PROGNOSTIC VALUE OF HUMAN MATURE ADRENOMEDULLIN IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION.

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ABSTRACT

Adrenomedullin correlates with adverse cardiovascular outcomes in patients with acute myocardial infarction (AMI) and in patients with heart failure. Measurement of human mature adrenomedullin (mADM) has been difficult and recent studies have used its surrogate, mid-regional pro-adrenomedullin (MRproADM). Our objective was to determine whether mADM measured by a novel sandwich immunoassay using the anti-C-terminal and an anti-mid-regional monoclonal antibody was prognostic of 30-day, 90-day, 1-year, and 2-year major adverse cardiovascular events (MACE) in 1111 consecutive patients who have suffered an AMI. We also compared it with the effect of MRproADM in the same patient population. A total of 311 (27.0%) patients experienced the primary endpoint at 2 years follow-up. The median (interquartile range) of mADM was significantly higher in patients who experienced a 2-year MACE (60.90 (44.00-86.97))pg/mL compared to event-free survivors (49.59 (36.20-68.15)) pg/mL, $P < 0.001$. mADM taken as one standard deviation of the continuous variable was predictive of MACE in multivariate analysis with hazard ratios (95% confidence intervals) ((HR (95% CI)) at 90 days (1.28 (1.01-1.62)), 1 year (1.31 (1.08-1.59)), and 2 years (1.42 (1.07-1.64))). It was also independently predictive of death at 1 year follow-up (1.52 (1.12-2.05)), and 2 years (1.42 (1.07-1.89)). mADM was a better predictor of these outcomes than MRproADM apart from death at 90 days and combined death and heart failure hospitalisation at 1 and 2 years respectively. Human mature adenomedullin can be reliably measured and predicts MACE events at medium-term follow-up, and confirms the paradigm of risk stratification using MRproADM, a surrogate for the active hormone. The relationship between mADM and MACE appears to be a continuum.

Keywords: Adrenomedullin, myocardial infarction, death, heart failure, assay

INTRODUCTION

The stratification of risk of adverse outcomes after an acute myocardial infarction (AMI) is crucial in the endeavours to curb mortality, and a multiple risk marker strategy is emerging. In addition to established risk stratification tools such as the GRACE score and others(1), biomarkers such as the natriuretic peptides have now been firmly established as having significant diagnostic and prognostic values for heart failure and in post-AMI patients(2, 3). Recently, adrenomedullin (ADM) has emerged as a novel peptide with diagnostic and prognostic significance independently of natriuretic peptides in patients who have suffered an AMI(4-13), patients presenting with acute chest pain (14, 15), patients with established heart failure(16, 17), and patients presenting with acute dyspnoea in emergency settings (18, 19).

Human mature adrenomedullin (mADM) is produced from a large peptide precursor molecule (preproadrenomedullin) which consists of 185 amino acids via a two-staged enzymatic process. Firstly the precursor is converted into an inactive C-terminally glycine-extended peptide intermediate, which is then subsequently converted into the mature form with 52 amino acids, through enzymatic amidation (20). The gene encoding for adrenomedullin is located within the short arm of chromosome 11 (21). Measurement of total mADM as biomarker has been previously hampered by its short plasma half-life of 22 ± 1.6 minutes (22), the presence of a binding protein called human adrenomedullin binding protein (AMBP-1) identified as complement factor H (23), and the immediate binding of ADM to local receptors, which had rendered its accurate measurement difficult to achieve (23, 24). A possible solution to this problem came from the development of assays to identify mid-regional pro-adrenomedullin

(MRproADM), which is a more stable and apparently non-functional fragment of initial precursor peptide, consisting of 45 to 92 amino acids. MRproADM is secreted in stoichiometric amounts to mADM and therefore acts as a reliable surrogate of mADM levels (24). However, conceptually, using MR-proADM as a surrogate for mADM has limitations: It does not reflect potential imbalances between the non-functional glycine-extended and the mature ADM, and it must be assumed to have different clearing kinetics than mADM.

Previous analytical laboratory methods could only measure free mADM, but reliability of assays was problematic (23). Earlier studies showed significant associations between free mADM and cardiovascular outcomes in patients with AMI (7, 9, 10, 25, 26). However, recent studies have shown the prognostic values of MRproADM in this patient group (4-6, 11, 13-15, 27, 28). There are currently no reliable assays for the measurement of total human mature adrenomedullin. We therefore undertook this study to determine the prognostic significance of human mature adrenomedullin (mADM) measured with the novel sandwich immunoassay using anti-C-terminal and an anti-mid-regional monoclonal antibody, which specifically detects the mature adrenomedullin, but not the non-functional glycine-extended variant nor the unprocessed prohormone, in relation major adverse cardiovascular events (MACE) at one year follow-up, in ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI) patients. We also compared mADM and MRproADM in relation to MACE in the same group of patients.

