C-Terminal Provasopressin (Copeptin) as Prognostic Marker after Acute Non ST Elevation Myocardial Infarction - Leicester Acute Myocardial Infarction Peptide II (LAMP II) study.

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Clinical Investigations

C-Terminal Provasopressin (Copeptin) as Prognostic Marker after Acute Non ST Elevation Myocardial Infarction - Leicester Acute Myocardial Infarction Peptide II (LAMP II) study.

Hafid Narayan[a], Onkar S Dhillon[a], Pauline A Quinn[a], Joachim Struck[b], Iain B Squire[a], Joan E Davies[a], Leong L Ng[a]

a. University of Leicester, Department of Cardiovascular Sciences, University of Leicester, and Leicester National Institute for Health Research Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester, United Kingdom
b. BRAHMS GmbH / Thermo Fisher Scientific, Hennigsdorf, Germany

Corresponding author: Hafid Narayan

Department of Cardiovascular Sciences
Clinical Sciences Building
Leicester Royal Infirmary
Leicester LE2 7LX, UK
And Leicester National Institute for Health Research Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester
Phone:+441162523132 ; fax:+441162523108; e-mail:hn31@le.ac.uk

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Abstract

Background

Copeptin, the 39 amino acid C-terminal portion of provasopressin, has been shown to be an independent predictor for adverse events following ST elevation myocardial infarction. We hypothesized that plasma copeptin was an independent predictor for adverse outcomes following acute non ST elevation myocardial infarction (NSTEMI) and evaluated whether copeptin added prognostic information to the GRACE score compared to NTproBNP.

Methods

Plasma copeptin and NTproBNP were measured in 754 consecutive patients admitted to hospital with chest pain and diagnosed as having NSTEMI in this prospective observational study. The endpoint was all cause mortality at 6 months.

Results: Upper median levels of copeptin were strongly associated with all cause mortality at 6 months. Copeptin was a significant predictor of time to mortality (HR = 5.98 [3.75 to 9.53], $p < 0.0005$) in univariate analysis and remained a significant predictor in multivariate analysis (HR = 3.03 [1.32 to 6.98], $p = 0.009$). There were no significant differences between the area under ROC curves of copeptin, NTproBNP and the GRACE score. Copeptin improved accuracy of risk classification when used in combination with the GRACE score as determined by net reclassification improvement whereas NTproBNP did not. The relative utility of the GRACE score was increased more by copeptin than by NTproBNP over a wide range of risks.

Conclusions: Plasma copeptin is elevated after NSTEMI and higher levels are associated with worse outcomes. Copeptin used in conjunction with the GRACE score improves risk stratification enabling more accurate identification of high risk individuals.

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Introduction

Recent guidelines recommend risk stratification of all patients following admission with non- ST elevation myocardial infarction (NSTEMI) in order to identify those individuals at maximum risk of adverse outcomes who may benefit from early aggressive therapy [1]. Currently clinical risk assessment tools such as the TIMI (Thrombolysis in Myocardial Infarction) [2] and GRACE (Global Registry of Acute Coronary Events) [3] scores are the most commonly used method of risk stratification, although more recently plasma biomarkers have emerged as a potentially alternative or complementary technique [4].

Copeptin is the 39 amino acid C-terminal portion of provasopressin, the precursor of arginine vasopressin (AVP) [5]. Copeptin has the advantages of a longer plasma half life and reduced propensity for protein binding compared to AVP making its measurement more accurate [6]. Copeptin has recently been demonstrated to be an independent predictor for adverse events following ST elevation myocardial infarction (STEMI) [7].

We hypothesized that plasma copeptin is an independent predictor for adverse outcomes following acute NSTEMI and further sought to evaluate whether copeptin provided additional prognostic information to NTproBNP (N Terminal pro B Type Natriuretic Peptide) and the GRACE score, established risk stratification markers.

This paper presents a post hoc analysis of a subset of patients with NSTEMI recruited to the original LAMP (Leicester Acute Myocardial Infarction Peptide) cohort study of patients with acute myocardial infarction supplemented with a new cohort of NSTEMI patients.

Methods

Study population

We analyzed patients admitted to the Leicester Royal Infirmary and Glenfield Hospital, Leicester with acute myocardial infarction (MI). The population for this LAMP II study was comprised of patients recruited as follows:- (1) 239 NSTEMI patients who were included in the original LAMP study which included both NSTEMI and STEMI patients, who were recruited between March 2000 and July 2005 and (2) a new group of 515 NSTEMI patients who were recruited between August 2005 and April 2007. As a control group plasma copeptin and NTproBNP were also measured in 82 healthy males over 65 and 41 females over 70 years of age.

This study abided by the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from all the patients. Acute NSTEMI was diagnosed if the patient described a history of cardiac sounding chest pain lasting greater than 20 minutes not associated with ST segment elevation with a plasma creatine kinase- MB level twice the upper limit of normal or cardiac troponin I level > 0.1ng/mL (8). NSTEMI was confirmed in all patients prior to inclusion. 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 with a 99th percentile of 0.04 ng/mL. We chose this cut-off to ensure patients had a definite diagnosis of NSTEMI.

Patients with known malignancy or surgery in the previous month were excluded. The estimated glomerular filtration rate (eGFR) of the study patients was calculated from the simplified formula derived from the Modification of Diet and Renal Disease (MDRD) study validated in patients with heart failure [9].

Plasma samples

Blood samples were taken within 24 hours of admission (at a median time of 25.75 h following onset of chest pain) for determination of plasma levels of copeptin and NTproBNP. After 15

minutes of bed rest 20mL of blood was collected into tubes containing EDTA and aprotinin. All plasma was stored at -70°C until assayed in a blinded fashion in a single batch.

