รายงานวิจัยฉบับสมบูรณ์

โครงการการศึกษาระดับ urocortin ในเลือดของผู้ป่วยไทย ที่มีภาวะหัวใจล้มเหลว

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ชื่อโครงการ: การศึกษาระดับ urocortin ในเลือดของผู้ป่วยไทยที่มีภาวะหัวใจล้มเหลว

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บทคัดย่อ: สาร urocortin เป็นสารที่หลั่งออกมาจากกล้ามเนื้อหัวใจเมื่อกล้ามเนื้อหัวใจเกิดภาวะ stress เช่นกล้ามเนื้อหัวใจขาดเลือด โดยมีผลในการป้องกันเซลล์กล้ามเนื้อหัวใจ อย่างไรก็ตามระดับ urocortin อาจบ่งถึงความรุนแรงของภาวะ stress ดังนั้นระดับ urocortin จึงเหมาะสมที่จะใช้พยากรณ์โรคหัวใจในภาวะต่างๆ เช่นกล้ามเนื้อหัวใจขาดเลือดเฉียบพลัน หรือหัวใจล้มเหลวเฉียบพลัน

การศึกษา ประกอบด้วย 2 การศึกษา ได้แก่ การศึกษาระดับ urocortin ในผู้ป่วยกล้ามเนื้อหัวใจตายเฉียบพลันเพื่อศึกษาประโยชน์ของการใช้ระดับ urocortin ในการพยากรณ์โรค และการศึกษาระดับ urocortin ในผู้ป่วยหัวใจล้มเหลวเฉียบพลันเพื่อศึกษาประโยชน์ของการใช้ระดับ urocortin ในการพยากรณ์โรค

ผู้ป่วยกล้ามเนื้อหัวใจตายเฉียบพลัน 61 รายได้รับการเจาะเลือด (พลาสมา) หาระดับ urocortin ที่วันที่แรก (วันที่ 0), วันที่ 1, 3, 5, เดือนที่ 3 และ 6 เดือน มีการเปรียบเทียบกับกลุ่มควบคุมซึ่งเป็นอาสาสมัครที่แข็งแรง 21 ราย และมีการติดตามผู้ป่วยกล้ามเนื้อหัวใจล้มเหลวประมาณ 1 ปี เพื่อศึกษาอัตราการเสียชีวิต การนอน รพ. เนื่องจากภาวะฉุกเฉินทางโรคหัวใจ เช่นการเกิดกล้ามเนื้อหัวใจขาดเลือดเฉียบพลันซ้ำ หัวใจล้มเหลว พบว่าผู้ป่วยกล้ามเนื้อหัวใจขาดเลือดเฉียบพลันมีระดับ urocortin สูงขึ้นกว่าอาสาสมัครที่แข็งแรงตั้งแต่วันที่ 0ถึงวันที่ 5 เมื่อติดตาม 1 ปีมีผู้ป่วยเสียชีวิต 17 ราย (ร้อยละ 25.8) ระดับ urocortin ในผู้ป่วยที่เสียชีวิตสูงกว่าผู้ป่วยที่รอดชีวิตอย่างมีนัยสำคัญ และระดับ urocortin สามารถพยากรณ์การเสียชีวิตได้โดยมีขนาดพื้นที่ใต้กราฟ (area under curve (AUC)) ของ receiver operating characteristic curve (ROC) ที่ 0.75 (95% confidence interval 0.619–0.881, P = 0.004) ในขณะที่ระดับ NT-proBNP ซึ่งเป็น biomarker มาตรฐานที่ใช้ในการพยากรณ์โรคมี AUC 0.857 (95% CI, 0.722–0.992; P = 0.003) ความไวในการพยากรณ์การเสียชีวิตโดยใช้ระดับ urocortin, ระดับNT-pro BNP และระดับ urocortin ร่วมกับระดับ NT-proBNP มีค่าร้อยละ0. 81 (95% CI, 0.54–0.95), 0.86 (95% CI, 0.42–0.99) และ 1.00 (95% CI, 0.56–1.00) ตามลำดับ

