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## **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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1 **Clinical Investigations**

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

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38 **Abstract**

39

40 **Background**

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

46

47 **Methods**

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

51

52 **Results:** Upper median levels of copeptin were strongly associated with all cause mortality at 6  
 53 months. Copeptin was a significant predictor of time to mortality (HR = 5.98 [3.75 to 9.53],  $p <$   
 54 0.0005) in univariate analysis and remained a significant predictor in multivariate analysis (HR =  
 55 3.03 [1.32 to 6.98],  $p = 0.009$ ). There were no significant differences between the area under ROC  
 56 curves of copeptin, NTproBNP and the GRACE score. Copeptin improved accuracy of risk  
 57 classification when used in combination with the GRACE score as determined by net  
 58 reclassification improvement whereas NTproBNP did not. The relative utility of the GRACE score  
 59 was increased more by copeptin than by NTproBNP over a wide range of risks.

60

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

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Accepted Manuscript

90 **Introduction**

91 Recent guidelines recommend risk stratification of all patients following admission with non- ST  
92 elevation myocardial infarction (NSTEMI) in order to identify those individuals at maximum risk of  
93 adverse outcomes who may benefit from early aggressive therapy [1]. Currently clinical risk  
94 assessment tools such as the TIMI (Thrombolysis in Myocardial Infarction) [2] and GRACE  
95 (Global Registry of Acute Coronary Events) [3] scores are the most commonly used method of risk  
96 stratification, although more recently plasma biomarkers have emerged as a potentially alternative  
97 or complementary technique [4].  
98

99 Copeptin is the 39 amino acid C-terminal portion of provasopressin, the precursor of arginine  
100 vasopressin (AVP) [5]. Copeptin has the advantages of a longer plasma half life and reduced  
101 propensity for protein binding compared to AVP making its measurement more accurate [6].  
102 Copeptin has recently been demonstrated to be an independent predictor for adverse events  
103 following ST elevation myocardial infarction (STEMI) [7].  
104

105 We hypothesized that plasma copeptin is an independent predictor for adverse outcomes following  
106 acute NSTEMI and further sought to evaluate whether copeptin provided additional prognostic  
107 information to NTproBNP (N Terminal pro B Type Natriuretic Peptide) and the GRACE score,  
108 established risk stratification markers.  
109

110 This paper presents a post hoc analysis of a subset of patients with NSTEMI recruited to the  
111 original LAMP (Leicester Acute Myocardial Infarction Peptide) cohort study of patients with acute  
112 myocardial infarction supplemented with a new cohort of NSTEMI patients.  
113

114 **Methods**

115 **Study population**

116 We analyzed patients admitted to the Leicester Royal Infirmary and Glenfield Hospital, Leicester  
117 with acute myocardial infarction (MI). The population for this LAMP II study was comprised of  
118 patients recruited as follows:- (1) 239 NSTEMI patients who were included in the original LAMP  
119 study which included both NSTEMI and STEMI patients, who were recruited between March 2000  
120 and July 2005 and (2) a new group of 515 NSTEMI patients who were recruited between August  
121 2005 and April 2007. As a control group plasma copeptin and NTproBNP were also measured in 82  
122 healthy males over 65 and 41 females over 70 years of age.  
123

124 This study abided by the Declaration of Helsinki and was approved by the local ethics committee.  
125 Written informed consent was obtained from all the patients. Acute NSTEMI was diagnosed if the  
126 patient described a history of cardiac sounding chest pain lasting greater than 20 minutes not  
127 associated with ST segment elevation with a plasma creatine kinase- MB level twice the upper limit  
128 of normal or cardiac troponin I level  $> 0.1$  ng/mL (8). NSTEMI was confirmed in all patients prior  
129 to inclusion. The Centaur cTnI Ultra immunoassay (Siemens Healthcare Diagnostics) was used to  
130 measure troponin I which has a coefficient of variation 10% at 0.03 ng/mL with a 99th percentile of  
131 0.04 ng/mL. We chose this cut-off to ensure patients had a definite diagnosis of NSTEMI.  
132

133 Patients with known malignancy or surgery in the previous month were excluded. The estimated  
134 glomerular filtration rate (eGFR) of the study patients was calculated from the simplified formula  
135 derived from the Modification of Diet and Renal Disease (MDRD) study validated in patients with  
136 heart failure [9].  
137

138 **Plasma samples**

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

141 minutes of bed rest 20mL of blood was collected into tubes containing EDTA and aprotinin. All  
142 plasma was stored at  $-70^{\circ}\text{C}$  until assayed in a blinded fashion in a single batch.

143

#### 144 Copeptin assay

145 Copeptin was detected with a novel commercial assay. Briefly, tubes were coated with a purified  
146 sheep polyclonal antibody raised against a peptide that represented amino acids 132 to 147 of  
147 preproAVP. A purified sheep polyclonal antibody raised against a peptide representing amino acids  
148 149 to 164 of preproAVP was labeled with methyl acridinium N-hydroxysuccinimide ester (In Vent  
149 GmbH, Bielefeld, Germany) and used as tracer. Dilutions of a peptide representing amino acids 132  
150 to 164 of preproAVP in normal horse serum served as standards. The immunoassay was performed  
151 by incubating 50  $\mu\text{L}$  of samples/standards and 200  $\mu\text{L}$  of tracer in coated tubes for 2 hours at room  
152 temperature. Tubes were washed 4 times with 1 mL of wash solution (BRAHMS AG), and bound  
153 chemiluminescence was measured with an LB952T luminometer (Berthold, Bad Wildbad,  
154 Germany). The assay limit of detection was 0.4 pmol/L, with functional assay sensitivity  $< 1$   
155 pmol/L. The interassay coefficient of variation was 10% at 3 pmol/L and  $< 7\%$  for values  $> 10$   
156 pmol/L.

157

#### 158 NTproBNP assay

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

166

#### 167 Endpoints

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

173

#### 174 Statistical analysis

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

184

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

188

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

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

194

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

198

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

201

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

205

## 206 **Results**

### 207 Patient Characteristics

208 754 consecutive patients were recruited of whom 519 were male, median age 70 [37 to 97] years.  
209 No patients were lost to follow up and there was a minimum length of follow up of 6 months. In 6  
210 months, 56 patients (7.4%) had died and 49 (6.5%) had a recurrent myocardial infarction. The  
211 baseline characteristics of patients and 123 healthy controls are shown in Table 1. The median time  
212 from symptom onset to blood draw in patients was 25 hours 45 minutes. Both copeptin and  
213 NTproBNP were significantly raised in the NSTEMI patients compared to the healthy controls  
214 (Table 1).

215

### 216 Factors Associated with Elevated Plasma Copeptin and NTproBNP

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

219

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

225

226 Copeptin level was significantly positively correlated with age and Killip class, creatinine and  
227 NTproBNP levels in NSTEMI patients, while being inversely correlated with eGFR (Table 2). For  
228 comparison in healthy controls, copeptin was significantly higher in males compared to females  
229 (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 ( $r_s =$   
230  $.05$ ,  $p = 0.602$ ) or age ( $r_s = -.047$ ,  $p = 0.614$ ).

231

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

235

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

239

### 240 Prognostic Utility of Plasma Copeptin and NTproBNP

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

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

248

#### 249 Kaplan-Meier Analysis

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

252

#### 253 Prognostic Utility of the GRACE score, Copeptin and NTproBNP

254

##### 255 *a. Comparison of area under ROC curves*

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

260

261 The combined prognostic accuracy of the GRACE score & copeptin (AUC = 0.84 [0.78 to 0.89])  
262 was greater than for the GRACE score & NTproBNP (AUC = 0.80 [0.74 to 0.87]) and copeptin &  
263 NTproBNP (AUC = 0.81 [0.76 to 0.87]) with an overall significant difference between them ( $p =$   
264 0.016) but no significant pairwise differences (Figure 4b).

265

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

268

##### 269 *b. Comparison of Relative Utilities*

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

275

#### 276 Additional Prognostic Utility of Copeptin and NTproBNP

277

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

280

##### 281 *a. Comparison of area under ROC curves*

282 Copeptin combined with the GRACE score improved prognostic accuracy (AUC = 0.84 [0.78 to  
283 0.89]) compared to the GRACE score alone (AUC = 0.81 [0.75 to 0.87]), but this difference did not  
284 achieve statistical significance ( $p = 0.079$ ). NTproBNP combined with the GRACE score (AUC =  
285 0.80 [0.74 to 0.87]) again showed no significant increase in AUC ( $p = 0.871$ ) compared to the  
286 GRACE score alone.