Copeptin assay

Copeptin was detected with a novel commercial assay. Briefly, tubes were coated with a purified sheep polyclonal antibody raised against a peptide that represented amino acids 132 to 147 of preproAVP. A purified sheep polyclonal antibody raised against a peptide representing amino acids 149 to 164 of preproAVP was labeled with methyl acridinium N-hydroxysuccinimide ester (InVent GmbH, Bielefeld, Germany) and used as tracer. Dilutions of a peptide representing amino acids 132 to 164 of preproAVP in normal horse serum served as standards. The immunoassay was performed by incubating 50 µL of samples/standards and 200 µL of tracer in coated tubes for 2 hours at room temperature. Tubes were washed 4 times with 1 mL of wash solution (BRAHMS AG), and bound chemiluminescence was measured with an LB952T luminometer (Berthold, Bad Wildbad, Germany). The assay limit of detection was 0.4 pmol/L, with functional assay sensitivity < 1 pmol/L. The interassay coefficient of variation was 10% at 3 pmol/L and < 7% for values > 10 pmol/L.

NTproBNP assay

The NTproBNP assay used was based on a non-competitive assay [10]. Sheep antibodies were raised to the N-terminal of human NTproBNP, and monoclonal mouse antibodies were raised to the C-terminal. Samples or NTproBNP standards were incubated in C-terminal IgG-coated wells with the biotinylated N-terminal antibody for 24 hours at 4°C. Detection was with methyl-acridinium ester–labeled streptavidin on an MLX plate luminometer (Dynex Technologies Ltd, Worthing, UK). The lower limit of detection was 0.3pmol/L. There was no cross-reactivity with atrial natriuretic peptide, B-type natriuretic peptide, or C-type natriuretic peptide.

Endpoints

We assessed the utility of plasma copeptin and NTproBNP level for prediction of the endpoint of all cause mortality at 6 months. Endpoints were obtained by reviewing the Office of National Statistics Registry and by contacting each patient. The Office of National Statistics Registry collates death certificate records. The endpoints were not adjudicated separately. There was a minimum 6 months follow-up of all surviving patients.

Statistical analysis

R 2.12 [11] was used to conduct statistical analyses. Non parametric variables were expressed as median [range] and parametric variables as mean [95% Confidence Interval]. Associations between copeptin and NTproBNP levels with demographic data, risk factors and clinical status were analysed using the Mann Whitney U test and Spearman correlations. Independent predictors for time to mortality were identified using Cox proportional hazards regression, with significant univariate predictors used as covariates in multivariate analysis. Troponin, copeptin and NTproBNP were included as continuous $\log_{10}$ transformed variables. Thus hazard ratios refer to a 10-fold rise in levels. The impact of copeptin levels on prognosis was visualised using Kaplan-Meier curves stratified for median of plasma concentration and compared using the log rank test.

The prognostic accuracy of plasma copeptin was compared to the GRACE score by comparison of area under curve (AUC) of receiver operating characteristic (ROC) curves. The AUC of ROC curves was derived by the method of Hanley and McNeil [12].

The GRACE score was calculated for each patient derived from clinical and demographic data using the algorithm for estimation of risk for 6 month all cause mortality [3]. The additional prognostic utility of copeptin and NTproBNP to the GRACE score was assessed using

reclassification tables with calculation of Net Reclassification Improvement (NRI). Calculation of NRI is described in detail by Pencina *et al* [13].

Relative utility was calculated according to the method described by Baker *et al* [14]. The relevant region was the risk greater than the prevalence of the endpoint in our cohort at 6 months. 95% confidence intervals were derived from 25 bootstrap replications of the original data.

The prognostic accuracy of using combinations of the GRACE score, copeptin or NTproBNP was estimated using a logistic regression model for the outcome of total mortality at 6 months.

A 2-tailed probability value of less than 0.05 was deemed to be statistically significant. All authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Patient Characteristics

754 consecutive patients were recruited of whom 519 were male, median age 70 [37 to 97] years. No patients were lost to follow up and there was a minimum length of follow up of 6 months. In 6 months, 56 patients (7.4%) had died and 49 (6.5%) had a recurrent myocardial infarction. The baseline characteristics of patients and 123 healthy controls are shown in Table 1. The median time from symptom onset to blood draw in patients was 25 hours 45 minutes. Both copeptin and NTproBNP were significantly raised in the NSTEMI patients compared to the healthy controls (Table 1).

Factors Associated with Elevated Plasma Copeptin and NTproBNP

Univariate associations between demographic and risk factors and copeptin and NTproBNP levels in NSTEMI patients are shown in Table 2.

Elevated copeptin levels were significantly associated with a past history of MI, heart failure, diabetes, Killip class > 1 on admission and left ventricle ejection fraction (LVEF) < 40%, ST segment deviation at admission as well as prior prescription of aspirin, betablockers, angiotensin converting enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARB). Copeptin was strongly associated with the endpoint of all cause mortality at 6 months.

Copeptin level was significantly positively correlated with age and Killip class, creatinine and NTproBNP levels in NSTEMI patients, while being inversely correlated with eGFR (Table 2). For comparison in healthy controls, copeptin was significantly higher in males compared to females (4.2 pmol/L [2.4 to 28.7] vs 3.5 pmol/L [1.5 to 11.8], $p = 0.006$) but not correlated with eGFR ($r_s = .05$, $p = 0.602$) or age ($r_s = -.047$, $p = 0.614$).

NTproBNP levels were significantly higher in females compared to males and were further associated with a past history of MI, heart failure, diabetes, higher Killip class, LVEF < 40%, ST segment deviation at admission as well as previous aspirin, ACEi/ARB, betablocker and statin use.