METHODS

Study population

The study population of this cohort consisted of 1111 consecutive patients with acute myocardial infarction (STEMI and NSTEMI), admitted to the University Hospitals of Leicester NHS Trust between 2005 and 2008. Written informed consent was obtained from patients, and the study complied with the Declaration of Helsinki and was approved by the local ethics committee. Acute myocardial infarction was diagnosed if a patient had a plasma cardiac troponin I level >0.1 ng/ml with at least one of the following: chest pain lasting >20 min or diagnostic serial ECG changes consisting of new pathological Q waves or ST segment and T wave changes. The Centaur cTnI Ultra immunoassay (Siemens Healthcare Diagnostics) was used to measure troponin I which has a coefficient of variation 10% at 0.03 ng·ml⁻¹ with a 99th percentile of 0.04 ng·ml⁻¹. Hypertension was defined as a blood pressure measurement of greater than 140/90 mmHg on two or more occasions or if the patient was on medication for hypertension. Hypercholesterolaemia was defined as total cholesterol of greater than 5.0 mmol/l, or treatment with a statin. Diabetes was defined as random plasma glucose of 11.1 mmol/l, fasting plasma glucose of 7 mmol/l or a history of diabetes on treatment. Past medical history of ischaemic heart disease (angina or myocardial infarction), heart failure, and smoking history (current or ex-smoking) were obtained directly from patients or from their medical records. Data on demographics, clinical including treatment, laboratory, and echocardiography were obtained. The estimated glomerular filtration rate (eGFR) was calculated from the simplified formula derived from the Modification of Diet in Renal Disease study. All patients received standard secondary prevention therapy for ischemic heart disease, and revascularization was at the discretion of the attending physician.

Blood sampling

Blood samples were drawn at day 1 to day 5 days after admission with AMI. Blood samples were taken after 15 minutes of bed rest. 20 ml of venous blood was taken from the antecubital vein into tubes containing EDTA (1 ml/10 ml blood) and aprotinin (500 international units/ml blood). The blood samples were immediately immersed in ice and then centrifuged at 3000 rpm 10°C for 15 minutes. The plasma was aspirated and stored at -80°C until assayed in a blinded fashion in a single batch.

mADM assay

mADM was detected using a novel research assay provided by Sphingotec GmbH (Hennigsdorf, Germany), as previously described (29, 30). The ADM assay is a one-step sandwich coated tube chemiluminescence immunoassay, based on Acridinium NHS-ester labelling for the detection of human ADM in unprocessed, neat plasma. It employs two mouse monoclonal antibodies, one directed against the midregion (solid phase), the other directed against the C-terminal moiety of ADM (labeled antibody). Polystyrene tubes (Greiner) were coated with the anti-midregional antibody (per tube 1.5 µg antibody in 300 µL of 50 mmol/L Tris, 100 mmol/L NaCl, pH 7.8) overnight at 22 °C. Tubes were then blocked with 10 mmol/L Na-phosphate (pH 6.5) containing 3% Karion FP (Merck) and 0.5% BSA (protease-free) and lyophilized. The anti-C-terminal antibody (1 g/L) was labeled by incubation with MACN-acridinium-NHS (N-hydroxysuccinimide)-ester (1 g/L; InVent GmbH, Hennigsdorf, Germany) in a 1:5 molar ratio for 20 min at 22°C. The reaction was stopped by addition of 1/5 volume of 50 mmol/L glycine for 10 min at 22°C. Labelled antibody was separated from free label by size exclusion chromatography first on a Centri Pure P5 Hydrated gel filtration column

(emp Biotech GmbH, Berlin, Germany) and followed by a Bio-Silect® SEC 400-5 column (Bio-Rad) for HPLC. For the immunoassay, the labelled antibody was diluted into assay buffer (300 mmol/L K-phosphate; 100 mmol/L NaCl; 10 mmol/L sodium EDTA; 5 g/L BSA (protease-free) (Sigma); 1 g/L each of nonspecific bovine and mouse IgG; 0.9 g/L Na-azide; 20 tabs/L Protease Inhibitor Cocktail (Roche AG); 10 µmol/L Amastatin; 20 µmol/L Leupeptin; pH 7.0). Dilutions of full-length human ADM peptide (American Peptide Company, Sunnyvale, CA, USA) in Calibrator Dilution Buffer (10 mmol/L Tris; 250 mmol/L NaCl; 2 g/L Triton X-100; 50 g/L BSA (protease-free); 20 tabs/L Protease Inhibitor Cocktail (Roche AG); pH 7.0) served as calibrators. The immunoassay was performed as follows: 50 µL of plasma samples/calibrators and 200 µL of labelled detection antibody (800,000 relative light units (RLU) per 200 µL) were pipetted in the coated tubes. The assay was incubated 18 hours at 4°C. Unbound tracer was removed by washing 5 times with wash solution (1 mL each). Tube-bound chemiluminescence was measured for 1s by using the LB953 Multi-Tube Luminometer (Berthold Technologies GmbH & Co. KG, Germany). The analytical assay sensitivity was 2 pg/ml. The median ADM concentration of 200 healthy subjects was 20.7 pg/mL; the 99th percentile was 43 pg/mL.

