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Pro-Substance P for Evaluation of Risk in Acute Myocardial Infarction

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ABSTRACT

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

OBJECTIVES This study assessed whether ProSP was associated with poor prognosis after acute myocardial infarction (AMI) to identify novel pathophysiological mechanisms.

METHODS ProSP was measured in 1,148 AMI patients (825 men, mean age 66.2 ± 12.8 years). Endpoints were major adverse cardiac events (composite of death, reinfarction, and heart failure [HF] hospitalization), death/reinfarction, and death/HF. GRACE (Global Registry of Acute Coronary Events) scores were compared with ProSP for death and/or reinfarction at 6 months.

RESULTS During 2-year follow-up, there were 140 deaths, 112 HF hospitalizations, and 149 re-AMI. ProSP levels were highest on the first 2 days after admission and related to estimated glomerular filtration rate, age, history of diabetes, ischemic heart disease or hypertension, Killip class, left ventricular 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% confidence interval [CI]: 1.10 to 1.54; $p < 0.002$), death and/or AMI (HR: 1.42; 95% CI: 1.20 to 1.68; $p < 0.0005$), death and/or HF (HR: 1.38; 95% CI: 1.14 to 1.67; $p < 0.001$). ProSP levels with GRACE scores were independent predictors of 6-month death and/or reinfarction ($p < 0.0005$ for both). ProSP-adjusted GRACE scores reclassified patients significantly (overall category-free net reclassification improvement of 31.6 (95% CI: 14.3 to 49.0; $p < 0.0005$) mainly by down-classifying those without endpoints.

CONCLUSIONS ProSP levels post-AMI are prognostic for death, recurrent AMI, or HF, and they improve risk prediction of GRACE scores, predominantly by down-classifying risk in those without events. (J Am Coll Cardiol 2014;64:1698–707)

Over the last few decades, treatment of acute myocardial infarction (AMI) has improved substantially with the introduction of thrombolysis, percutaneous coronary intervention, beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, statins,

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antiplatelet agents (such as aspirin and adenosine diphosphate receptor antagonists), and aldosterone antagonists. Although patient prognosis has improved, there remains a need to identify new pathways that may further improve outcomes after infarction.

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Substance P (SP) and the neurokinins (NK) belong to the tachykinin family and are widely distributed in the central and peripheral nervous system (1). Although levels of SP are low in the myocardium, SP still can affect the heart via its role in nociception, inflammation, plasma extravasation, platelet and leukocyte aggregation in post-capillary venules, and leukocyte chemotactic migration through vessel walls (1). As for NK receptors, they are mainly present in coronary vessels and intracardiac ganglia, but not on the ventricular or atrial myocardium (2). One direct action on the NK1 receptor in coronary arteries may be nitric oxide–mediated vasodilation (1), although this effect may be impaired in patients with coronary artery disease (3), leading to a dominant NK2-mediated vasoconstriction. Both SP and neurokinin A are negatively inotropic and chronotropic, acting via cholinergic neurons (2), whereas NK1 antagonists improve inotropy and lusitropy in rat AMI models. SP attenuates the positive inotropic effect of norepinephrine (4) and, via the NK1 receptor, has been implicated in myocardial stunning post-AMI in guinea pigs (5).

SP is also expressed in platelets, where it has a pro-aggregatory effect (6). Furthermore, NK1 receptor inhibition reduces thrombus formation. Administration of an NK1 receptor antagonist reduced fibrinous adhesion formation and increased tissue plasminogen activator messenger ribonucleic acid and activity, suggesting that SP has a role in fibrinolysis (7). Also, SP strengthens clots formed in blood, an effect that may be mediated via leucocytes, the magnitude of which depends 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, although investigations in humans have been hampered by the very short half-life of SP (12 min) (11), so there are no large studies examining the role of SP in AMI. The recent development of an assay for stable pro-tachykinin A (Pro-substance P [ProSP]), 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 reinfarction.

METHODS

STUDY POPULATION. We studied 1,148 ST-segment elevation myocardial infarction (STEMI) and non-STEMI patients admitted to University Hospitals of Leicester National Health Service trust between August 1, 2004 and April 30, 2007. This observational cohort study complied with the Declaration of Helsinki and was approved by the local ethics committee; patients gave written informed consent. AMI was diagnosed if a patient had a cardiac troponin I level above the 99th percentile with ≥1 of the following: chest pain lasting >20 min 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 revascularization at the discretion of the attending physician.