Higher NTproBNP levels were significantly associated with the endpoints of mortality at 6 months in addition to being strongly positively correlated with age, Killip class and creatinine levels while being inversely correlated with eGFR.

Prognostic Utility of Plasma Copeptin and NTproBNP

Plasma copeptin and NTproBNP were significant predictors of all cause mortality at 6 months in univariate analysis (HR = 5.98 [3.75 to 9.53], $p < 0.0005$ and 6.07 [2.98 to 12.37], $p < 0.0005$ respectively) along with increasing age, LVEF < 40%, GRACE score, ST segment deviation at

admission, past history of MI, hypertension, Killip Class >1 and eGFR. Only copeptin retained significant independent prognostic utility (HR = 3.03 [1.32 to 6.98], p = 0.009) when entered into a multivariate model (Table 3). Stratification by inpatient PCI produced only minor differences in hazard ratios (Table 3, * values).

Kaplan-Meier Analysis

Upper median levels of copeptin (>7.9 pmol/L) at admission significantly indicated a worse outcome (p <0.0005) (Figure 1).

Prognostic Utility of the GRACE score, Copeptin and NTproBNP

a. Comparison of area under ROC curves

The prognostic accuracy of plasma copeptin for all cause mortality at 6 months (AUC = 0.79 [0.73 to 0.86]) was not significantly different from the GRACE score (AUC = 0.81 [0.75 to 0.87]) or plasma NTproBNP (AUC = 0.75 [0.67 to 0.82]), p = 0.126 for overall differences between them (Figure 4a).

The combined prognostic accuracy of the GRACE score & copeptin (AUC = 0.84 [0.78 to 0.89] was greater than for the GRACE score & NTproBNP (AUC = 0.80 [0.74 to 0.87] and copeptin & NTproBNP (AUC = 0.81 [0.76 to 0.87] with an overall significant difference between them (p = 0.016) but no significant pairwise differences (Figure 4b).

The AUC of the combination of copeptin & NTproBNP was significantly better than that of NTproBNP alone (p = 0.020) but not copeptin alone (p = 0.285).

b. Comparison of Relative Utilities

Relative utility curves for the GRACE score, NTproBNP and copeptin are shown in Figure 2. Over the range of baseline risks from 0.15 to 0.6, copeptin had a consistently greater relative utility for prediction of the outcome compared to the GRACE score or NTproBNP. When used in combination, the relative utility of the GRACE score & copeptin was consistently greater than the GRACE score & NTproBNP as well as copeptin & NTproBNP (Figure 3).

Additional Prognostic Utility of Copeptin and NTproBNP

We further evaluated the additional prognostic utility of copeptin and NTproBNP when used in combination with the GRACE score compared to the GRACE score used alone as a baseline model.

a. Comparison of area under ROC curves

Copeptin combined with the GRACE score improved prognostic accuracy (AUC = 0.84 [0.78 to 0.89]) compared to the GRACE score alone (AUC = 0.81 [0.75 to 0.87], but this difference did not achieve statistical significance (p = 0.079). NTproBNP combined with the GRACE score (AUC = 0.80 [0.74 to 0.87]) again showed no significant increase in AUC (p = 0.871) compared to the GRACE score alone.

b. Difference in Relative Utility

At a baseline risk of 0.15, using copeptin combined with the GRACE score increased the relative utility of the GRACE score by 0.097 [-0.106 to 0.127] compared to 0.009 [-0.103 to 0.097] for NTproBNP, at a risk of 0.2 copeptin increased the relative utility of the GRACE score by 0.089 [-0.095 to 0.132] compared to 0.007 [-0.080 to 0.087] for NTproBNP and at a risk of 0.4 copeptin increased the relative utility by 0.048 [-0.043 to 0.082] compared to 0.0002 [-0.005 to 0.047] for NTproBNP.

These translate into test thresholds for copeptin of 139, 151 and 283 compared to 1562, 2058 and 56314 for NTproBNP for the added use of these biomarkers to the GRACE score at baseline risks of 15%, 20% and 40% respectively.

For comparison, when copeptin is used as the baseline model, the additional relative utility of adding NTproBNP was 0.038 [0.025 to 0.216], 0.017 [0.004 to 0.197] and 0.001 [-0.013 to 0.099] at baseline risks of 0.15, 0.2 and 0.4, while the reverse of adding copeptin to a baseline model of NTproBNP adds 0.194 [0.180 to 0.372], 0.154 [0.141 to 0.334] and 0.034 [0.021 to 0.133] of relative utility for the same baseline risks.

These translate into test thresholds of 351, 782 and 21295 for the addition of NTproBNP to a baseline model of copeptin and test thresholds 70, 88 and 393 for the addition of copeptin to a baseline model of NTproBNP at baseline risks of 15%, 20% and 40% respectively.

c. Reclassification

Integer GRACE scores were used to assign patients into categories at low, intermediate and high risk of mortality at 6 months as per the GRACE website (www.outcomes-umassmed.org/GRACE/). A binary logistic regression model with GRACE score as a single continuous covariate was then used to calculate the range of probabilities associated with each category. New predicted risks of mortality with either copeptin or NTproBNP added to the GRACE score were then calculated which were used to reclassify individuals. Changes in classification group were then used to calculate the net reclassification improvement (NRI).

In those who did not experience the endpoint at 6 months, the addition of copeptin improved risk classification in 100 individuals but made it worse in 44, while in those who did have the endpoint, copeptin reduced the risk classification in 1 individual while increasing the classification in 2. The NRI was 13.3% (95% C.I. [3.5 to 23.1]), p = 0.008, demonstrating a significant overall improvement in accuracy of risk classification compared to using the GRACE score alone (Table 4).