MRproADM assay

MRproADM was detected using a novel commercial assay (BRAHMS AG, Hennigsdorf, Germany), as previously described (31). Briefly, tubes were coated with a purified sheep polyclonal antibody raised against a peptide representing amino acids 83 to 94 of pre-pro-AM. A purified sheep methylacridinium *N*-hydroxysuccinimide ester-labeled polyclonal antibody directed against amino acids 68 to 86 of pre-pro-AM was used as a tracer. The immunoassay was performed by incubating 10 µl of samples/standards and

200 µl of tracer in coated tubes for 2 h at room temperature and measuring the bound chemiluminescence using an LB952T luminometer (Berthold, Bad Wildbad, Germany). The lower detection limit of the assay is 0.08 nmol/l. The intra-assay (interassay) coefficients of variation at 0.5 and 5 nmol/l were 3% (8.5%) and 3.5% (6.5%), respectively.

NT-proBNP assay

Plasma NT-proBNP assay was measured using a non-competitive immunoluminometric assay as previously published(3).

Endpoints

The primary endpoint was MACE at 30 days, 90 days, 1 year and 2 years follow-up, which was a composite of all-cause mortality, myocardial re-infarction, and hospitalization for heart failure. Secondary endpoints were: death, hospitalization for heart failure, myocardial re-infarction, a combination of death and hospitalization for heart failure, a combination of death and myocardial re-infarction, a combination of myocardial infarction and hospitalization for heart failure. Deaths were validated by reviewing the hospital record management systems and the Office of National Statistics Registry. 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 with echocardiographic evidence of heart failure. New myocardial infarction was defined as for the initial AMI. New myocardial infarction and heart failure endpoints were ascertained using computerised hospital patients records for University Hospitals of Leicester and validated by contacting each patient.

Statistical analyses

Statistical analyses were done using STATA version 11. Quartiles of mADM were created. Hypothesis testing was done using ANOVA for trend across quartiles of mADM for normally distributed continuous variables, Kruskal- Wallis tests for skewed variables, and Chi squared test for trend for categorical variables. Independent correlates of mADM were estimated using the generalized linear model. Univariate and multivariate hazard ratios for the predictive impact of baseline mADM on endpoints were estimated by the Cox proportional hazard model. The Likelihood ratio χ^2 test was used to test for statistical significance of each variable in the Cox regression model. Curves of Kaplan-Meier MACE-free and survival estimates were obtained and the Log-Rank test was used to compare MACE-free and survival estimates across quartiles of mADM.

RESULTS

Patients characteristics

Table 1 shows patients characteristics across quartiles of mADM. The cohort consisted of 519 STEMI patients (46.7%). Males had lower levels of mADM levels compared to females. There was a significant positive trend for age, past medical history of ischemic heart disease, hypertension, diabetes mellitus, and smoking history across tertiles of mADM, but not for history of hyperlipidaemia and chronic obstructive airway disease. The same pattern was seen for MRproADM.

Daily variation of human mature adrenomedullin (mADM) and relationship with endpoint (MACE at 1year).

Daily samples of mADM were collected for five days. As shown in Figure 1, mADM rises up to day 2 ($p < 0.0005$), and then remains elevated on repeated measures analysis with Bonferroni correction, with MACE at 1 year as a factor. Adrenomedullin levels were higher in patients with MACE compared to those without MACE ($p < 0.0005$). There was no interaction between MACE and time of sampling. There was no significant effect of daily variation of mADM on outcome measures.

Correlates of mADM and MRproADM.

There was a strong correlation between mADM and MRproADM as shown in Figure 2, with coefficient of 0.63 ($p < 0.001$). Using the generalized linear model, independent multivariate correlates of mADM were NTproBNP ($p = 0.001$), eGFR ($P < 0.001$), past medical history of diabetes mellitus on admission ($p = 0.039$), and history of heart failure ($p = 0.014$). The relationship between eGFR and mADM was inverse and appeared very strong, while mADM was positively correlated to the rest of afore-mentioned covariates. Independent correlates of MRproADM were NTproBNP ($P < 0.001$), mADM ($p < 0.001$), eGFR ($p < 0.001$), and past medical history of diabetes mellitus ($p = 0.007$).

Human mature adrenomedullin and follow-up outcomes.