287

##### 288 *b. Difference in Relative Utility*

289 At a baseline risk of 0.15, using copeptin combined with the GRACE score increased the relative  
290 utility of the GRACE score by 0.097 [-0.106 to 0.127] compared to 0.009 [-0.103 to 0.097] for  
291 NTproBNP, at a risk of 0.2 copeptin increased the relative utility of the GRACE score by 0.089 [-  
292 0.095 to 0.132] compared to 0.007 [-0.080 to 0.087] for NTproBNP and at a risk of 0.4 copeptin  
293 increased the relative utility by 0.048 [-0.043 to 0.082] compared to 0.0002 [-0.005 to 0.047] for  
294 NTproBNP.

295

296 These translate into test thresholds for copeptin of 139, 151 and 283 compared to 1562, 2058 and  
297 56314 for NTproBNP for the added use of these biomarkers to the GRACE score at baseline risks  
298 of 15%, 20% and 40% respectively.

299

300 For comparison, when copeptin is used as the baseline model, the additional relative utility of  
301 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]  
302 at baseline risks of 0.15, 0.2 and 0.4, while the reverse of adding copeptin to a baseline model of  
303 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  
304 relative utility for the same baseline risks.

305

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

309

### 310 *c. Reclassification*

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

318

319 In those who did not experience the endpoint at 6 months, the addition of copeptin improved risk  
320 classification in 100 individuals but made it worse in 44, while in those who did have the endpoint,  
321 copeptin reduced the risk classification in 1 individual while increasing the classification in 2. The  
322 NRI was 13.3% (95% C.I. [3.5 to 23.1]),  $p = 0.008$ , demonstrating a significant overall  
323 improvement in accuracy of risk classification compared to using the GRACE score alone (Table  
324 4).

325

326 NTproBNP combined with the GRACE score failed to significantly improve risk stratification  
327 compared to using the GRACE score alone for outcome of mortality at 6 months (NRI = -4.9% [-  
328 12.5 to 2.8],  $p = 0.211$ ) (Table not shown).

329

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

334

### 335 **Discussion**

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

346



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

352  
353 The inclusion of copeptin levels to a logistic regression risk model including the GRACE score  
354 produced significant incremental benefit in prognostic accuracy for mortality at 6 months, making  
355 copeptin a strong candidate for inclusion into a multimarker model, which has been suggested as the  
356 optimal approach for combining information from different risk markers [16].

357  
358 This analysis has shown that adding copeptin to the GRACE score or NTproBNP resulted in only  
359 minor increases in ROC performance in contrast to our previous study in a predominantly STEMI  
360 population [7]. However ROC curves have been shown to have poor sensitivity in evaluating the  
361 incremental benefit of risk predictors [17]. Furthermore in order for a biomarker to be clinically  
362 useful it must show an incremental benefit over previous risk stratification methods sufficient to  
363 alter treatment decisions, which comparison of ROC curves is not able to quantify in an easily  
364 understandable manner [18].

365  
366 Thus in this paper we have used the newer methods of reclassification and relative utility to  
367 evaluate the potential benefit of using copeptin in addition to more established risk stratification  
368 measures, the GRACE score and NTproBNP.

369  
370 Reclassification showed that copeptin was particularly useful in down classifying patients from  
371 higher to lower risk groups in those that did not go on to reach the endpoint compared to using the  
372 GRACE score alone. In contrast, NTproBNP did not show any significant incremental benefit  
373 above the GRACE score in risk stratifying individuals into low, intermediate and high risk groups.