NTproBNP combined with the GRACE score failed to significantly improve risk stratification compared to using the GRACE score alone for outcome of mortality at 6 months (NRI = -4.9% [-12.5 to 2.8], p = 0.211) (Table not shown).

The addition of copeptin to baseline model of NTproBNP improved risk classification by tertiles (NRI = 10.1 [-5.1 to 25.2], p = 0.193) to a greater extent than the reverse of adding NTproBNP to a baseline model of copeptin (NRI = 3.4 [-10.4 to 17.2], p = 0.631) although both improvements were non-significant (Tables not shown).

Discussion

We have shown that an elevated plasma copeptin following admission with NSTEMI is strongly associated with adverse outcomes and has independent prognostic value when included in a multivariate risk model. This study demonstrates that copeptin could be used as a prognostic biomarker following admission with NSTEMI, which supports our previous work in a predominantly STEMI population [7]. In this previous study plasma copeptin levels were observed to decline significantly in the days following admission, which likely accounts for the slightly higher levels observed here (7.9 pmol/L) which were sampled at admission compared to levels measured in the STEMI population (7.2 pmol/L) which were sampled at 3-5 days post admission. This may also account for the significant association between copeptin levels and past history of MI observed here but not shown in the predominantly STEMI cohort.

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Copeptin levels in the healthy controls were not only significantly less than in NSTEMI patients but were also distributed over a narrower range, resulting in no significant correlations with either age or eGFR in contrast to the case in NSTEMI patients. Similarly, the wider variation of copeptin levels in NSTEMI patients results in there being no significant difference in levels between men and women unlike in the healthy controls here and a previously reported healthy cohort [15].

The inclusion of copeptin levels to a logistic regression risk model including the GRACE score produced significant incremental benefit in prognostic accuracy for mortality at 6 months, making copeptin a strong candidate for inclusion into a multimarker model, which has been suggested as the optimal approach for combining information from different risk markers [16].

This analysis has shown that adding copeptin to the GRACE score or NTproBNP resulted in only minor increases in ROC performance in contrast to our previous study in a predominantly STEMI population [7]. However ROC curves have been shown to have poor sensitivity in evaluating the incremental benefit of risk predictors [17]. Furthermore in order for a biomarker to be clinically useful it must show an incremental benefit over previous risk stratification methods sufficient to alter treatment decisions, which comparison of ROC curves is not able to quantify in an easily understandable manner [18].

Thus in this paper we have used the newer methods of reclassification and relative utility to evaluate the potential benefit of using copeptin in addition to more established risk stratification measures, the GRACE score and NTproBNP.

Reclassification showed that copeptin was particularly useful in down classifying patients from higher to lower risk groups in those that did not go on to reach the endpoint compared to using the GRACE score alone. In contrast, NTproBNP did not show any significant incremental benefit above the GRACE score in risk stratifying individuals into low, intermediate and high risk groups.

Relative utility is defined as the fraction of perfect prediction that is achieved at the optimal cut off point for the risk model, where utility refers to the sum of harms and benefits measured in the same units [19]. A relative utility of 1 indicates perfect prediction of the outcome by the prognostic model. Relative utility curves illustrate a risk model's performance over a range of risk thresholds and allow comparison with other prognostic models. The increment in relative utility produced by a new risk factor added to the baseline model can be used to calculate the test threshold, the minimum number of tests that would have to be traded for a true positive prediction. For example a test threshold of 100 means that 100 tests would need to performed in order to identify one person who will go on to have the endpoint. Our analysis showed that NTproBNP added only marginal utility to the GRACE score, with the number of tests required to identify a true positive ranging between 1562 and 56314 for baseline risks between 15% and 40%. In contrast copeptin added consistently more utility, with test thresholds between 139 and 283 for the same baseline risks. This means that far fewer copeptin tests than NTproBNP would need to be performed in order to identify true positives for the outcome.

Evidence of the superior prognostic value of copeptin compared to NTproBNP was further supported by finding that the additional relative utility and NRI of adding copeptin to a baseline model of NTproBNP was greater than the reverse of adding NTproBNP to a baseline model of copeptin.

Thus in this analysis we have shown that measurement of plasma copeptin levels adds important prognostic information to the GRACE score as well as NTproBNP. We suggest more accurate assignment of patients into different risk groups following admission to hospital with NSTEMI

would be helpful in guiding subsequent treatment decisions, although prospective clinical trials are needed to test this hypothesis.

Study Limitations

The diagnostic and prognostic performance of copeptin would need to be compared against existing risk stratification tools such as exercise testing, and would require external validation in further patient cohorts. We acknowledge the low rates of in-hospital percutaneous coronary intervention (PCI), treatment with GPIIb/IIIa inhibitors and clopidogrel as this cohort was recruited at a period when these treatments were not so frequently used.

Conclusion

Plasma copeptin is a novel independent prognostic biomarker in patients following non-ST elevation acute coronary syndrome and improves the early prognostic accuracy of the GRACE score compared to NTproBNP.

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Dr. Struck is an employee of BRAHMS GmbH, part of Thermo Fisher Scientific.

Author Contributions

Hafid Narayan analysed the data and wrote and the manuscript, Onkar Dhillon recruited and followed up patients, Pauline Quinn performed laboratory analyses of the plasma samples, Joachim Struck prepared the copeptin assays, Iain Squire assisted with manuscript revision, Joan Davies performed echocardiography, Leong Ng designed the study and assisted with data analysis.

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Figure 1. Kaplan Meier curves comparing time to total mortality in patients stratified to above or below median plasma copeptin, median copeptin = 7.9 pmol/L, $p < 0.0005$ for difference (Log Rank test).

Figure 2 Comparison of relative utility curves of the GRACE score, copeptin and NTproBNP for endpoint of total mortality at 6 months. The relevant region is the risk > 0.074, the prevalence of the endpoint at 6 months.