A total of 311 (27.0%) patients experienced the primary endpoint at 2 years follow-up. The median (interquartile range) of mADM was significantly higher in patients who experienced a 2-year MACE (60.90 (44.00-86.97))pg/mL compared to event-free survivors (49.59 (36.20-68.15) pg/mL, $P < 0.001$). The same pattern was observed with MRproADM at 1.00 (0.66-1.58) nmol/l for MACE versus 0.66 (0.49-0.90) nmol/l for no MACE, $P < 0.001$. Table 2 shows multivariate predictors of 1 year MACE by Cox proportional hazard regression. mADM taken as one standard deviation of the

continuous variable was predictive of MACE in multivariate analysis with hazard ratios (95% confidence intervals) ((HR (95% CI)) at 90 days 1.28 (1.01-1.62), 1 year 1.31 (1.08-1.59), and 2 years 1.42 (1.07-1.64). It was also independently predictive of death at 1 year follow-up (HR 1.52 (1.12-2.05)), and 2 years 1.42 (1.07-1.89). Hazard ratios (95% confidence intervals) of mADM and MRproADM for various outcomes are depicted in Table 3. Overall, results indicate that mADM was a better predictor of primary and secondary endpoints than MRproADM, apart from death at 90 days and combined death and heart failure hospitalisation at 1 and 2 years respectively where MRproADM was a better predictor. This was confirmed by added value analysis of nested regression models, and the reason appears to be that MRproADM is correlated to NTproBNP, while mADM is not (data not shown).

Taking adrenomedullin as a categorical variable, hazard ratios (95% confidence intervals) for 2-year MACE in the upper tertile of mADM compared to the lower two tertiles were 1.76 (1.41-2.21), $p < 0.001$ in univariate analysis, but was not significant in multivariate analysis, 1.42 (0.95-2.13), $p = 0.092$, suggesting a continuum of risk across levels of mADM. Hazard ratios (95% CI) for MACE in the upper tertile of MRproADM compared to the lower tertiles were 3.33 (2.65-4.71), $p < 0.001$ univariate analysis, and 1.68 (1.19-2.39), $p < 0.004$ in multivariate analysis.

Human mature adrenomedullin and survival

Figures 3 and 4 show Kaplan-Meier estimates for MACE-free and survival respectively at two years of follow-up. There was a significant trend of these outcomes across tertiles

of mADM. For MRproADM, a significant decrease in MACE-free and survival estimates was observed in patients at the upper tertile as evident by the Log-Rank test.

Receiver operator characteristic curve

The area under the curve (AUC) and 95 % confidence intervals for two-year MACE was 0.63 (0.59-0.66) for mADM, compared to MRproADM 0.69 (0.66-0.73), and 0.69 (0.65-0.73) for NTproBNP, $p = 0.0002$. The AUC for two year all-cause mortality was 0.64 (0.60-0.69) for mADM and 0.76 (0.71-0.80) for NTproBNP, $p = 0.0004$. Although NTproBNP had a better AUC, mADM was a more significant predictor of outcomes in multivariate Cox regression analysis.

DISCUSSION

We have shown that using the novel Sandwich immunoassay using anti-C-terminal and an anti-mid-regional monoclonal antibody, total mature mADM can be reliably measured and that levels are fairly stable over five days of serial measurements. Our study showed that mADM is predictive of MACE events at 90 days, 1 year, and 2 years, as well as predictive of death at 1 year and 2 years. This study also depicted that MRproADM is a significant predictor of death at 90 days, one year and two years of follow-up. We observed that MRproADM has similar predictive effects with regard to MACE and death as mADM, although when introduced into same stepwise multivariate prediction model as mADM, its significance tended to fade out in favour of mADM. Our findings are significant as they confirm that the prognostic effects of mADM in AMI patients are similar to MRproADM which has been established as its stable and reliable surrogate (24). However, we observed that the relationship between mADM and MACE was a continuum, while there appears to be a threshold effect in our study with MRproADM, which has been observed in other studies (5, 15). To the best of our knowledge, this is the first study to look at the active mature peptide and its inactive

propeptide surrogate in same study population in relation to MACE. Our findings are significant as they confirm that the prognostic effects of mADM in AMI patients are similar to MRproADM which has been established as its stable and reliable surrogate as observed by the LAMP Study and the LAMP Study II (5, 8), and others (4, 6, 11-13).

ADM is a known independent predictor of future MACE in coronary disease. It significantly predicts fatal and non-fatal cardiovascular events and all-cause mortality in patients with stable angina (4, 27, 28, 32), patients with acute coronary syndromes(4-11, 13), and patients presenting with acute chest pain (14, 15). Our findings corroborate the above observations and help to enhance the importance of ADM as prognostic marker in acute coronary syndromes whether as the mature mADM or its surrogate MRproADM, independent of other established risk markers like the GRACE score and natriuretic peptides. Initial studies used free ADM to relate to cardiovascular outcomes (7, 9, 10), while more recent studies have used the surrogate MRproADM to study this relationship(4-6, 8, 14, 15, 27, 33).