374  
375 Relative utility is defined as the fraction of perfect prediction that is achieved at the optimal cut off  
376 point for the risk model, where utility refers to the sum of harms and benefits measured in the same  
377 units [19]. A relative utility of 1 indicates perfect prediction of the outcome by the prognostic  
378 model. Relative utility curves illustrate a risk model's performance over a range of risk thresholds  
379 and allow comparison with other prognostic models. The increment in relative utility produced by a  
380 new risk factor added to the baseline model can be used to calculate the test threshold, the minimum  
381 number of tests that would have to be traded for a true positive prediction. For example a test  
382 threshold of 100 means that 100 tests would need to be performed in order to identify one person who  
383 will go on to have the endpoint. Our analysis showed that NTproBNP added only marginal utility to  
384 the GRACE score, with the number of tests required to identify a true positive ranging between  
385 1562 and 56314 for baseline risks between 15% and 40%. In contrast copeptin added consistently  
386 more utility, with test thresholds between 139 and 283 for the same baseline risks. This means that  
387 far fewer copeptin tests than NTproBNP would need to be performed in order to identify true  
388 positives for the outcome.

389  
390 Evidence of the superior prognostic value of copeptin compared to NTproBNP was further  
391 supported by finding that the additional relative utility and NRI of adding copeptin to a baseline  
392 model of NTproBNP was greater than the reverse of adding NTproBNP to a baseline model of  
393 copeptin.

394  
395 Thus in this analysis we have shown that measurement of plasma copeptin levels adds important  
396 prognostic information to the GRACE score as well as NTproBNP. We suggest more accurate  
397 assignment of patients into different risk groups following admission to hospital with NSTEMI

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

400

401

402 **Study Limitations**

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

408

409 **Conclusion**

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

413

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416

417 **Author Contributions**

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

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428

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430

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509 **Figure 1.** Kaplan Meier curves comparing time to total mortality in patients stratified to above or  
510 below median plasma copeptin, median copeptin = 7.9 pmol/L,  $p < 0.0005$  for difference (Log Rank  
511 test).  
512

513 **Figure 2** Comparison of relative utility curves of the GRACE score, copeptin and NTproBNP for  
514 endpoint of total mortality at 6 months. The relevant region is the risk  $> 0.074$ , the prevalence of the  
515 endpoint at 6 months.  
516

517 **Figure 3** Comparison of relative utility curves of the GRACE score & NTproBNP, GRACE score  
518 & copeptin and copeptin & NTproBNP for endpoint of total mortality at 6 months. The relevant  
519 region is the risk  $> 0.074$ , the prevalence of the endpoint at 6 months.  
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521 **Figure 4** ROC curves for outcome of all cause mortality at 6 months. **a)** GRACE score AUC = 0.81  
522 [0.75 to 0.87]), copeptin AUC = 0.79 [0.73 to 0.86], NTproBNP AUC = 0.75 [0.67 to 0.82].  $p =$   
523 0.126 for overall difference between them. **b)** GRACE Score & NTproBNP AUC = 0.80 [0.74 to  
524 0.87], GRACE score & copeptin AUC = 0.84 [0.78 to 0.89], copeptin & NTproBNP AUC = 0.81  
525 [0.76 to 0.87].  $p = 0.016$  for overall difference between them.

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**Table 1 Baseline Characteristics**

	<b>NSTEMI Patients n = 754</b>	<b>Healthy Controls n = 123</b>	<b>P</b>
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 <sup>-1</sup> .1.73m <sup>-2</sup> 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)	-	
<b>Past History (% of n)</b>			
MI	209 (27.7)	-	
Hypertension	435 (57.7)	-	
Diabetes	200 (26.5)	-	
Smoker	255 (33.8)	-	
HF	17 (2.3)	-	
<b>Admission Medication (% of n)</b>			
Aspirin	300 (39.8)	-	
BetaBlocker	228 (30.2)	-	
Statin	273 (36.6)	-	
ACEi or ARB	264 (35.0)	-	
Diuretics	150 (19.9)	-	
<b>Inpatient Medication (% of n)</b>			
GPIIa/IIIb Inhibitor Use	34 (6.6)‡	-	
<b>Discharge Medication (% of n)</b>			
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

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

	<b>Copeptin Levels (pmol/L)</b>	<b>p</b>	<b>NTproBNP (pmol/L)</b>	<b>p</b>
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
<b>Previous medical history:</b>				
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
<b>Admission medications:</b>				
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
<b>End point at 6 Months:</b>				
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
<b>Spearman correlation</b>				
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				