PLASMA SAMPLES. Blood samples (anticoagulated with ethylenediaminetetraacetic acid and aprotinin) were drawn after 15 min 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 N-terminal pro-B-type natriuretic peptide (NT-proBNP).

ECHOCARDIOGRAPHY. Transthoracic echocardiography was performed in 895 patients (77.9%) during index admission, using either a Sonos 5500 or IE 33 instrument (Philips Medical Systems, Reigate, United Kingdom). A 16-segment left ventricular wall motion index score was performed on the basis of the American Society of Echocardiography method (15). In suitable patients, left ventricular ejection fraction was calculated using the biplane method of disks formula. Left ventricular systolic dysfunction was defined as either a left ventricular ejection fraction <40% or a left ventricular wall motion index >1.8.

GRACE (GLOBAL REGISTRY OF ACUTE CORONARY EVENTS) SCORING. On the basis of 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 MACE, defined as death and/or reinfarction (17). We used GRACE scores on discharge for comparison with later outcomes.

ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

AUC = area under the curve

CI = confidence interval(s)

eGFR = estimated glomerular filtration rate

HF = heart failure

HR = hazard ratio(s)

MACE = major adverse cardiac event(s)

NK = neurokinin(s)

NT-proBNP = N-terminal pro-B-type natriuretic peptide

ProSP = Pro-substance P

SP = substance P

STEMI = ST-segment elevation myocardial infarction

TABLE 1 Characteristics of Acute Myocardial Infarction Patients According to ProSP Quartiles on Admission

	All (n = 1,148)	ProSP Quartiles 1 <52.0 pmol/l (n = 288)	2 52.0–65.19 pmol/l (n = 286)	3 65.19–89.1 pmol/l (n = 288)	4 >89.1 pmol/l (n = 286)	p Value
ProSP, pmol/l	77.2 ± 55.7	42.2 ± 7.43	58.4 ± 4.0	75.6 ± 7.1	132.9 ± 87.4	<0.0005
NT-proBNP, pmol/l	1,849 ± 2,108	891.3 ± 1,062	1,339 ± 1,641	1,874 ± 2,030	3,300 ± 2,569	<0.0005
Demographics						
Age, yrs	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-segment 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/1.73 m^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	1,002 (87)	270 (94)	258 (90)	260 (90)	214 (75)	<0.0005
Loop diuretic agent	289 (25)	39 (14)	59 (21)	69 (24)	122 (43)	<0.0005
Revascularization	343 (30)	95 (33)	99 (35)	79 (27)	70 (24)	0.027
Endpoints, 2 yrs						
MACE	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
Nonfatal MACE	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
Reinfarction	149 (13)	29 (10)	35 (12)	33 (11)	52 (18)	0.021

Values are mean ± SD or n (%). The p values are quoted for the Kruskal-Wallis or chi-square tests for continuous or categorical variables, respectively.

ACE = angiotensin-converting enzyme; AMI = acute myocardial infarction; ARB = angiotensin II receptor blocker; eGFR = estimated glomerular filtration rate; GRACE = Global Registry of Acute Coronary Events; IHD = ischemic heart disease; LV = left ventricular; LVSD = left ventricular systolic dysfunction; MACE = major adverse cardiac event(s); NS = not significant; NT-proBNP = N-terminal pro-B-type natriuretic peptide; ProSP = pro-substance P.

BIOMARKER ASSAYS. The NT-proBNP assay was on the basis of a noncompetitive assay as previously published (18). Troponin I was measured using the Centaur cTnI Ultra immunoassay (Siemens Healthcare Diagnostics, Munich, Germany), which has a 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: a mouse monoclonal anti-ProSP antibody (against amino acid sequence 21 to 36 of ProSP) was used to coat polystyrene tubes. Polyclonal antibodies against amino acids 3 to 22 of the ProSP sequence were labelled with methyl-acridinium ester and served as the detector antibody. Standards (ProSP peptide; amino acids 1 to 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.