Abundant evidence has accrued suggesting the important role ADM might be playing in cardiovascular disease. ADM plasma levels are increased in atherosclerosis in coronary and peripheral circulation, and there is augmented expression with severity of plaque formation/stenosis and degree of ischemic heart disease syndromes, e.g. less expression in patients with stable angina compared to unstable angina (34).

ADM is significantly raised in acute myocardial infarction(25), and correlates with severity of AMI (26). Intravenous ADM infusion to AMI patients significantly reduces

infarct size and prospectively reduces regional wall motion abnormality on cardiac magnetic resonance imaging(35).

An expanding number of studies have shown diagnostic and prognostic significance of ADM in heart failure patients. Initial cross-sectional studies demonstrated that adrenomedullin levels were significantly raised in patients with established heart failure compared to controls (36). ADM is elevated in cardiac failure proportionally to the severity of the disease assessed by NYHA and to the degree of left ventricular impairment (6, 16). ADM also has diagnostic value alongside the natriuretic peptides in acute dyspnoea and in chronic heart failure (16, 18, 19, 37), and diastolic dysfunction (38). In post-AMI patients with new heart failure (6), patients with established heart failure (16, 17), and patients presenting with acute dyspnoea in emergency settings(18, 19, 37, 39), MRproADM has been shown to have independent prognostic significance for major adverse cardiovascular outcomes. It is also predictive of adverse outcomes in community patients with symptoms of heart failure (40), although one large population study did not show its predictive utility for incident heart failure (41).

The receptors of ADM are a complex of receptor-activity modifying proteins (RAMP) and calcitonin receptor-like receptor (CRLR)(42). The mechanisms of action of ADM are thought so far to be through the generation of nitric oxide via nitric oxide-cyclic guanine monophosphate pathway or through increased intracellular cyclic adenosine monophosphate which activates protein kinase A which then activates nitric oxide synthase or through phosphatidylinositol kinase -3 activation and Akt phosphorylation resulting in enhanced stimulation of endothelial nitric oxide synthase (21, 34).

LIMITATIONS

The study findings are from a single centre and will need to be replicated in other patient populations. Only 5 daily measures of mADM were done after initial presentation and we cannot account for the variability of these peptides during the follow-up period. Measurements of peptides were done on samples that were frozen, but lack of differential treatment of samples should uphold the internal validity of our findings.

CONCLUSIONS

- Human mature adrenomedullin (mADM) can be reliably measured, and levels are stable for up to five days post acute myocardial infarction.
- mADM is an independent predictor of major adverse cardiac events at short and medium term of follow-up. The relationship between mADM and MACE appears to be a continuum.

CLINICAL PERSPECTIVES

- Reliable measurement of human mature adrenomedullin (mADM) has been difficult.
- Mid regional pro-adrenomedullin (MRproADM) has been a reliable surrogate of mADM .
- MRproADM predicts adverse outcomes in myocardial infarction patients.
- We reliably measured mature human adrenomedullin (mADM) using the novel sandwich immunoassay and showed that mADM is predictive of major adverse cardiovascular events in STEMI and NSTEMI patients.

- Both mADM and MRproADM are predictive of outcomes confirming the surrogacy of MRproADM for mADM in relation to outcome, but mADM is a better predictor of outcomes in this study than MRproADM.
- This confirms the paradigm of risk stratification using MRproADM, a surrogate for the active hormone.

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Conflicts of interest: None declared.

TABLE 1: Characteristics of patients according to admission levels of human mature adrenomedullin (mADM) and Mid-regional pro-adrenomedullin (MRproADM)