**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*
<b>Previous History</b>				
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

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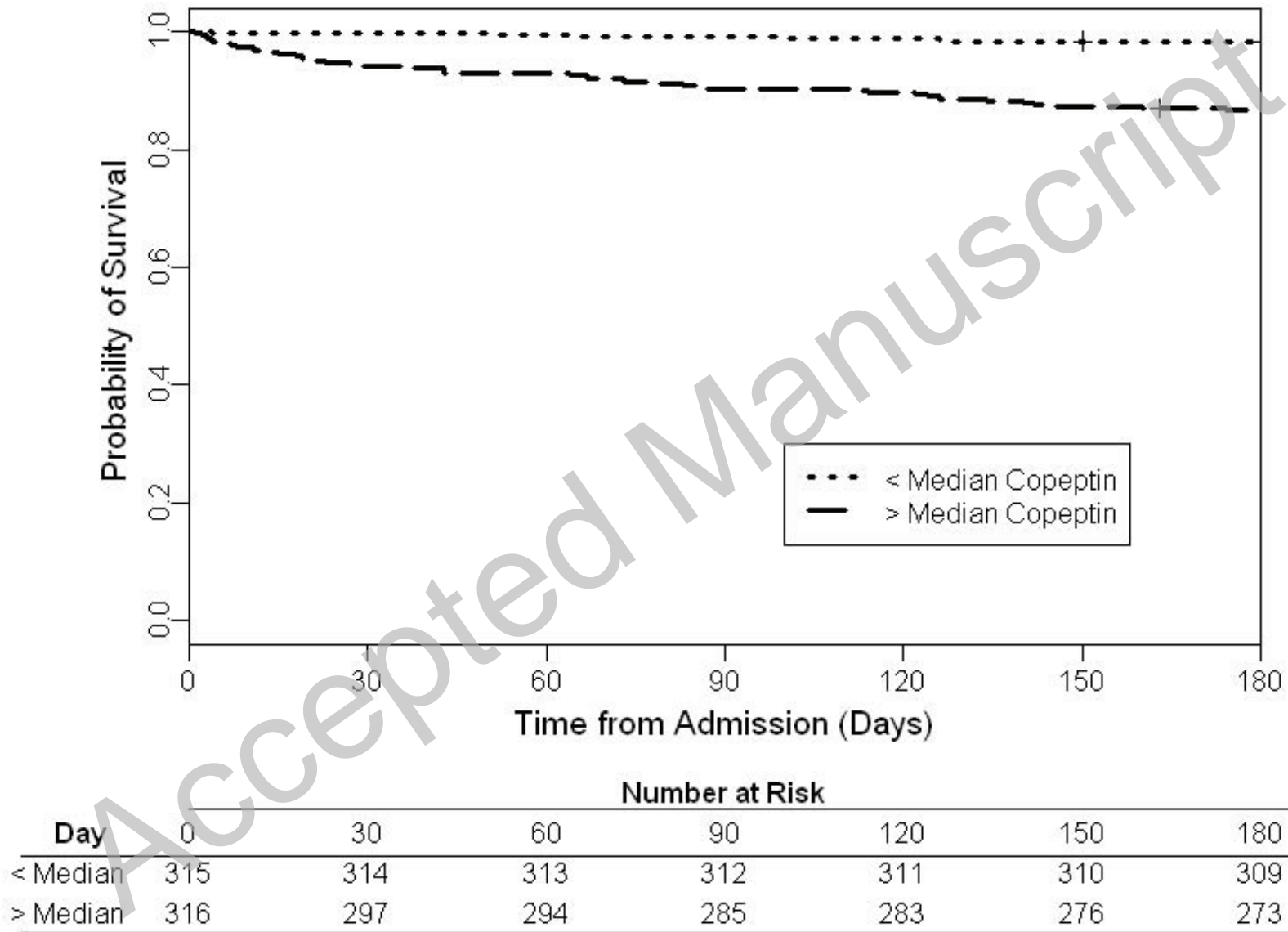
**Table 4 Reclassification for 6 Month Risk All Cause Mortality in NSTEMI Patients**