ENDPOINTS. The primary composite endpoint was MACE, defined as all-cause mortality, HF hospitalization, or recurrent 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 diuretic agents, inotropes, or intravenous nitrate. Reinfarction was determined using the universal definition for MI (13). Other secondary composite endpoints were death and/or reinfarction and death and/or HF readmission. The endpoint of death and/or recurrent AMI at 6 months was used in analyses involving the GRACE score as this time point was used in developing the risk score. Endpoints were obtained by reviewing the local hospital databases and patient 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, IBM Corp., Armonk, New York) and Stata (version 12.1, StataCorp LP, College Station, Texas). 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. Nonparametric 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 2,000 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 endpoints on univariate analysis (age, sex, previous history of ischemic heart disease, hypertension or diabetes, Killip class, eGFR, and biomarkers [log troponin I and log NT-proBNP]). 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 NT-proBNP, ProSP, and the GRACE score. To demonstrate independence from clinical variables and NT-proBNP or the GRACE score with and without NT-proBNP, the added value of ProSP was evaluated on the basis of the likelihood ratio chi-square 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 as described by Pencina et al. (19). We constructed classification trees using chi-square Automatic Interaction Detection (analysis performed using SPSS), which detects which biomarker has the strongest interaction with the dependent variable in stepwise 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, and more likely to have histories of hypertension, ischemic heart disease, diabetes, or HF. Higher ProSP levels also were associated with higher GRACE scores, NT-proBNP, 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 blood pressure (-0.178), NT-proBNP (0.428), wall motion score index

TABLE 2 General Linear Model Showing Independent Predictors of ProSP Levels

	Coefficient	Standard Error	Lower 95% CI	Upper 95% CI	p Value
eGFR	-0.00345	0.00045	-0.004	-0.003	<0.001
Age	0.003272	0.000548	0.002	0.004	<0.001
Killip class >1	0.045352	0.012334	0.021	0.070	<0.001
Male	0.011859	0.013149	-0.014	0.038	NS
History of IHD	0.015876	0.012434	-0.008	0.040	NS
History of hypertension	-0.00233	0.011583	-0.025	0.020	NS
History of 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
LVSD	0.004572	0.012434	-0.020	0.029	NS

Coefficients reported for 2,000 bootstrapped samples.
BP = blood pressure; CI = confidence interval; other abbreviations as in Table 1.

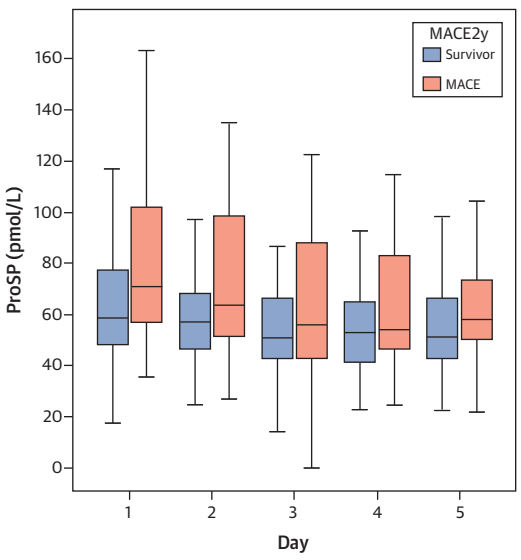


FIGURE 1 Profile of Plasma ProSP Over 5 Days After AMI

The profile includes those with post-AMI MACE at 2 years **(salmon)** or those without MACE at 2 years **(periwinkle)**. AMI = acute myocardial infarction; MACE = major adverse cardiac event(s); ProSP = pro-substance P.

TABLE 3 Cox Regression Analysis for MACE at 2 Years Post-AMI

	Univariable, HR (95% CI)	p Value	Multivariable Model 1, HR (95% CI)	p Value	Multivariable Model 2, HR (95% CI)	p Value
Age, yrs	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	0.62 (0.50–0.78)	0.001	1.09 (0.83–1.45)	NS	1.07 (0.81–1.42)	NS
ST-segment 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/1.73 m^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	1.01 (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						
IHD 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 NT-proBNP, 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 chi-square			152.39		171.30	0.0001*

Multivariable analysis results in model 1 included variables and biomarkers (except ProSP) that were significant on univariable analysis. Multivariable Model 2 used the variables in model 1 with the addition of ProSP as a continuous variable. *This p value is for the increment in log likelihood chi-square for models.

HR = hazard ratio; other abbreviations as in Tables 1 and 2.

(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 2,000 bootstrap samples showed eGFR, age, and Killip class > 1 as independent predictors of ProSP level (Table 2).