Variable		mADM (admission tertiles) pg/mL				MRproADM (admission tertiles) nmol/l			
Size	All (n=1111)	1 < 42.5 (n=372)	2 42.6-64.0 (n=369)	3 > 64.0 (n=370)	P value	1 < 0.58 (n=371)	2 0.58-0.91 (n=370)	3 >0.91 (n=370)	P value
STEMI	519 (46.7%)	243 (65.3)	163 (44.2)	113 (30.5)	<0.001	233 (62.8)	152 (41.1)	134 (36.2)	<0.001
Age	66.3 (12.7)	62.2 (12.2)	66.2 (13.1)	70.1 (11.7)	<0.001	59.7 (11.3)	64.4 (11.6)	74.9 (10.3)	<0.001
Male sex	799 (72.1%)	298 (80.3)	272 (73.7)	229 (62.1)	<0.001	299 (80.8)	275 (74.3)	225 (61.0)	<0.001
Past medical history									
Current/Ex-smoker	456 (41.2%)	176 (47.4)	172 (46.8)	108 (29.2)	<0.001	189 (51.2)	160 (43.4)	107 (28.9)	<0.001
IHD	370 (33.3%)	83 (22.3)	119 (32.3)	168 (45.4)	<0.001	78 (21.1)	116 (31.3)	176 (47.6)	<0.001
Heart failure	40 (3.6%)	15 (4.0)	15 (4.1)	10 (2.7)	0.328	15 (4.0)	9 (2.4)	16 (4.3)	0.844
Hypertension	577 (52%)	157 (42.2)	188 (51.0)	232 (62.7)	<0.001	150 (40.5)	191 (51.7)	236 (63.8)	<0.001
Hyperlipidaemia	384 (34.6%)	114 (30.7)	133 (36.0)	137 (37.0)	0.072	115 (31.1)	140 (37.8)	129 (34.9)	0.279
Diabetes mellitus	251 (22.6%)	42 (11.3)	76 (20.7)	133 (35.9)	<0.001	40 (10.8)	88 (23.9)	123 (33.2)	<0.001
COPD	43 (9.0%)	9 (9.4)	13 (7.9)	21 (9.7)	0.821	7 (6.3)	15 (8.7)	21 (10.9)	0.821

Risk markers during admission									
GRACE score	150.7 (40.1)	143.2 (35.3)	147.9 (39.7)	160.4(42.9)	0.002	135.1 (37.4)	141.9 (33.7)	175.4 (37.3)	0.109
Killip class >1	411 (40.2%)	104 (32.3)	125 (36.1)	182 (51.4)	<0.001	86 (25.9)	119 (34.3)	206 (59.9)	<0.001
Glucose (mmol/l)*	7.5 (6.2-9.9)	7.2 (6.1-9.1)	7.4 (6.1-9.4)	8.3 (6.5-11.8)	<0.001	7.3 (6.1-9.1)	7.2 (6.1-9.6)	8.4 (6.6-11.8)	<0.001
Cholesterol (mol/l)	4.8 (2.9)	4.9 (1.8)	5.1 (3.3)	4.3 (1.7)	<0.001	5.2 (4.1)	4.8(1.7)	4.4 (1.6)	0.002
eGFR	65.6 (20.0)	72.1 (17.9)	66.7 (19.0)	57.8 (20.3)	<0.001	75.2 (16.8)	70.1 (16.9)	51.4 (17.7)	<0.001
NT-proBNP (pmol/l)*	3.0 (2.5-3.4)	2.8 (2.2-3.2)	3.0 (2.5-3.4)	3.3 (2.8-3.6)	<0.001	2.6 (1.9-3.0)	2.9 (2.5-3.3)	3.4 (3.2-3.6)	<0.001
Ejection fraction (%)	42.0 (15.1)	43.2 (12.9)	42.1 (15.7)	40.2 (16.6)	<0.001	44.2 (13.2)	43.2 (15.7)	37.9 (15.4)	<0.001
Medications on discharge									
Aspirin	929 (83.7%)	327 (88.1)	315 (85.4)	287 (77.6)	<0.001	332 (89.7)	319 (86.2)	278 (75.1)	<0.001
Statin	970 (87.4%)	340 (91.6)	328 (88.9)	302 (81.6)	<0.001	347 (93.8)	337 (91.1)	286 (77.3)	<0.001
Betablocker	891 (80.3%)	323 (87.3)	301 (81.6)	267 (72.2)	<0.001	329 (88.9)	304 (82.2)	258 (69.9)	<0.001
ACEI or ARB	912 (82.2%)	319 (85.9)	301 (81.6)	292 (78.9)	0.012	316 (85.4)	316 (85.4)	280 (75.7)	<0.001
Major outcomes									
MACE 90 days	176 (15.8%)	46 (12.4)	51 (13.8)	79 (21.4)	0.001	33 (8.8)	35 (9.5)	108 (29.2)	<0.001
MACE 30 days	125 (11.3%)	35 (9.4)	31 (8.4)	59 (15.9)	0.002	24 (6.5)	17 (4.6)	77 (20.8)	<0.001
MACE 1 year	276 (24.8%)	64 (17.2)	85 (23.4)	127 (33.9)	<0.001	57 (15.4)	57 (15.4)	162 (43.8)	<0.001
MACE 2 years	311 (27.0%)	71 (19.1)	101 (27.4)	139 (37.4)	<0.001	64 (17.3)	69 (18.7)	178 (48.1)	<0.001
Death 1 year	117 (10.5%)	22 (5.9)	36 (9.9)	59 (15.7)	<0.001	11 (3.0)	17 (4.60)	89 (24.1)	<0.001
Death 2 years	136 (12.2%)	25 (6.7)	46 (12.5)	65 (17.6)	<0.001	15 (4.0)	21 (5.7)	100 (27.3)	<0.001
Death/ HF 1 year	192 (17.3)	41 (11.0)	56 (15.4)	95 (25.3)	<0.001	25 (6.7)	34 (9.2)	133 (35.9)	<0.001
Death /HF 2 years	217 (19.5%)	43 (11.6)	68 (18.4)	106 (28.6)	<0.001	29 (7.8)	41 (11.08)	147 (39.7)	<0.001
MI 1 year	122 (10.9%)	33 (8.9)	42 (11.5)	47 (12.5)	0.006	40 (10.8)	31 (8.4)	51 (13.7)	0.062
MI 2 year	141 (12.7%)	39 (10.5)	51 (13.8)	51 (13.8)	0.181	46 (12.4)	37 (10.0)	58 (15.7)	0.117
HF 1 year	98 (8.8%)	23 (6.2)	29 (7.9)	46 (12.3)	0.011	18 (4.9)	22 (6.0)	58 (15.7)	<0.001
HF 2 years	107 (9.7%)	24 (6.5)	32 (8.7)	51 (13.8)	<0.001	19 (5.2)	25 (6.8)	63 (17.0)	<0.001

Values are mean (standard deviation) for normally distributed variables or number (percentages) for categorical variables or *Median (interquartile range) for skewed variables. Hypothesis testing was done using ANOVA for trend across tertiles of mADM and MRpoADM for normally distributed continuous variables, Kruskal- Wallis tests for skewed variables, and Chi squared tests for trend for categorical variables. mADM = mature human adrenomedullin.

MRproADM = Mid regional pro-adrenomedullin. NSTEMI = Non-ST elevation myocardial infarction. STEMI = ST elevation myocardial infarction. IHD = Ischaemic heart disease. COPD = Chronic obstructive pulmonary disease. GRACE = Global Registry of Acute Coronary Events. eGFR = estimated glomerular filtration rate in mls/min/1.73 m² surface area. NTproBNP = N-terminal pro-B-type natriuretic peptide. ACEI = Angiotensin converting enzyme inhibitor. ARB = Angiotensin II receptor blocker. MI = Myocardial infarction. HF = Heart failure

TABLE 2: Predictors of 1 year MACE from Cox regression.

	Univariate	P value	Multivariate	P value
mADM*	1.51 (1.34-1.70)	<0.001	1.31 (1.08-1.59)	0.006
Age	1.05 (1.04-1.06)	<0.001	1.03 (1.01-1.05)	0.005
Sex (male)	0.63 (0.49-0.79)	<0.001	NS	-
STEMI vs NSTEMI	1.20 (0.95-1.51)	0.129	2.02 (1.41-2.84)	<0.001
Past history				
Current/Ex-smoker	0.77 (0.61-0.98)	<0.001	NS	-
IHD	1.55 (1.23-1.96)	<0.001	NS	-
Heart failure	1.18 (0.66-2.09)	0.584	NS	-
Hyperlipidaemia	1.10 (0.86-1.39)	0.455	NS	-
Risk markers during admission				
GRACE score	1.02 (1.02-1.02)	<0.001	1.01 (1.01-1.02)	0.013
Killip class >1	2.73 (2.12-3.49)	<0.001	NS	-
Glucose ^{\$}	8.59 (4.40-16.76)	<0.001	5.03 (2.17-11.67)	<0.001
eGFR	0.97 (0.96-0.98)	<0.001	NS	-
NTproBNP*	2.00 (1.69-2.16)	<0.001	NS	-
SBP	0.99 (0.99-0.99)	0.005	NS	-
Heart rate	1.01 (1.01-1.01)	<0.001	NS	-
Troponin*	1.15 (1.01-1.30)	0.038	NS	-
Medications on discharge				
Aspirin	0.53 (0.41-0.69)	<0.001	NS	-
Statin	0.36 (0.27-0.48)	<0.001	0.54 (0.36-0.83)	0.004
Betablocker	0.48 (0.37-0.62)	<0.001	NS	-
ACEI or ARB	0.51 (0.39-0.65)	<0.001	0.63 (0.42-0.93)	0.020

*: One standard deviation increased in variable.

Above are hazard ratios (95% confidence intervals) with multivariate values derived from back stepwise Cox regression analysis. NS = Not significant and rejected out of the final multivariate regression model. mADM = mature human adrenomedullin. NSTEMI = Non-ST elevation myocardial infarction. STEMI = ST elevation myocardial infarction. IHD = Ischaemic heart disease. COPD = Chronic obstructive pulmonary disease. GRACE = Global Registry of Acute Coronary Events. eGFR = estimated glomerular filtration rate in mls/min/1.73 m² surface area. NTproBNP = N-terminal pro-B-type natriuretic

peptide. ACEI = Angiotensin converting enzyme inhibitor. ARB = Angiotensin II receptor blocker. SBP = Systolic blood pressure. Glucose^{\$} = Log to base of serum glucose.

TABLE 3: Cox Regression of human mature adrenomedullin (mADM) and Mid-regional pro-adrenomedullin (MRproADM) for various outcomes.

	mADM				MRproADM			
	<i>Univariate</i>	<i>P value</i>	<i>Multivariate</i>	<i>P value</i>	<i>Univariate</i>	<i>P value</i>	<i>Multivariate</i>	<i>P value</i>
MACE 30 days	1.48 (1.25-1.76)	<0.001	NS	-	1.58 (1.37-1.81)	<0.001	NS	-
MACE 90 days	1.46 (1.26-1.69)	<0.001	1.28 (1.01-1.62)	0.038	1.63 (1.45-1.83)	<0.001	1.19 (0.97-1.45)	0.088
MACE 1 year	1.51 (1.34-1.70)	<0.001	1.31 (1.08-1.59)	0.006	1.55 (1.41-1.71)	<0.001	NS	-
MACE 2 years	1.50 (1.35-1.69)	<0.001	1.37 (1.14-1.64)	0.001	1.54 (1.40-1.68)	<0.001	1.16 (1.01-1.34)	0.040
Death 30 days	2.03 (1.61-2.56)	<0.001	1.60 (0.93-2.76)	0.091	2.66 (1.85-2.78)	<0.001	1.78 (0.99-3.21)	0.053
Death 90 days	2.07 (1.75-2.44)	<0.001	NS	-	1.84 (1.56-2.27)	<0.001	1.42 (1.01-2.01)	0.041
Death 1 year	1.83 (1.54-2.18)	<0.001	1.55 (1.14-2.12)	0.006	2.15 (1.87-2.47)	<0.001	1.49 (1.15-1.93)	0.002
Death 2 years	1.73 (1.47-2.04)	<0.001	1.42 (1.07-1.89)	0.016	2.01 (1.76-2.28)	<0.001	1.23 (1.03-1.59)	0.028
Death/HF 1 year	1.68 (1.46-1.93)	<0.001	NS	-	1.94 (1.73-2.16)	<0.001	1.32 (1.07-1.63)	0.009
Death/HF 2 years	1.69 (1.48-1.93)	<0.001	1.29 (1.03-1.61)	0.024	1.91 (1.72-2.11)	<0.001	1.28 (1.05-1.56)	0.013
HF 1 year	1.47 (1.20-1.79)	<0.001	NS	-	1.56 (1.33-1.82)	<0.001	NS	-
MI 1 year	1.17 (0.97-1.40)	0.104	NS	-	1.02 (0.90-1.21)	0.764	NS	-

Above are hazard ratios (95% confidence intervals) derived from back stepwise Cox regression analysis. NS = Not significant and rejected out of the final multivariate regression model. mADM = mature human adrenomedullin. MRproADM = Mid regional pro-adrenomedullin. MACE = Major adverse cardiovascular events. MI = Myocardial infarction. HF = Heart failure.

N/B: mADM and MRproADM were introduced separately into the models. Introduction of both variables into various predictive models of the above outcomes invariably excluded MRproADM from the model, leaving mADM as a better predictor for most of the outcomes, apart from death at 90 days and combined death and heart failure hospitalisation at 1 year and 2 years where MRproADM was a better predictor. Above multivariate analyses were adjusted for age, sex, N-terminal pro-B-type natriuretic peptide (NTproBNP), hyperlipidaemia, ischaemic heart disease history, smoking, Killip class, GRACE score on admission, systolic blood pressure, serum glucose, eGFR, left ventricular ejection fraction, class of myocardial infarction (STEMI vs NSTEMI), and discharge medications.

FIGURE 1: Day curves for mADM vs endpoint (MACE at 1 year)

Above are box and whisker plots of daily curves of human mature adrenomedullin (mADM) compared between patients who experienced the primary endpoint which was MACE (major adverse cardiovascular events) at 1 year, and those who did not. **Central horizontal line** = Median level. **Box** = Interquartile range. **Whiskers** = 5th and 95th centiles.

Figure 2: Scatter plot of human mature adrenomedullin (mADM) and mid-regional pro-adrenomedullin (MRproADM).

FIGURE 3: MACE-free Kaplan-Meier estimates across tertiles of adremomedullin

Above are MACE-free Kaplan-Meier estimates at 2 years for human mature adrenomedullin(mADM), Log-Rank test 29.84, $p < 0.001$; and mid-regional pro-adrenomedullin (MRproADM), Log-Rank 123.98, $p < 0.001$

FIGURE 4: Kaplan-Meier survival estimates across tertiles of adremomedullin

Above are Kaplan-Meier survival estimates at 2 years for human mature adrenomedullin(mADM), Log-Rank 18.64, $p < 0.001$; and mid-regional pro-adrenomedullin (MRproADM), Log-Rank 118.49, $p < 0.001$.

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