Figure 3 Comparison of relative utility curves of the GRACE score & NTproBNP, GRACE score & copeptin and copeptin & NTproBNP for endpoint of total mortality at 6 months. The relevant region is the risk > 0.074, the prevalence of the endpoint at 6 months.

Figure 4 ROC curves for outcome of all cause mortality at 6 months. **a)** GRACE score AUC = 0.81 [0.75 to 0.87]), copeptin AUC = 0.79 [0.73 to 0.86], NTproBNP AUC = 0.75 [0.67 to 0.82]. p = 0.126 for overall difference between them. **b)** GRACE Score & NTproBNP AUC = 0.80 [0.74 to 0.87], GRACE score & copeptin AUC = 0.84 [0.78 to 0.89], copeptin & NTproBNP AUC = 0.81 [0.76 to 0.87]. p = 0.016 for overall difference between them.

Table 1 Baseline Characteristics

	NSTEMI Patients n = 754	Healthy Controls n = 123	P
Male (% of n)	519 (68.8)	82 (66.7)	0.620
Age * (yrs)	70 (37 to 97)	71.5 (65 to 81)	0.032
Creatinine * (µmol/L)	99 (39 to 455)	89 (25 to 146)	< 0.005
eGFR † (ml.min^{-1}.1.73m^{-2} surface area)	63.5 (62 to 65)	65.8 (64 to 67)	0.142
LV Ejection Fraction*(%)	39 (13 to 61)	-	
LV Ejection Fraction < 40% (% of n)	227 (44.2)‡	-	
Killip Class > 1 (% of n)	271 (36.0)	-	
TIMI score*	4 (0 to 10)	-	
6 Month GRACE score†	122 (119 to 125)	-	
Troponin I* (µg/L)	1.9 (0 to 67)	-	
Copeptin* (pmol/L)	7.9 (0.3 to 523)	3.9 (1.4 to 28.7)	< 0.005
NTproBNP* (pmol/L)	1135 (0 to 11779)	51 (6 to 992)	< 0.005
ST Deviation at Admission (% of n)	334 (44.1)	-	
In Hospital PCI	132 (17.5)	-	
Past History (% of n)			
MI	209 (27.7)	-	
Hypertension	435 (57.7)	-	
Diabetes	200 (26.5)	-	
Smoker	255 (33.8)	-	
HF	17 (2.3)	-	
Admission Medication (% of n)			
Aspirin	300 (39.8)	-	
BetaBlocker	228 (30.2)	-	
Statin	273 (36.6)	-	
ACEi or ARB	264 (35.0)	-	
Diuretics	150 (19.9)	-	
Inpatient Medication (% of n)			
GPIIa/IIIb Inhibitor Use	34 (6.6)‡	-	
Discharge Medication (% of n)			
Aspirin	622 (82.5)	-	
Clopidogrel	310 (60.2)‡	-	
Betablocker	563 (74.7)	-	
Statin	587 (77.9)	-	
ACEi or ARB	523 (69.4)	-	
Diuretics	239 (31.7)	-	

* median (range), † mean (95% C.I.), eGFR = estimated Glomerular Filtration Rate, PCI = Percutaneous Coronary Intervention, LV = Left Ventricle, MI = Myocardial Infarction, HF = Heart Failure, ACEi = Angiotensin Converting Enzyme Inhibitor, ARB = Angiotensin II Receptor Blocker ‡ % Data available for 515 patients out of total 754

Table 2 Univariate Analysis of Plasma Copeptin and NTproBNP Levels in NSTEMI Patients

	Copeptin Levels (pmol/L)	p	NTproBNP (pmol/L)	p
Males vs Females, median (range)	7.2 (0.8 to 523.0) vs 8.7 (0.3 to 233.0)	0.340	932 (0 to 10474) vs 1773 (4 to 9734)	<0.0005
Previous medical history:				
MI vs None	13.1 (1.4 to 523.0) vs 7.0 (0.3 to 485.0)	<0.0005	2365 (73 to 10474) vs 873 (0 to 10354)	<0.0005
HF vs None	26.6 (4.0 to 233.0) vs 7.7 (0.3 to 523.0)	<0.0005	4025 (325 to 6776) vs 1149 (0 to 10474)	0.030
Diabetes vs None	12.0 (1.0 to 523.0) vs 7.0 (0.3 to 485.0)	<0.0005	1643 (2 to 11779) vs 990 (0 to 10904)	0.030
Killip class > 1 vs Killip class 1	15.9 (1.0 to 523.0) vs 6.0 (0.3 to 485.0)	<0.0005	2743 (30 to 10474) vs 729 (0 to 6034)	<0.0005
LVEF<40% vs LVEF >40%	9.1 (1.0 to 523.0) vs 6.4 (0.3 to 485.0)	<0.0005	2174 (22 to 10474) vs 644 (0 to 10354)	<0.0005
ST Deviation at Admission vs None	8.6 (0.8 to 523.0) vs 7.2 (0.3 to 485)	0.014	1439 (11 to 11779) vs 1004 (0 to 8111)	0.01
Admission medications:				
Aspirin vs None	9.5 (0.8 to 330.0) vs 6.9 (0.3 to 523.0)	0.003	990 (0 to 10354) vs 2360 (7 to 10474)	<0.0005
ACEi or ARB vs None	8.7 (0.3 to 523.0) vs 6.9 (0.6 to 485.0)	0.004	1104 (4 to 10474) vs 1427 (0 to 10249)	0.016
Betablocker vs None	9.1 (0.3 to 523.0) vs 7.2 (0.6 to 485.0)	0.003	1027 (4 to 10354) vs 1846 (0 to 10474)	<0.0005
Statin vs None	8.5 (0.9 to 523.0) vs 7.7 (0.3 to 485.0)	0.073	1059 (0 to 10474) vs 2816 (7 to 8314)	<0.0005
End point at 6 Months:				
Mortality vs Event free survival	32.0 (2.4 to 330.0) vs 7.2 (0.3 to 523.0)	<0.0005	3669 (184 to 10249) vs 1067 (0 to 10474)	<0.0005
Spearman correlation				
Age	0.328	<0.0005	0.556	<0.0005
eGFR	-0.477	<0.0005	-0.481	<0.0005
Creatinine	0.480	<0.0005	0.374	<0.0005
Killip class	0.377	<0.0005	0.426	<0.0005
NTproBNP	0.467	<0.0005	-	-

MI =Myocardial Infarction, HF = Heart Failure, LVEF = Left Ventricle Ejection Fraction, ACEi = Angiotensin Converting Enzyme Inhibitor, ARB = Angiotensin II Receptor Blocker, eGFR = estimated glomerular filtration rate

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Table 3 Cox Proportional Hazards Regression Analysis in NSTEMI Patients

	Univariate Analysis		Multivariate Analysis	
	HR (95% C.I.)	**p**	**HR (95% C.I.)**	**p**
Age	1.09 (1.06 to 1.12) 1.09 (1.06 to 1.12)*	<0.0005 <0.0005*	1.05 (0.98 to 1.13) 1.05 (0.98 to 1.13)*	0.141 0.135*
Gender	0.88 (0.51 to 1.53) 0.91 (0.52 to 1.58)*	0.650 0.732*		
LVEF <40%	2.34 (1.22 to 4.50) 2.31 (1.20 to 4.45)*	0.011 0.012*	1.34 (0.60 to 2.99) 1.35 (0.60 to 3.02)*	0.472 0.470*
GRACE Score	1.03 (1.02 to 1.04) 1.03 (1.02 to 1.04)*	<0.0005 <0.0005*	1.01 (0.98 to 1.04) 1.01 (0.98 to 1.03)*	0.659 0.612*
ST Segment Deviation	1.85 (1.09 to 3.15) 1.86 (1.09 to 3.17)*	0.024 0.022*	1.32 (0.57 to 3.05) 1.48 (0.67 to 3.28)*	0.512 0.332*
Previous History				
MI	2.56 (1.51 to 4.32) 2.54 (1.50 to 4.29)*	<0.0005 <0.0005*	0.85 (0.38 to 1.91) 0.90 (0.40 to 2.05)*	0.700 0.809*
Heart Failure	1.55 (0.38 to 6.37) 1.47 (0.36 to 6.03)*	0.540 0.595*		
Hypertension	2.03 (1.13 to 3.67) 2.00 (1.11 to 3.62)*	0.019 0.021*	1.07 (0.49 to 2.33) 1.03 (0.47 to 2.26)*	0.863 0.939*
Diabetes	1.58 (0.91 to 2.72) 1.55 (0.90 to 2.69)*	0.103 0.114*		
Killip class ≥1	3.32 (1.92 to 5.74) 3.27 (1.89 to 5.65)*	<0.0005 <0.0005*	2.56 (0.94 to 6.99) 2.56 (0.94 to 6.97)*	0.067 0.065*
Troponin I	1.15 (0.74 to 1.78) 1.13 (0.73 to 1.76)*	0.543 0.579*		
Copeptin	5.98 (3.75 to 9.53) 5.94 (3.72 to 9.50)*	<0.0005 <0.0005*	3.03 (1.32 to 6.98) 3.13 (1.38 to 7.09)*	0.009 0.006*
NTproBNP	6.07 (2.98 to 12.37) 6.00 (2.90 to 12.44)*	<0.0005 <0.0005*	1.24 (0.41 to 3.73) 1.22 (0.41 to 3.60)*	0.698 0.724*
eGFR	0.96 (0.94 to 0.97) 0.96 (0.94 to 0.97)*	<0.0005 <0.0005*	1.01 (0.99 to 1.04) 1.01 (0.98 to 1.03)*	0.426 0.493*
* Stratified by Inpatient PCI				

Table 4 Reclassification for 6 Month Risk All Cause Mortality in NSTEMI Patients

	GRACE Score + Copeptin						
GRACE Score Risk Group	Number of Individuals			Reclassification		% Correctly Reclassified	p
	Low	Intermediate	High	Increased	Decreased		
Individuals without Endpoint at 6 Months n = 523							
Low	77	24	1				
Intermediate	32	90	19	44	100	10.7	
High	2	66	212				
Individuals with Endpoint at 6 Months n = 39							
Low	0	0	0				
Intermediate	0	2	2	2	1	2.6	
High	0	1	34				
% Net Reclassification Improvement [95%]						**13.3 [3.5 to 23.1]**	**0.008**

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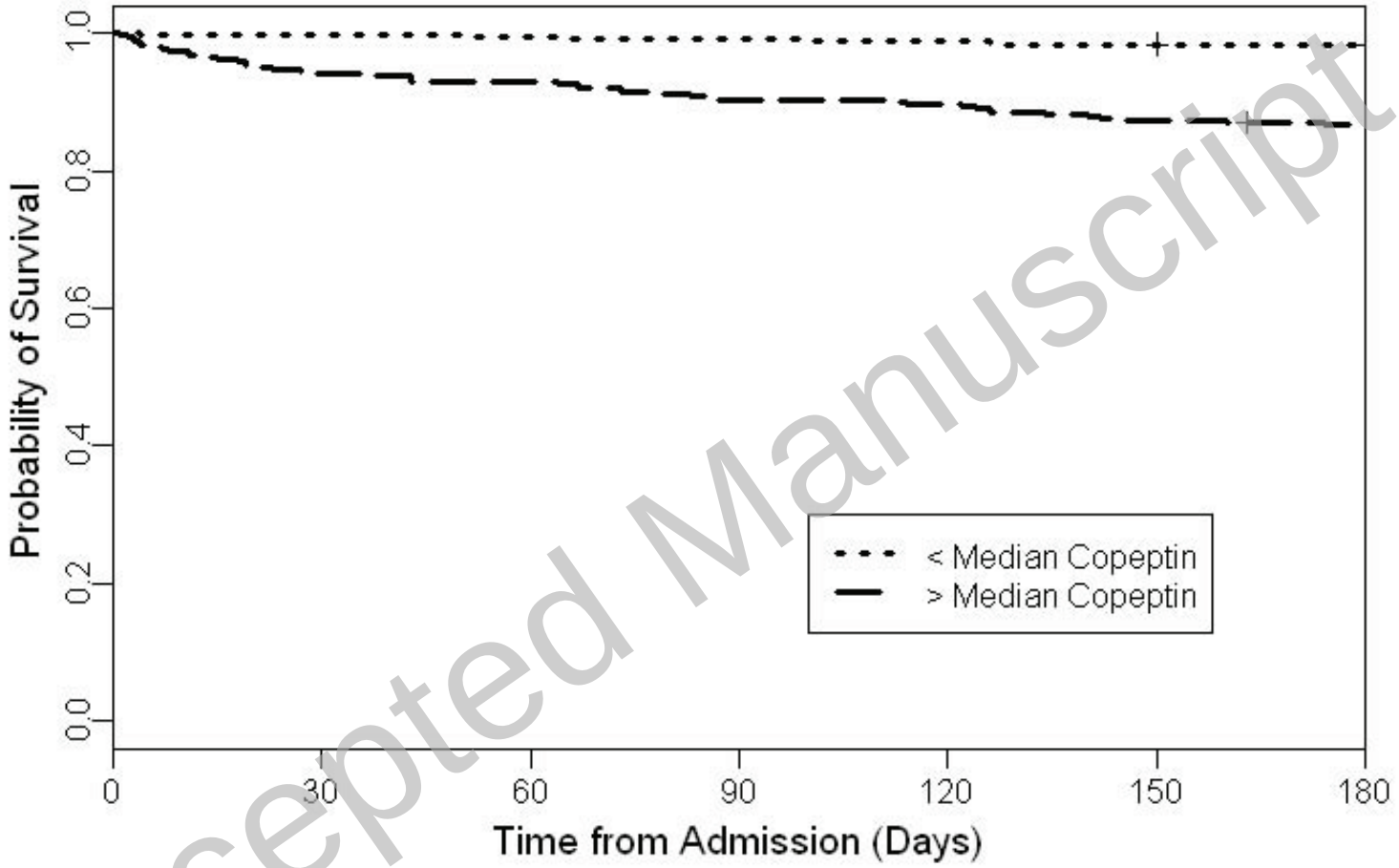

Number at Risk

Day	0	30	60	90	120	150	180
< Median	315	314	313	312	311	310	309
> Median	316	297	294	285	283	276	273

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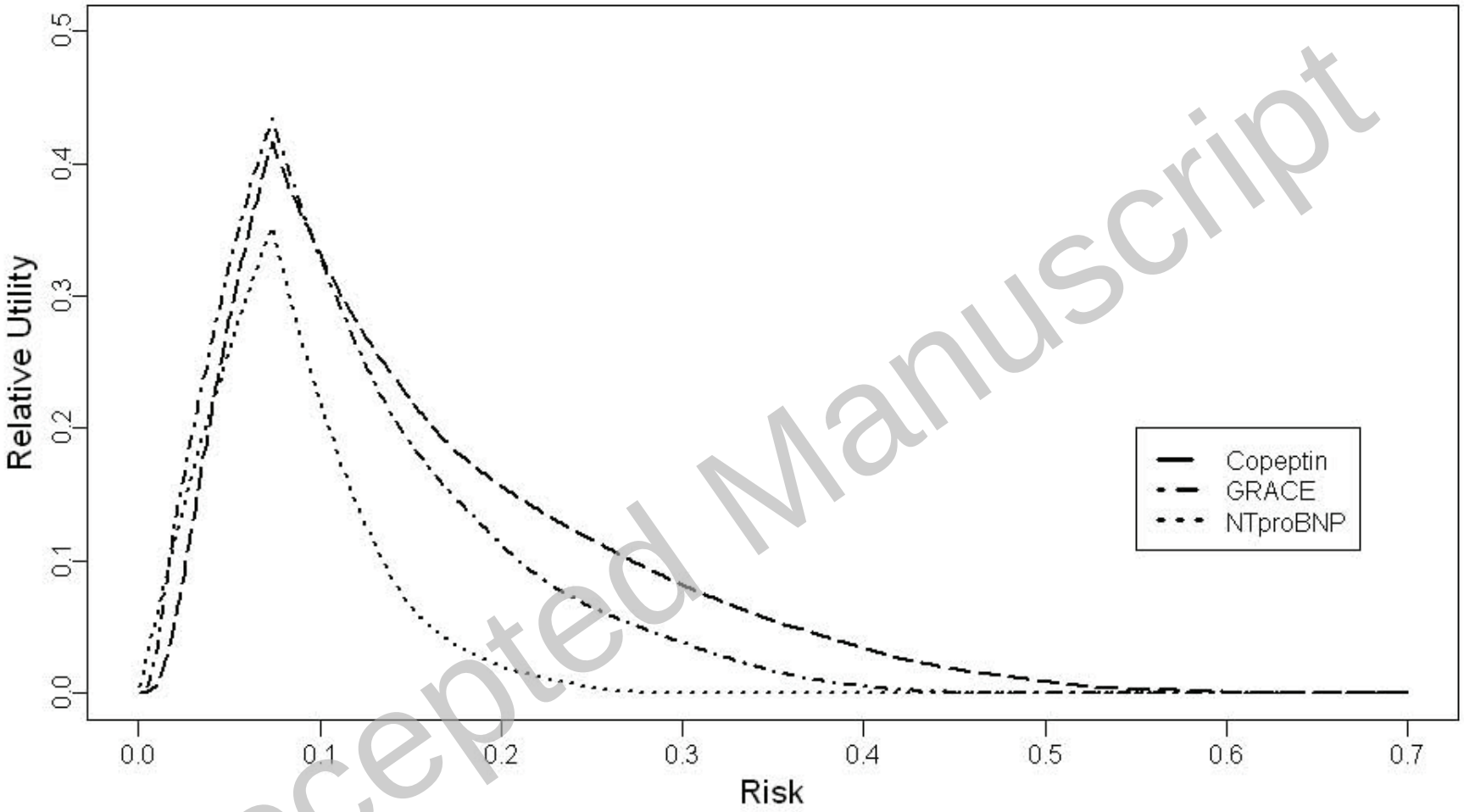

	Relative Utility					
Risk	0.1	0.2	0.3	0.4	0.5	0.6
Copeptin	0.332	0.156	0.082	0.034	0.009	0.001
GRACE	0.331	0.112	0.038	0.005	0	0
NTproBNP	0.223	0.020	0	0	0	0

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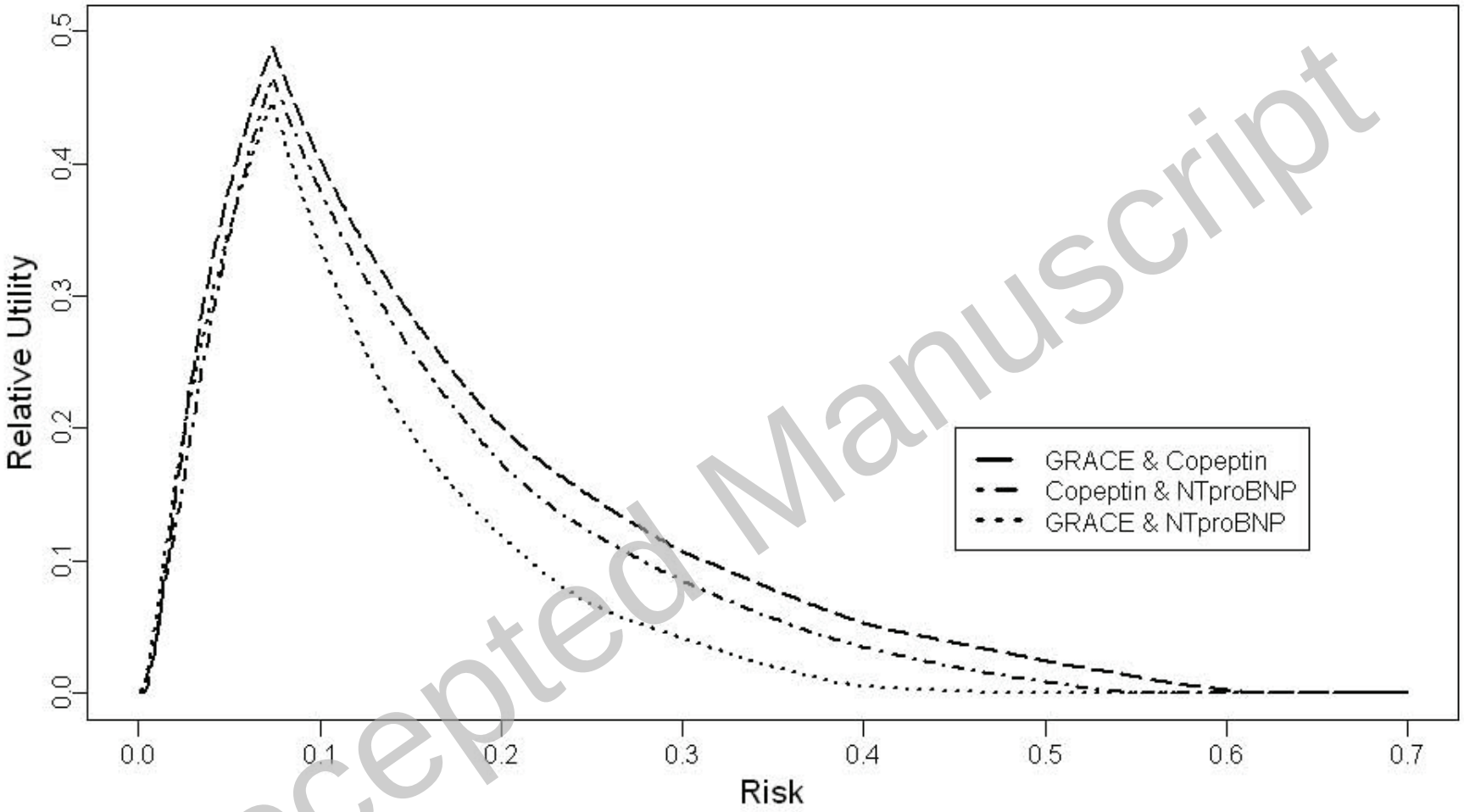

	Relative Utility					
Risk	0.1	0.2	0.3	0.4	0.5	0.6
GRACE & Copeptin	0.404	0.202	0.107	0.053	0.024	0.002
Copeptin & NTproBNP	0.381	0.174	0.085	0.034	0.008	0
GRACE & NTproBNP	0.340	0.119	0.041	0.005	0	0

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