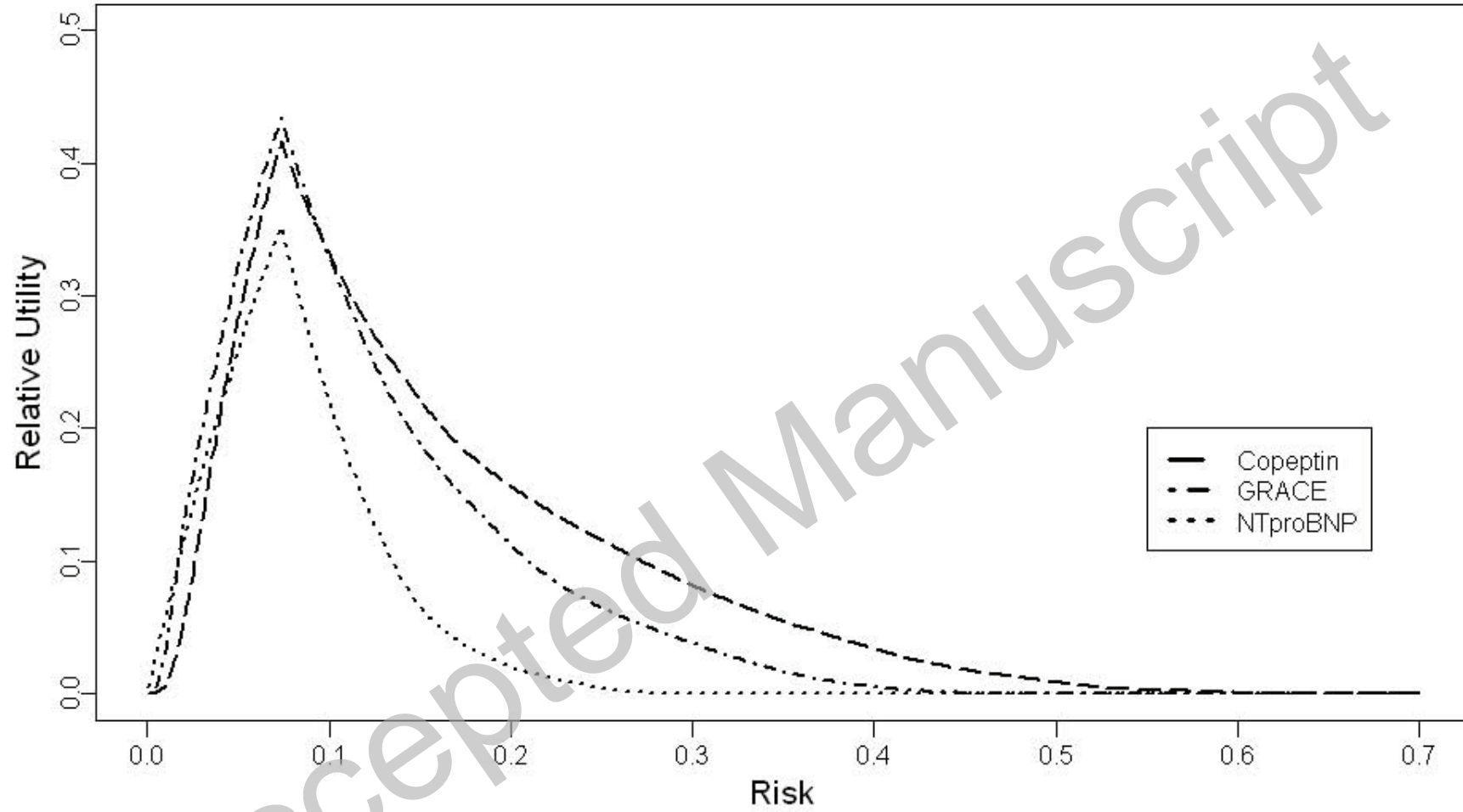
GRACE Score Risk Group	GRACE Score + Copeptin			Reclassification		% Correctly Reclassified	p
	Number of Individuals			Increased	Decreased		
	Low	Intermediate	High				
<b>Individuals without Endpoint at 6 Months n = 523</b>							
<b>Low</b>	77	24	1				
<b>Intermediate</b>	32	90	19	44	100	10.7	
<b>High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

