Cox regression analysis for endpoints at 6 months (MACE, death and/or re-AMI, death and/or HF). LLχ^2 refers to the log likelihood χ^2 of the model with associated p value for added value of the biomarker(s) (* compared to GRACE only; † compared to GRACE and NTproBNP model). ‡.

MACE	**Univariable HR (95% CI)**	**p**	**Multivariable HR (95% CI)**	**p**	**Multivariable HR (95% CI)**	**p**	**Multivariable HR (95% CI)**	**p**
GRACE	1.02 (1.02-1.03)	0.0005	1.02 (1.01-1.02)	0.0005	1.02 (1.01-1.02)	0.0005	1.02 (1.01-1.02)	0.0005
NTproBNP	2.02 (1.67-2.44)	0.0005	1.29 (1.04-1.60)	0.002	Excluded		1.29 (1.04-1.60)	0.02
ProSP	1.76 (1.57-1.96)	0.0005	Excluded		1.38 (1.18-1.61)	0.0005	1.31 (1.11-1.54)	0.001
LLχ^2	101.13 (GRACE only)		108.57	0.006*	122.84	0.0001*	126.63	0.0001*†
Death and/or re-AMI								
GRACE	1.02 (1.01-1.02)	0.0005	1.02 (1.01-1.02)	0.0005	1.01 (1.01-1.02)	0.0005	1.01 (1.01-1.02)	0.0005
NTproBNP	1.89 (1.53-2.33)	0.0005	1.38 (1.08-1.75)	0.009	Excluded		1.24 (0.98-1.57)	NS
ProSP	1.70 (1.52-1.90)	0.0005	Excluded		1.47 (1.26-1.72)	0.0005	1.42 (1.21-1.67)	0.0005
LLχ^2	58.36 (GRACE only)		63.89	0.019*	85.53	0.0001*	88.42	0.0001*†
Death and/or HF								
GRACE	1.03 (1.02-1.04)	0.0005	1.03 (1.02-1.03)	0.0005	1.03 (1.02-1.03)	0.0005	1.02 (1.02-1.03)	0.0005
NTproBNP	3.22 (2.47-4.20)	0.0005	2.06 (1.50-2.82)	0.0005	Excluded		1.85 (1.34-2.55)	0.0005
ProSP	1.95 (1.74-2.19)	0.0005	Excluded		1.43 (1.19-1.71)	0.0005	1.28 (1.05-1.56)	0.01
LLχ^2	130.66 (GRACE only)		142.55	0.0001*	152.05	0.0001*	159.27	0.0001*†

Table 7.

Reclassification analysis using continuous reclassification showing the net reclassification improvement (NRI) and the significance of the NRI, of adding NTproBNP or ProSP to the classification using GRACE scoring only and for adding ProSP to the classification using GRACE scoring with NTproBNP, for the endpoints of death and/or re-AMI, MACE and death and/or HF at 6 months.

Outcome	**6m Death/MI**		**6m MACE**		**6m Death/HF**	
Adding NTproBNP to GRACE						
Endpoint	**NRI (95% CI)**	**p**	**NRI (95% CI)**	**p**	**NRI (95% CI)**	**p**
No	-7.7 (-14.5,-1.0)	.025	-7.5 (-14.5,-0.6)	.033	7.2 (0.5,13.9)	.034
Yes	37.3 (21.3, 53.3)	.0005	33.7(19.6, 47.8)	.0005	37.4 (20.3, 54.5)	.0005
Total	29.6 (12.2, 47.0)	.001	26.1(10.4, 41.9)	.001	44.6 (26.2, 63.0)	.0005
Adding ProSP to GRACE						
Endpoint	**NRI (95% CI)**	**p**	**NRI (95% CI)**	**p**	**NRI (95% CI)**	**p**
No	22.3 (15.5, 29.1)	.0005	18.6 (11.6, 25.5)	.0005	20.0 (13.4, 26.7)	.0005
Yes	9.3 (-6.7, 25.3)	NS	4.7 (-9.4, 18.8)	NS	8.4 (-8.7, 25.5)	NS
Total	31.6 (14.2, 49.0)	.0005	23.3 (7.5, 39.0)	.004	28.4 (10.1, 46.8)	.002
Adding ProSP to GRACE and NTproBNP						
Endpoint	**NRI (95% CI)**	**p**	**NRI (95% CI)**	**p**	**NRI (95% CI)**	**p**
No	18.0 (11.2, 24.8)	.0005	13.6 (6.6, 20.5)	.0005	3.3 (-3.4, 10.0)	NS
Yes	9.3 (-6.7, 25.3)	NS	2.6 (-11.5, 16.7)	NS	6.9 (-10.3, 24.0)	NS
Total	27.3 (10.0, 44.7)	.002	16.2 (0.4, 31.9)	.044	10.1 (-8.3, 28.5)	NS