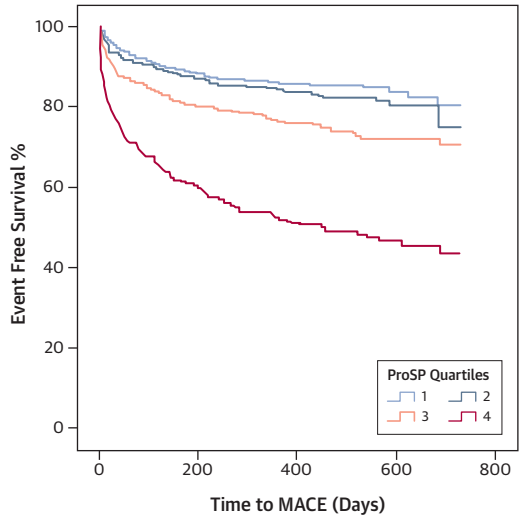


FIGURE 2 Kaplan-Meier Plots for the Endpoint of MACE According to ProSP Quartiles

Event-free survival according to ProSP quartiles are plotted, with follow-up over 2 years. Patients in the highest ProSP quartiles have the highest rates of death, heart failure, and reinfarction (MACE). Abbreviations as in Figure 1.

DAY CURVES FOR PROSP. Sequential plasma sampling over 5 days was available for 110 patients, of whom 29 had a MACE within 2 years. Figure 1 demonstrates the plasma profile along with a general linear model with repeated measures that show 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 were higher than on 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 2-year follow-up, patients with elevated ProSP levels (log_{10} transformed and standardized by 1 SD) had more MACE, deaths, and rehospitalizations with HF or reinfarction (Table 1). Table 3 shows the univariate hazard ratios (HR) of various factors that affected MACE. In multivariate analysis for predicting 2-year MACE, significant independent predictors included age, Killip class >1, eGFR, and NT-proBNP. Addition of ProSP to the model (model 2 in Table 3) showed ProSP had an independent predictive value (HR: 1.30; 95% confidence interval [95% CI]: 1.10 to 1.54; $p < 0.002$), and the added value of ProSP as evaluated by the likelihood ratio chi-square test for nested regression models was $p < 0.0001$. Kaplan-Meier survival analysis visualizes the MACE rates in ProSP quartiles (Figure 2), showing quartile 4 was significantly different from all other quartiles ($p < 0.0005$, log rank

TABLE 4 Cox Regression Analysis for Death and/or Reinfarction at 2 Years Post-AMI

	Univariable, HR (95% CI)	p Value	Multivariable Model 1, HR (95% CI)	p Value	Multivariable Model 2, HR (95% CI)	p Value
Age, yrs	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	0.66 (0.51–0.85)	0.001	1.15 (0.83–1.58)	NS	1.11 (0.81–1.52)	NS
ST-segment 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/1.73 m^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	1.01 (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						
IHD	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 NT-proBNP, 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 chi-square			93.45		119.72	0.0001*

Multivariable analysis results are reported for model 1, which included variables and biomarkers (except ProSP) that were significant on univariable analysis. Multivariable model 2 used the variables in model 1 with the addition of ProSP. *This p value is for the increment in log likelihood chi-square for models.

Abbreviations as in Tables 1, 2, and 3.

test [Mantel-Cox]), and quartile 3 was significantly different from quartiles 4 ($p < 0.0005$), 2 ($p < 0.022$), and 1 ($p < 0.001$).

There was no significant benefit with the inclusion of glucose in Cox survival models for MACE (HR: 1.11; 95% CI: 0.97 to 1.26; p = NS), but there was for ProSP (HR: 1.24; 95% CI: 1.03 to 1.49; p = 0.02). Inclusion of white cell count in survival models for MACE showed no effect either (HR: 0.98; 95% CI: 0.95 to 1.01; p = NS) compared with ProSP, which did (HR: 1.27; 95% CI: 1.06 to 1.53; p = 0.01).

In other models for prediction of the secondary composite endpoints of death and/or recurrent 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.

TABLE 5 Cox Regression Analysis for Death and/or HF at 2 Years Post-AMI

	Univariable, HR (95% CI)	p Value	Multivariable Model 1, HR (95% CI)	p Value	Multivariable Model 2, HR (95% CI)	p Value
Age, yrs	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	0.51 (0.39–0.66)	0.001	1.01 (0.72–1.41)	NS	0.98 (0.70–1.37)	NS
ST-segment 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/1.73 m^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	1.01 (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						
IHD	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 NT-proBNP, 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 chi-square			201.25		227.63	0.0001*

Multivariable analysis results are reported for model 1, which included variables and biomarkers (except ProSP) that were significant on univariable analysis. Multivariable model 2 used the variables in model 1 with the addition of ProSP. *This p value is for the increment in log likelihood chi-square for models.

HF = heart failure; other abbreviations as in Tables 1, 2, and 3.

TABLE 6 Cox Regression Analysis for Endpoints at 6 Months (MACE, Death and/or Reinfarction, Death and/or HF)

	Univariable, HR (95% CI)	p Value	Multivariable (ProSP Excluded), HR (95% CI)	p Value	Multivariable (NTproBNP Excluded), HR (95% CI)	p Value	Multivariable (ProSP and NTproBNP Included), HR (95% CI)	p Value
MACE								
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
NT-proBNP	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
LL chi-square	101.13 (GRACE only)		108.57	0.006*	122.84	0.0001*	126.63	0.0001*†
Death and/or reinfarction								
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
NT-proBNP	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
LL chi-square	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
NT-proBNP	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
LL chi-square	130.66 (GRACE only)		142.55	0.0001*	152.05	0.0001*	159.27	0.0001*†

LL chi-square refers to the log likelihood chi-square of the model with associated p value for added value of the biomarker(s). *Compared with GRACE only. †Compared with GRACE and NT-proBNP model. Abbreviations as in Tables 1, 2, 3, and 5.

In both models, ProSP showed added value to the clinical variables and NT-proBNP (log likelihood chi-square test $p < 0.0001$ for both composite endpoints).

COMPARISON WITH GRACE SCORES

The GRACE risk score (17) was originally derived for prediction of death and/or reinfarction at 6 months. We investigated the utility of the biomarkers NT-proBNP and ProSP for prediction of death and/or reinfarction as well as other composite endpoints (MACE, death, and/or HF). In univariate analysis, GRACE scores and the biomarkers NT-proBNP and ProSP predicted all composite endpoints (Table 6). In multivariate analysis for MACE, death, and/or recurrent AMI, and death and/or HF at 6 months, both NT-proBNP and ProSP demonstrated added value to the GRACE score. Moreover, ProSP showed added value to models with GRACE and NT-proBNP for all composite endpoints analyzed ($p < 0.0001$ for all) (Table 6).

Using receiver-operating characteristic curve analysis for death and/or reinfarction at 6 months, the area under the curve (AUC) increased from 0.69

TABLE 7 Reclassification Analysis Using Continuous Reclassification

Outcome	6-Month Death/MI		6-Month MACE		6-Month Death/HF	
Endpoint	NRI (95% CI)	p Value	NRI (95% CI)	p Value	NRI (95% CI)	p Value
Adding NT-proBNP to GRACE						
No	-7.7 (-14.5 to -1.0)	0.025	-7.5 (-14.5 to -0.6)	0.033	7.2 (0.5 to 13.9)	0.034
Yes	37.3 (21.3 to 53.3)	0.0005	33.7 (19.6 to 47.8)	0.0005	37.4 (20.3 to 54.5)	0.0005
Total	29.6 (12.2 to 47.0)	0.001	26.1 (10.4 to 41.9)	0.001	44.6 (26.2 to 63.0)	0.0005
Adding ProSP to GRACE						
No	22.3 (15.5 to 29.1)	0.0005	18.6 (11.6 to 25.5)	0.0005	20.0 (13.4 to 26.7)	0.0005
Yes	9.3 (-6.7 to 25.3)	NS	4.7 (-9.4 to 18.8)	NS	8.4 (-8.7 to 25.5)	NS
Total	31.6 (14.2 to 49.0)	0.0005	23.3 (7.5 to 39.0)	0.004	28.4 (10.1 to 46.8)	0.002
Adding ProSP to GRACE and NT-proBNP						
No	18.0 (11.2 to 24.8)	0.0005	13.6 (6.6 to 20.5)	0.0005	3.3 (-3.4 to 10.0)	NS
Yes	9.3 (-6.7 to 25.3)	NS	2.6 (-11.5 to 16.7)	NS	6.9 (-10.3 to 24.0)	NS
Total	27.3 (10.0 to 44.7)	0.002	16.2 (0.4 to 31.9)	0.044	10.1 (-8.3 to 28.5)	NS

Analysis shows the NRI and the significance of the NRI, of adding NT-proBNP or ProSP to the classification using GRACE scoring only, and for adding ProSP to the classification using GRACE scoring with NT-proBNP, for the endpoints of death and/or reinfarction, MACE, and death and/or HF at 6 months.

MI = myocardial infarction; NRI = net reclassification improvement; other abbreviations as in Tables 1, 2, and 5.

(95% CI: 0.65 to 0.74) for GRACE scoring only to 0.72 (95% CI: 0.68 to 0.77) with the addition of ProSP ($p = 0.01$). Addition of NT-proBNP to the GRACE score yielded a higher area (AUC: 0.70; 95% CI: 0.65 to 0.75; p = NS). Comparison of the areas for GRACE score and NT-proBNP (AUC: 0.70; 95% CI: 0.65 to 0.75) and that of GRACE score, NT-proBNP, and ProSP (AUC: 0.72; 95% CI: 0.68 to 0.77) was not significant ($p = 0.06$).

RECLASSIFICATION ANALYSIS. Category-free reclassification analysis (with no arbitrary cutoff probabilities) was employed to calculate the net reclassification improvement for the effect of adding NT-proBNP or ProSP to the probabilities derived from the GRACE score in predicting the endpoints of death and/or reinfarction, MACE, and death and/or HF (Table 7). NT-proBNP up-classified risk in all those with events for all these composite endpoints. However, it wrongly (and significantly) up-classified risk in those without events for the death and/or reinfarction and MACE endpoints, although it correctly down-classified risk in those without events for the death and/or HF endpoint. However, ProSP correctly down-classified risk in those without events for all composite endpoints. When ProSP was added to a composite risk score composed of GRACE and NT-proBNP, ProSP down-classified risk in those without events for the endpoints of MACE and death and/or MI, but did not significantly reclassify those with the specific endpoint of death and/or HF (Table 7).

DECISION TREE ANALYSIS. To determine optimal cut points for biomarkers, we constructed decision trees (using ProSP and NT-proBNP levels and GRACE scores) to classify patients for event-free survival or those with an endpoint of death and/or reinfarction at 6 months. Using ProSP as an initial classifier (Figure 3), a ProSP level <72.08 pmol/l and GRACE score <137 defines a low-risk group of patients (n = 512, 44.6% of the total) who had an event rate that was 3.0%. Of these, only 1 (0.09%) had died within 30 days, and 3 patients (0.26%) had died within 6 months. ProSP levels >121.6 pmol/l defined a high-risk group of patients with a death/recurrent AMI rate of 37.7% and a death rate of 30.7% (Figure 3).

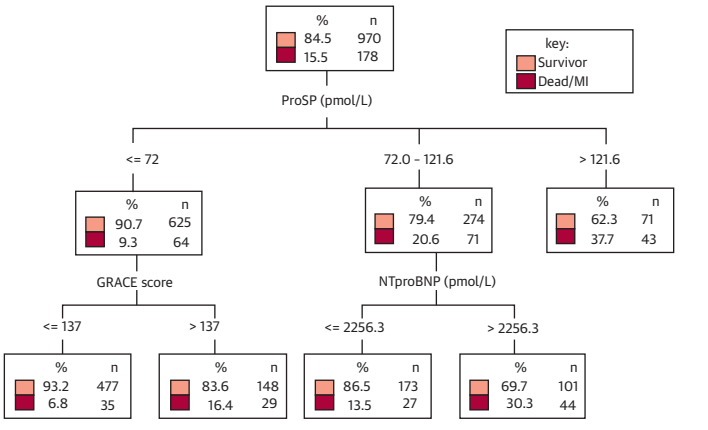


FIGURE 3 Classification Tree for the Endpoint of Death and/or Reinfarction at 6 Months

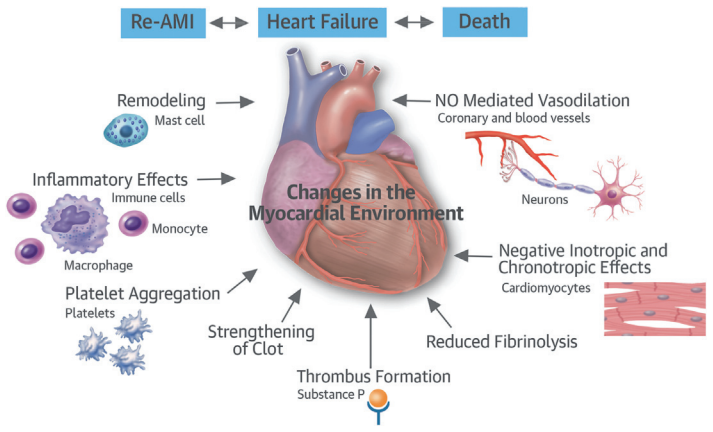
A classification tree using plasma ProSP as the initial classifier, followed by the GRACE score or plasma NT-proBNP enables effective selection of low- and high-risk groups of patients after acute myocardial infarction. GRACE = Global Registry of Acute Coronary Events; MI = myocardial infarction; NT-proBNP = N-terminal pro-B-type natriuretic peptide; ProSP = pro-substance P.

DISCUSSION

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 ischemic heart disease, Killip class, wall motion index, sex, and blood pressure. There was no relation to infarct size. ProSP may therefore closely reflect a patient's renal function at AMI presentation. ProSP levels peaked at days 1 and 2 after 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 NT-proBNP mainly predicted mortality and HF, with poorer detection of death and/or reinfarction. In contrast, ProSP provided independent prognostic information for the composite of MACE, death and/or reinfarction, and also death and/or HF. These analyses suggest that SP may potentially have a role in the pathophysiology of outcomes after AMI.

Analysis of the increment in receiver-operating characteristic AUC showed that addition of ProSP had small effects on this area. However, such analyses are relatively insensitive to the addition of novel biomarkers (20) and reclassification analyses should also be performed. For example, NT-proBNP demonstrated a small nonsignificant increase in receiver-operating characteristic 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 the 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



CENTRAL ILLUSTRATION **Pathophysiological Pathways Involving Substance P After Myocardial Infarction**

Substance P may affect cardiac function through effects on vasculature, platelets, clot strength, inflammation, and myocardial remodeling and contractility, resulting in adverse outcomes (death, reinfarction, and heart failure). AMI = acute myocardial infarction; NO = nitric oxide.

would be especially useful in detecting patients with low risk, which was confirmed on decision tree analysis. ProSP levels <72.1 pmol/l may 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 NT-proBNP could up- or down-classify risk in those assessed with the GRACE score, but ProSP only showed added value for the endpoints of MACE and death and/or MI not death and/or HF. However, because 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 (Central Illustration). The association with HF rehospitalization could be due to the known negative inotropic and lusitropic effects of SP that have been demonstrated in animal models of AMI (4,5). SP is expressed in monocytes and macrophages (21) and may play a role in inflammation (22) as well as leukocyte chemotaxis and egress from vessels (1,2), which may also affect myocardial function. 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 tumor necrosis factor alpha (23), and remodelling also was reduced in TAC1 gene knockout mice. Mast cells are co-localized 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 for 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, but SP strengthens a clot after formation (8), 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 also may have some potentially beneficial roles in ischemia post-conditioning (26) and in mobilization of progenitor cells that may play a role in angiogenesis within ischemic tissue (27). Our findings are hypothesis-generating for investigating the role of SP on outcomes after AMI, as it is uncertain whether beneficial effects of SP could be outweighed by their deleterious effects.

STUDY LIMITATIONS. Our findings are on the basis of a population from a single center, with 2 admitting hospitals, and should be verified in other larger populations. The rate of early revascularization in our non-STEMI population was low and may not reflect the more contemporary invasive approach of revascularization within 72 h of presentation. One advantage is that the relation of ProSP with poor outcomes would not have been confounded by higher early revascularization rates.

CONCLUSIONS

After AMI, circulating ProSP levels provide added value to the prognostic information determined by the GRACE score and the prognostically important biomarker NT-proBNP. 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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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Pro-substance P provides information beyond that of existing clinical risk assessment tools and may have prognostic value in patients with myocardial infarction as a predictor of heart failure, reinfarction, and survival.

TRANSLATIONAL OUTLOOK 1: Larger multicenter studies are needed to explore the value of Pro-substance P in clinical risk stratification among survivors of acute coronary syndromes.

TRANSLATIONAL OUTLOOK 2: The effect of neurokinin receptor inhibitors on clinical outcomes after myocardial infarction requires further investigation.

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KEY WORDS acute coronary syndrome(s), B-type natriuretic peptide, GRACE score, myocardial infarction, neurokinins, substance P