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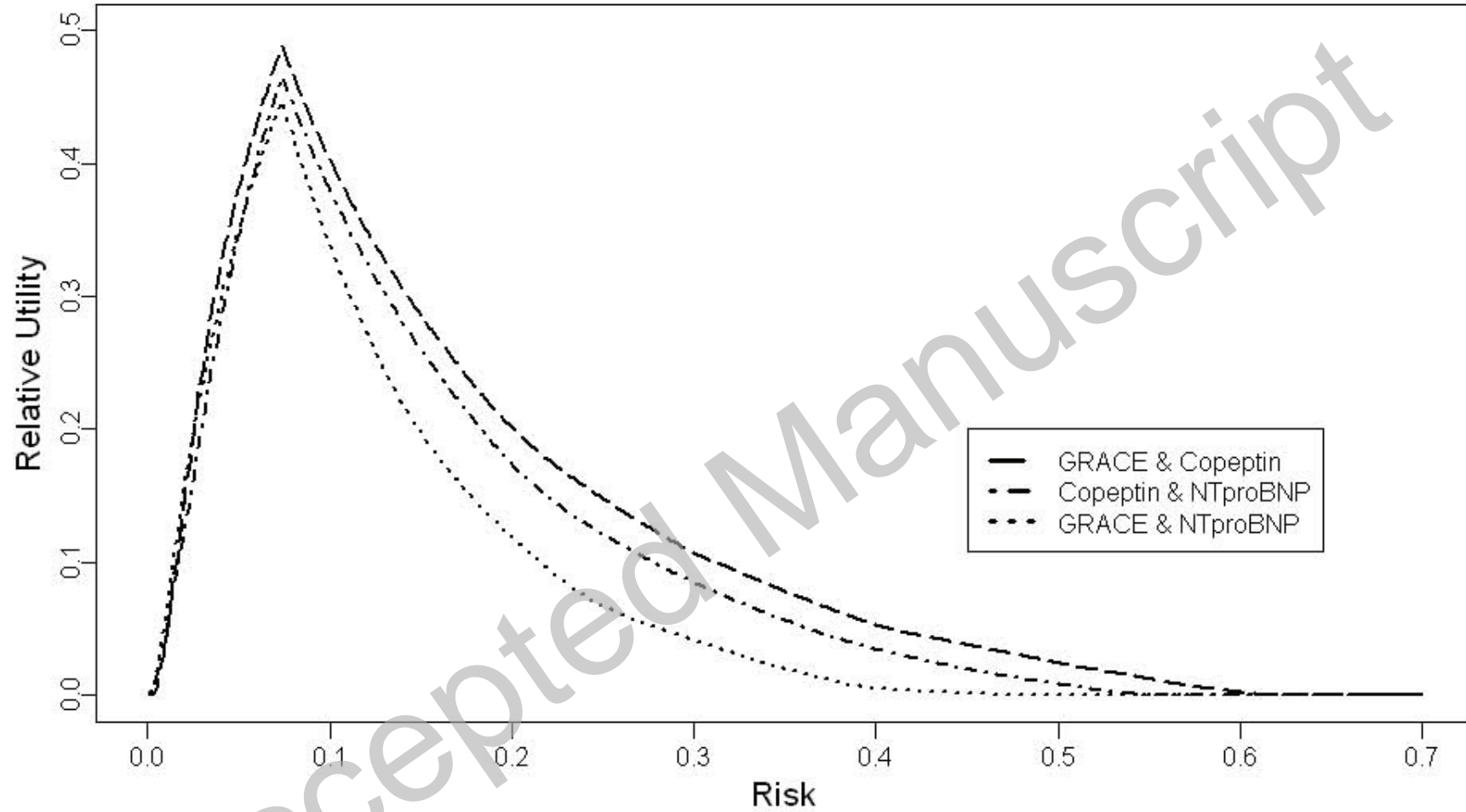
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Risk	Relative Utility					
	0.1	0.2	0.3	0.4	0.5	0.6
Copeptin	0.332	0.156	0.082	0.034	0.009	0.001
GRACE	0.331	0.112	0.038	0.005	0	0
NTproBNP	0.223	0.020	0	0	0	0



Risk	Relative Utility					
	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