การศึกษาในผู้ป่วยหัวใจล้มเหลวเฉียบพลัน 61 ราย พบว่าระดับ ระดับ urocortin สามารถพยากรณ์การเสียชีวิตได้โดยมีขนาดพื้นที่ใต้กราฟ (area under curve (AUC)) ของ receiver operating characteristic curve (ROC) ที่ 0.679 (95% confidence interval 0.522–0.835, P = 0.021)

โดยสรุป ระดับพลาสมา urcorotin สามารถพยากรณ์การเสียชีวิตในผู้ป่วยกล้ามเนื้อหัวใจตายเฉียบพลันและผู้ป่วยหัวใจล้มเหลว

คำหลัก: ระดับ urocortin พยากรณ์โรค กล้ามเนื้อหัวใจตายเฉียบพลัน หัวใจล้มเหลว

Abstract

Project Code: MRG5280169

Project Title: Study of plasma profile of urocortin in Thai patients with Heart Failure

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Project Period: 2 years

Abstract: Urocortin is a peptide belonging to the corticotrophin-releasing factor peptide family and released when the heart is under stress (such as ischaemia or heart failure). It has been shown to cause coronary vasodilatation, positive inotropic effect, and also has an anti-apoptotic effect in the myocardium that has undergone ischaemia–reperfusion injury. Despite the proposed cardioprotective effect, the role of plasma urocortin in AMI and its prognostic value remains unknown.

We investigated plasma profile of urocortin AMI patients. The long-term prognostic performance of plasma urocortin was investigated in 2 groups of patients; patients with AMI and patients with acute heart failure (AHF).

In AMI patients; sixty-six AMI patients were included in this study. Blood samples for urocortin were collected on days 0 (onset), 1, 3 and 5 and at 3 and 6 months. Primary endpoint was mortality within 1 year of follow-up. Secondary endpoint was combined death and nonfatal adverse cardiac events (i.e. myocardial reinfarction, urgent revascularization or hospitalization due to heart failure) within 1 year. During 1 year follow-up, 17 (25.8%) patients died. Plasma urocortin in AMI patients were increased on days 0, 1, 3 and 5 ($P < 0.05$ vs. control). The receiver-operating characteristic curve of plasma urocortin level in predicting 1 year mortality showed an area under curve (AUC) of day 0 urocortin to be 0.75 with 95% confidence interval (CI) of 0.619–0.881 ($P = 0.004$), whereas AUC of NTproBNP was 0.857 (95% CI, 0.722–0.992; $P = 0.003$). Sensitivity values for predicting the mortality of urocortin NT-proBNP and a combined urocortin and NT-proBNP were 0.81 (95% CI, 0.54–0.95), 086 (95% CI, 0.42–0.99) and 1.00 (95% CI, 0.56–1.00), respectively.

In AHF patients, plasma urocortin level was significantly higher in patients who died during 1 year of follow up. The receiver-operating characteristic curve of plasma urocortin level in predicting 1 year mortality showed an area under curve (AUC) of 0.679 with 95% confidence interval (CI) of 0.522–0.835 ($P = 0.021$).

Conclusions. Plasma urocortin level shows prognostic value in patients with AMI and AHF. High plasma urocortin level at admission is associated with high mortality rate.

Keywords. Urocortin, Prognosis, Acute myocardial infarction, Heart failure, Cardiac markers

Executive Summary

Objectives

Primary endpoint was mortality within 1 year of follow-up. Secondary endpoint was combined major cardiovascular events including death, nonfatal myocardial reinfarction, urgent revascularization and hospitalization due to heart failure within 1 year.

Methods

This study was approved by an institutional ethical review committee at the Faculty of Medicine, Chiang Mai University, and reporting of the study conforms to STROBE [18]. Sixty-six AMI patients admitted to Maharaj Nakorn Chiang Mai Hospital from June 2006 to September 2006 were enrolled. The AMI patients were defined by the presence of typical prolonged chest pain (> 30 min) accompanied by electrocardiogram changes indicating new ischaemia (new ST-T changes or new left bundle branch block) and significant (> 99th percentile of the upper reference limit) increase in myocardial damage markers [i.e. creatinine kinase-MB mass (CK-MB mass) or cardiac troponin T (cTnT)] [19]. Patients undergoing chronic dialysis, and those with decompensated liver disease and cancer were excluded from the study. