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Proteinase 3 and prognosis of patients with acute myocardial infarction

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Keywords Myocardial infarction, peptides, proteinase 3, myeloperoxidase, neutrophil, prognosis

Conflicts of interest:

Prof. L. Ng has submitted patents on behalf of the University of Leicester and British Heart Foundation on biomarkers in risk stratification of acute coronary syndromes.

Abstract**Background**

A multimarker approach may be useful for risk stratification in AMI patients, particularly utilising pathways that are pathophysiologically distinct.

Aim

Our aim was to assess the prognostic value of Proteinase 3 in patients post acute myocardial infarction (AMI). We compared the prognostic value of Proteinase 3, an inflammatory marker to an established marker N-terminal pro-B-type natriuretic peptide (NT-proBNP) post-AMI.

Method

We recruited 900 consecutive post-AMI patients (700 men, age 64.6 ± 12.4 years) in a prospective study with follow-up over 347[0-764] days.

Results

Plasma Proteinase 3 was significantly higher in patients who died (666.2 [226.8-4035.5] ng/ml, $P < 0.001$) or were readmitted with heart failure (598 [231.6-1803.9] ng/ml, $P < 0.004$) compared to event free survivors (486.9 [29.3-3118.2] ng/ml).

Using Cox modelling \log_{10} Proteinase 3 (HR 3.80) and \log_{10} NT-proBNP (HR 2.51) were significant independent predictors of death or heart failure. When patients were stratified by plasma NTproBNP (median 1023 pmol/L), Proteinase 3 gave additional predictive value for death or heart failure, in both the patients in whom NTproBNP level was above the median (log rank for trend 12.54, $p < 0.0004$) and those with NTproBNP level below the median (log rank for trend 3.83, $p < 0.05$). Neither marker predicted recurrent AMI.

Conclusion

This is the first report showing a potential role for the serine protease Proteinase 3 in determining mortality and incidence of heart failure following AMI, independent of established conventional risk factors. Proteinase 3 may represent a clinically useful marker of prognosis after an AMI as part of a multimarker strategy.

Introduction

Atherosclerosis is associated with inflammatory changes in the blood vessel wall [1], including macrophage and lymphocyte infiltration. There is an association between neutrophil count and the risk of future acute coronary syndromes [2], and recent evidence has documented elevated neutrophil activity in acute coronary syndromes [3,4]. Despite their role in plaque destabilisation and acute coronary syndromes, it is not known whether neutrophil activation, with degranulation of their proteolytic enzymes might play a role in development of chronic heart failure and the risk of mortality following an acute myocardial infarction (AMI).

Myeloperoxidase (MPO), an enzyme present within the azurophilic granules of neutrophils, may stimulate plaque destabilisation by activation of metalloproteinases [5]. Plasma levels of MPO are raised in coronary artery disease [6], and predict outcome of patients presenting with chest pain [7] and acute coronary syndromes [8,9]. Proteinase 3 (PR3) is another major component of neutrophil azurophilic granules, together with other serine proteases such as elastase and cathepsin G [10]. Azurophilic granules are released in response to powerful stimuli to neutrophils, whereas secretory granules (which also contain PR3 [11]) are released with weaker stimuli. PR3 may also be expressed on endothelial cells [12]. Neutrophil serine proteases may degrade extracellular matrix; in addition, PR3 has other deleterious effects which may be relevant to a role in pathogenesis of vasculitis in Wegener's granulomatosis and potentially in prognosis of patients post-AMI. These include induction of apoptosis through a caspase-like activity on endothelial cells [13], releasing activated TNF α from its nascent membrane bound precursor form [14,15], activation of the pro-inflammatory mediators interleukin-1 β [15] and interleukin-18 [16] and generation of angiotensin I and II from angiotensinogen [17]. Although PR3 and autoantibodies to it (anti-neutrophil cytoplasmic antibodies or ANCA) are prime candidates for pathogenesis of vasculitis and tissue damage in the relatively uncommon autoimmune condition of Wegener's granulomatosis, the association of PR3 with morbidity and mortality in common medical vascular presentations such as AMI has never been examined.

PR3 is rapidly inactivated by irreversible binding to α -1-antitrypsin (SERPIN A1) [18,19] where the serine protease reacts with the reactive centre loop of the serpin, generating a covalently bound intermediate resulting in the distortion and inactivation of the protease [20]. Thus, little free PR3 exists in plasma and it is mainly present as a PR3-SERPIN A1 complex [18].

We hypothesised that measurement of such PR3-SERPIN A1 complexes would provide prognostic information on heart failure and mortality post-AMI, even when used in conjunction with recognised markers of prognosis such as B-type natriuretic peptide or its precursor (N-terminal proB-type natriuretic peptide or NTproBNP) in order to establish a role for neutrophil activation in determining these adverse outcomes. Since PR3 is easily mobilised from secretory granules compared to azurophilic granules, it may also be a better marker of neutrophil activation than MPO.

Methods

Study population

We recruited 900 consecutive patients with acute myocardial infarction admitted to the Coronary Care Unit of Leicester Royal Infirmary between 1st September 2003 and 31st July 2007. Written informed consent was obtained from patients and the study complied with the Declaration of Helsinki and was approved by the local ethics committee. 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. Exclusion criteria included malignancy or recent surgery (within one month). Hypertension was defined as a blood pressure measurement of greater than 140/90 on 2 or more occasions or if the patient was on medication for hypertension. Hypercholesterolaemia was defined as a total cholesterol of greater than 5.0 mmol/L, or treatment with a statin. Diabetes was defined as a random plasma glucose of 11.1mmol/L, fasting plasma glucose of 7mmol/L or a history of diabetes on treatment.

Plasma samples

Blood samples were drawn at 2-5 days after the onset of chest pain for determination of plasma PR3-SERPIN A1 and NTproBNP. After 15 minutes bed rest, 20mL blood was collected into tubes containing EDTA and aprotinin. All plasma was stored at -80°C until assayed, blind to clinical details, in a single batch.

Echocardiography

Transthoracic echocardiography was performed in patients using a Sonos 5500 instrument (Philips Medical Systems, Reigate, UK), at 3-5 days post-admission. A 16-segment left ventricular wall motion index (LVWMI) based on the American Society of Echocardiography mode was derived by scoring each LV segment (1=normal, 2=hypokinesis, 3=akinesis and 4=dyskinesis (Paradoxical Motion), and dividing the total by the number of segments scored. Left ventricular ejection fraction (LVEF) was calculated using the biplane method of discs formula [21]. Impaired LV systolic function was defined as an EF<40% or a LVWMI >1.8.

NTproBNP assay

Plasma NTproBNP assay was measured using a non-competitive immunoluminometric assay as previously published [22]. Samples or NTproBNP standards (10µL) were incubated in ELISA plate wells coated with mouse monoclonal IgG directed to the C-terminal of NTproBNP. Detection was with biotinylated rabbit N-terminal antibody followed by 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. This highly specific assay shows no cross-reactivity with atrial natriuretic peptide, BNP, or C-type natriuretic peptide.

PR3-SERPIN A1 assay

Plasma PR3-SERPIN A1 was measured using a non-competitive immuno-luminometric assay modified from a previously published method [18]. ELISA plate wells were coated with 200ng of mouse monoclonal antibody to PR3 (clone 1B10, IgG_{2a}, HyTest Ltd., Turku, Finland). Standards were produced by incubating PR3 with a 20 fold molar excess of SERPIN A1 (both from Sigma Aldrich Co. Ltd., Gillingham, UK). Samples or standards (10µL) were incubated with assay buffer (100µL) in the coated wells for 24 h at 4°C. Following washes, a specific SERPIN A1 rabbit antibody (Dako UK Ltd., Ely, UK) was pipetted into the wells (20ng/100µL) and incubated for 3 h with shaking at 250 Hz at room temperature. Following washes, a goat biotinylated anti-rabbit IgG (Rockland Immunochemicals Inc., Gilbertsville, PA, USA, previously pre-adsorbed with human, rabbit, mouse serum proteins) at a dilution of 1:100000 was incubated within the wells for 1 h, followed by MAE-labelled streptavidin for another 1½ hours. Chemiluminescence was elicited with sequential injections of H₂O₂ in nitric acid, followed by sodium hydroxide containing cetyl trimethylammonium bromide, as described [22]. Intra and inter-assay coefficients of variation were found to be less than 10%.

Myeloperoxidase (MPO) assay

This was based on a 2 site non-competitive immuno-luminometric assay employed an ELISA plate immobilised monoclonal MPO antibody (100ng/100µL, Research Diagnostics Inc., New Jersey, USA) and a rabbit polyclonal antibody (50ng/100µL, Merck BioSciences, Nottingham, UK), as previously described [23]. Standards and 10 µL of plasma were incubated in plates overnight, before washing and detection using goat biotinylated anti-rabbit IgG and streptavidin-MAE, as described for the PR3-SERPIN A1 assay. The MPO assays had inter- and intra-assay coefficients of variation <10%, with a lower limit of detection of 0.3 ng/ml.

End points

The primary endpoint was the combination of all-cause mortality and hospitalisation for heart failure. Secondary endpoints included the occurrence of further myocardial infarction. Hospitalization for heart failure was defined as a hospital admission for which heart failure was the primary reason, requiring treatment with high dose diuretics, inotropes, or intravenous nitrate. Deaths were validated by reviewing the hospital record management systems and the Office of National Statistics Registry. Heart failure episodes were identified through the hospital record management systems and validated 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 14.0 (SPSS Inc, Chicago, Illinois). Continuous variables were compared using the Mann Whitney U test. ANOVA with the Bonferroni correction for multiple comparisons was employed when more than 2 groups were compared. Correlation analysis employed Spearman's rho, and binary logistic regression and Cox proportional hazard analyses were used to develop models in order to test the independent predictive power for factors and variables. Plasma levels of NTproBNP, MPO and PR3-SERPIN A1 were normalised by log transformation. Thus, odds ratios and hazard ratios refer to a tenfold rise in the levels of these markers. Kaplan-Meier survival analysis was used to assess dichotomised values of NTproBNP and PR3-SERPIN A1 and the log rank test for used for comparison of groups. Receiver-operating characteristic (ROC) curves were generated and the area under the curves (AUC) was calculated to assess the accuracy of predictors [24]. A 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. Roles of authors include:- design of study and assay construction LN; recruitment of patients SQK; echocardiography analysis SOK and JED; assays LN, PQ; analysis of data LN, SQK, IBS, HN; manuscript preparation, all authors.

Results

Patient characteristics

The demographic features of the patient population are shown in Table 1. Median length of follow-up was 347 days with a range of 0–764 days. No patient was lost to follow-up. The index AMI was classed as ST-elevation MI (STEMI) in 777 of the patients, 529 (68.1%) of whom received thrombolytic therapy. Echocardiographic data were available for 622 (69.1%) of the patients during the index admission. During follow-up, 92 (10.2%) of the 900 patients died, 66 (7.3%) experienced readmission with heart failure, 143 (15.9%) patients experienced either end-point and 62 (6.9%) experienced hospitalisation with recurrent AMI.

Plasma PR3-SERPIN A1, NTproBNP and MPO levels

Plasma levels of PR3-SERPIN A1 in patients with AMI ranged from 29.3-4035.5 ng/ml with a median of 504.6 ng/ml, and were elevated compared to the established normal range (median [range] 350 [110-580] ng/ml). PR3-SERPIN A1 was significantly higher in patients who died (666.2 [226.8-4035.5] ng/ml, $P < 0.001$ using Bonferroni's correction) or were readmitted with heart failure (598 [231.6-1803.9] ng/ml, $P < 0.004$) compared to event free survivors (486.9 [29.3-3118.2] ng/ml). In contrast, levels of PR3-SERPIN A1 in those readmitted with recurrent AMI (482.5 [168.9-1753.9] ng/ml) were similar to those in event free survivors. PR3-SERPIN A1 levels were similar in males and females, and in those with or without previous histories of hypertension, angina, AMI or heart failure, but were slightly elevated in those with diabetes (547.0 [76.9-4035.5] ng/ml) vs. non-diabetic patients (495.1 [29.3-3118.2] ng/ml, $P < 0.018$).

Plasma PR3-SERPIN A1 levels were modestly correlated with plasma glucose ($r_s = 0.08$, $p < 0.03$), log creatinine ($r_s = 0.13$, $p < 0.001$), Killip class ($r_s = 0.10$, $p < 0.002$), NTproBNP ($r_s = 0.25$, $p < 0.001$) and creatine kinase ($r_s = 0.13$, $p < 0.001$) but had no relationship to age, site of infarction, presence or absence of ST elevation and whether thrombolysis was administered or not.

In confirmation of previous work, plasma NTproBNP was higher in patients who died (5955.4 [9.02-14057.2] pmol/L) or were readmitted with heart failure (3106.6 [2.4 - 12282.9] pmol/L) compared to event free survivors (806.8 [0.3-28886] pmol/L; $p < 0.001$ for both). NTproBNP levels were higher in females (1951.7 [22.9-15732] pmol/L) compared to males (871.6 [0.3-28886] pmol/ml; $p < 0.001$) and correlated with age ($r_s = 0.46$, $p < 0.001$), plasma glucose ($r_s = 0.15$, $p < 0.001$), log creatinine ($r_s = 0.27$, $p < 0.001$), Killip class ($r_s = 0.29$, $p < 0.001$) and creatine kinase ($r_s = 0.13$, $p < 0.001$).

Plasma MPO was significantly higher in patients who died (36.7 [6.44-132.1] ng/ml, $P < 0.035$ using Bonferroni's correction) compared to event free survivors (24.6 [0.3-405.2] ng/ml). In contrast those who were readmitted with heart failure (27.6 [6.5-210.8] ng/ml) or with recurrent AMI (23.9 [5.3-189.9] ng/ml) had similar values of MPO to event free survivors. MPO levels were higher in females (30.3 [0.3-405.2] ng/ml) compared to males (23.9 [3.6-215.1] ng/ml; $p < 0.001$) and in patients with diabetes (30.4 [5.1-238.7] ng/ml) compared to non-diabetic patients (24.0 [0.3-405.2] ng/ml; $p < 0.001$) and were correlated with age ($r_s = 0.12$, $p < 0.001$), plasma glucose ($r_s = 0.10$, $p < 0.009$), log creatinine ($r_s = 0.11$, $p < 0.001$), and Killip class ($r_s = 0.11$, $p < 0.002$).

There was a modest correlation of Plasma PR3-SERPIN A1 with impaired LV systolic function ($r_s = 0.16$, $p = 0.001$) on the index admission (as defined by $EF < 40\%$ or $LVWMI > 1.8$ on echocardiography) with a stronger relationship demonstrated for NTproBNP ($r_s = 0.35$, $p = 0.001$). Plasma MPO was weakly correlated to PR3-SERPIN A1 and NTproBNP ($r_s = 0.11$ and 0.12 respectively, $p = 0.002$) but not to LV systolic function.

Primary Endpoints: PR3-SERPIN A1 and NTproBNP as predictors of death or heart failure

Binary logistic models were developed for prediction of death or heart failure, using the following clinical and demographic variables:- age, gender, past history of AMI, hypertension or diabetes, Killip Class > 1 , thrombolytic use, log creatinine, NTproBNP, MPO and PR3-SERPIN A1. Independent predictors are reported in Table 2 and include NTproBNP and PR3-SERPIN A1 as significant predictor variables. The Nagelkerke r^2 was 0.35 suggesting a good fit of the model.

The receiver-operating-characteristic curve for the predictive value of PR3-SERPIN A1 for the primary endpoint yielded an area under the curve (AUC) of 0.65 (95% CI: 0.60-0.70, $p < 0.001$); for NTproBNP the AUC was 0.78 (95% CI: 0.74-0.83, $p < 0.001$). The logistic

model combining the 2 markers yielded an AUC of 0.84 (95% CI: 0.80-0.88, $p < 0.001$), which exceeded that of either peptide alone ($p < 0.003$ by method of Hanley and McNeil [24]). Cox proportional hazards modelling confirmed these findings, identifying the same independent predictors of death or heart failure apart from gender (Table 3). In both logistic and Cox models, plasma MPO was not an independent predictor of death or heart failure. Kaplan-Meier survival analysis demonstrated a significantly better clinical outcome in patients with PR3-SERPIN A1 below the median (504.6 ng/ml) compared with those with PR3-SERPIN A1 above the median (log rank 28.15, $p < 0.0001$, figure 1). The event free survival curves diverge early and continue to diverge even after 2 years. This was also true for NTproBNP (log rank 64.72, $p < 0.0001$). When patients were stratified by plasma NTproBNP (median 1023 pmol/L), PR3-SERPIN A1 gave additional predictive value for death or heart failure, in both the patients in whom NTproBNP level was above the median (log rank for trend 12.54 $p < 0.0004$) and those with NTproBNP level below the median (log rank for trend 3.83 $p < 0.05$). Those patients with both markers elevated above their respective medians had higher event rates than those with either marker elevated above median, and these in turn had higher event rates than those with both markers below their respective medians ($p < 0.001$ for all comparisons, figure 2).

Troponin levels were available for 414 patients. Inclusion of troponin in the Cox model for death or heart failure led to the following hazard ratios (and p values) : age (1.07, $p < 0.001$), Killip Class > 1 (1.83, $p < 0.04$), log creatinine (6.05, $p < 0.06$), past history of AMI (1.70, $p < 0.07$), NTproBNP (1.74, $p < 0.08$), troponin (1.18, $p = 0.3$), PR3-SERPIN A1 (2.83, $p < 0.06$).

Echocardiography scans were analysable on 622 subjects, of which 203 showed evidence of LV systolic dysfunction (as defined by $EF < 40\%$ or $LVWMI > 1.8$). Inclusion of echocardiography evidence of systolic dysfunction into the Cox model for death or heart failure led to the following hazard ratios (and p values) : age (1.04, $p < 0.001$), Killip Class > 1 (2.17, $p < 0.005$), log creatinine (6.55, $p < 0.01$), past history of AMI (1.40, $p = 0.13$), NTproBNP (1.92, $p < 0.007$), LV systolic dysfunction (1.39, $p = 0.13$), PR3-SERPIN A1 (2.88, $p < 0.02$).

Reclassification analyses for predictors of death or heart failure

The GRACE score [25] is a recognised risk predictor in AMI, enabling patients to be classified into low, intermediate and high risk groups for major adverse cardiac events after an acute coronary syndrome, and incorporates many of the above predictors for death or heart failure e.g. age, history of AMI, elevated plasma creatinine, elevated biomarker levels, Killip class. In this present study, GRACE score was a significant predictor of death or heart failure (hazard ratio 1.03 (95% confidence interval 1.02-1.04, $p < 0.0005$). In a model for predicting death or heart failure using GRACE score, NTproBNP and PR3-SERPIN A1, all 3 remained significant predictors (hazard ratio [95% confidence interval] p values were respectively, GRACE score 1.02 [1.01-1.03], $p < 0.0005$; NTproBNP 2.67 [1.79-3.99], $p < 0.0005$; PR3-SERPIN A1 4.08 [1.89-8.79], $p < 0.0005$).

Reclassification analyses were therefore performed using GRACE score, with the addition of NTproBNP and PR3-SERPIN A1 as described by Pencina et al [26]. NTproBNP led to significant up-classification of patients with endpoints and down-classification of event free survivors (Table 4), when added to the GRACE score ($p < 0.0002$). PR3-SERPIN A1 in addition to the GRACE score also led to significant ($p < 0.02$) up-classification of patients with endpoints, although when added to GRACE score with NTproBNP, this up-classification was not significant ($p = 0.14$).

Secondary Endpoints: PR3-SERPIN A1 and NTproBNP as predictors of myocardial infarction

Compared to survivors with no endpoints, patients who were readmitted with further AMI during follow up had similar NTproBNP (median [range] 1480.7[2.6-10645.9] vs. 806.8[0.3-28886] pmol/L; p=NS), PR3-SERPIN A1 levels (median [range] 482.5[168.9-1753.9] vs. 486.9[29.3-3118.2] ng/ml; p=NS) and MPO levels (23.9 [5.3-189.9] vs. 24.6 [0.3-405.2] ng/ml; p=NS).

Discussion

This study reports for the first time the prognostic potential of PR3-SERPIN A1 in a large cohort of patients with AMI, recruited prospectively from a single centre. This complex of a serine protease and its inhibitor provided prediction of death or heart failure, independently of established conventional risk factors such as renal function and clinical demographic information and the GRACE score. Moreover this novel prognostic marker complements the information provided by an established marker of poor prognosis post AMI, namely NTproBNP. The use of PR3-SERPIN A1 for independent prediction of death or heart failure together with troponin levels or echocardiographic evidence of systolic dysfunction failed to achieve conventional levels of statistical significance due to the reduced number of subjects with data available for analysis. Reclassification analyses with the GRACE score suggested that PR3-SERPIN A1 had utility, mainly in up-classifying patients with endpoints, in contrast to NTproBNP which up-classified those with endpoints and down-classified those without endpoints. However, adding PR3-SERPIN A1 to models with GRACE score and NTproBNP led to insignificant up-classification of patients with endpoints. The study may have been underpowered to detect significant reclassification in this analysis.

The measurement of PR3 levels in the post AMI setting is supported by strong evidence of its damaging role on cells, such as its pro-apoptotic activity [13] and its pro-inflammatory effect, by releasing the active forms of both TNF α , interleukin-1 β , interleukin-18 [14-16] and the angiotensins [17]. These cytokines may be especially relevant to development of heart failure post-AMI, which still occurs despite therapy with inhibitors of the renin-angiotensin system. The involvement of such serine proteases in the development of heart failure and their association with increased risk of adverse outcome post-AMI may support the search for new agents that could attenuate the damaging effects of these enzymes. For example, there is preliminary evidence that elafin, a serine protease inhibitor could reduce the vascular and myocardial damage in experimental models of cardiac transplantation [27].

The source of circulating PR3 may be partly from degranulating, activated neutrophils, being present in both azurophilic and secretory granules. Neutrophils are known to infiltrate myocardium after infarction, although their direct contribution to the development of heart failure to mortality after AMI has not been clarified. Alternatively, or additionally, PR3 may be secreted from endothelial cells [12] or from lung parenchymal cells [28]. Pneumocytes in normal lung have been demonstrated to express PR3 [28], although expression is greatly upregulated in Wegener's Granulomatosis. Active PR3 is rapidly inactivated in the plasma by SERPIN A1 and alpha2-macroglobulin [10] the PR3-SERPIN A1 complex can therefore only be formed with an active protease and may therefore be a good measure of the amount of active enzyme that has been secreted. However, these plasma borne inhibitors may not have rapid access to extravascular sites where the enzyme could be released post-AMI. The levels of the PR3-SERPIN A1 complex that we have demonstrated in patients who have a poor outcome post-AMI are very similar to those described in patients with active vasculitis in Wegener's Granulomatosis (median 560 [range 110-3940] ng/ml) [29]. In normal subjects

and those with inactive vasculitis, levels of about 350 (median [range 110-580] ng/ml) have been reported [29], which are lower than our values for our event-free post-AMI survivors. In this cohort of AMI patients, MPO did not provide independent prediction of death or heart failure, when models included NTproBNP and PR3-SERPIN A1. MPO is localised within neutrophil azurophilic granules alone, which are released with strong stimuli [10], in contrast to the presence of easily mobilisable pool of PR3 in secretory granules [11]. In addition, previous studies with MPO have included patients with acute coronary syndromes or chest pain [7,8] which may be a different spectrum from those with more myocardial damage. Indeed, Baldus et al [8] reported prognostic potential of MPO in those with low or medium Troponin T elevations, with poorer predictive potential in those with high Troponin T levels. Survival analysis revealed that those at highest risk were those with NTproBNP elevated above the median of 1023 pmol/L and PR3-SERPIN A1 above the median of 504.6 ng/ml. It is uncertain whether intensification of therapy or provision of timely interventional procedures could have altered clinical outcome, but this remains to be investigated.

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, and the utility of PR3-SERPIN A1 in patients with other acute coronary syndromes should be investigated (eg in those with non-ST elevation AMI or chest pain). Larger studies may need to be performed to confirm the utility of PR3-SERPIN A1 in reclassification analyses.

Conclusion

This is the first report showing a potential role for the serine protease PR3 in determining mortality and incidence of heart failure following AMI, independent of established conventional risk factors and also the newly introduced biomarker NTproBNP. The involvement of PR3 in pathophysiology of Wegener's granulomatosis may extend to more common presentations of vascular disease. A multimarker approach with clinical risk scores and either or both of PR3-SERPIN A1 and NTproBNP may be useful for risk stratification in AMI patients, both markers providing complementary independent prediction, and the identification of such high risk patients may facilitate their subsequent management.

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Table 1 Characteristics of patients in the study. Values are means (SD) or numbers (%)

	AMI Patients
Number (male)	900 (700)
Age (in years)	64.6 ± 12.4
Previous Medical History	
Myocardial infarction	165 (18.3)
Angina Pectoris	195 (21.7)
Hypertension	391 (43.4)
Diabetes mellitus	192 (21.3)
Hypercholesterolaemia	221 (24.6)
Current/Ex-Smokers	560 (62.2)
ST-elevation AMI	777 (86.3)
Thrombolytic	529 (58.8)
Territory of Infarct	
Anterior	369 (41.0)
Inferior	373 (41.4)
Other/undetermined	158 (17.6)
Killip Class on Admission	
I	457 (50.7)
II	357 (39.7)
III	77 (8.6)
IV	9 (1.0)
Peak CK (IU/L)	1157 ± 1263
Creatinine (µmol/L)	103.9 ± 35.4
Medication	
Aspirin	794 (88.2)
ACE inhibitors/ Angiotensin receptor blockers	683 (75.9)
Beta blockers	721 (80.1)
Statins	701 (77.9)

Table 2 Binary Logistic regression model for prediction of death and heart failure

Variable	Odds Ratio	95% CI	p value
Age	1.04	1.02-1.07	0.001
Gender	.57	0.35-0.93	0.024
Past history AMI	2.28	1.43-3.74	0.001
Killip Class >1	1.66	1.05-2.63	0.032
Log Creatinine	18.55	3.02-113.9	0.001
Log NTproBNP	2.79	1.79-4.37	0.001
Log PR3-SERPIN A1	6.42	2.25-18.3	0.001

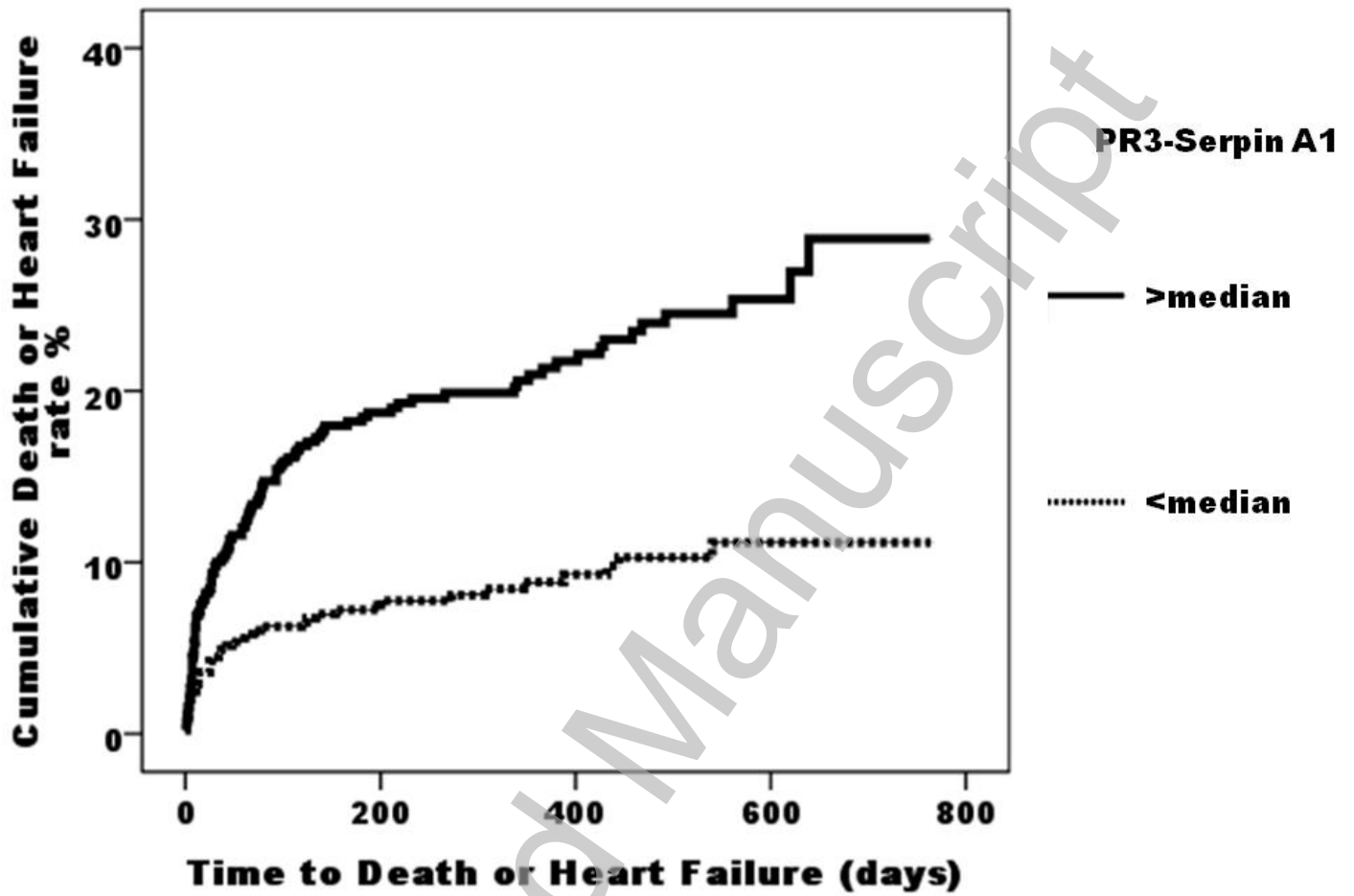
Table 3 Multivariate Cox proportional hazards regression model of significant predictors of death or heart failure

Variable	Hazard Ratio	95% CI	p value
Age	1.04	1.03-1.06	0.001
Past history AMI	1.52	1.06-2.17	0.023
Killip Class >1	1.60	1.08-2.37	0.02
Log Creatinine	4.75	1.39-16.19	0.013
Log NTproBNP	2.51	1.70-3.71	0.001
Log PR3-SERPIN A1	3.80	1.78-8.14	0.001

Table 4 Reclassification analysis for death or heart failure using GRACE score, with the addition of NTproBNP and/or PR3-SERPIN A1

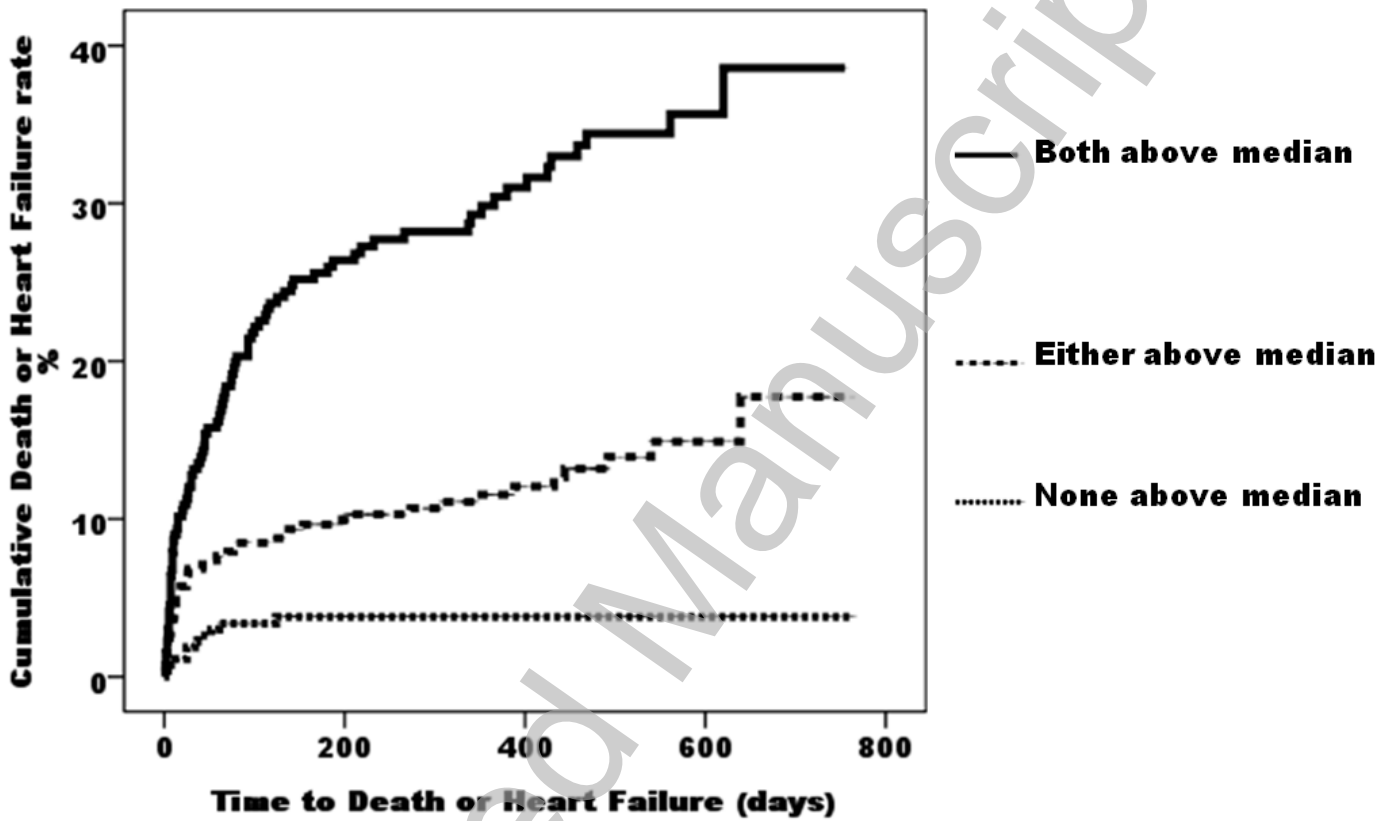
GRACE score vs GRACE score with NTproBNP				
	Reclassification		% Correctly Reclassified	p value
	Increased %	Decreased %		
Patients without endpoints	8.9	16.3	7.4	0.0002
Patients with endpoints	15.3	7.0	8.3	
GRACE score vs GRACE score with PR3-SERPIN A1				
Patients without endpoints	10.3	11.4	1.1	0.02
Patients with endpoints	9.1	2.3	6.8	
GRACE score with NTproBNP vs GRACE score with NTproBNP and PR3-SERPIN A1				
Patients without endpoints	8.6	7.9	-0.7	0.14
Patients with endpoints	7.6	2.3	5.3	

Figure 1: Cumulative incidence plots showing time to adverse events (death or heart failure) in patients stratified by PR3-SERPIN A1.



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Figure 2: Cumulative incidence plots showing time to adverse events (death or heart failure) in patients stratified according to whether both PR3-SERPIN A1 and NTproBNP are below their respective median values, either marker being elevated, or both markers elevated above median.



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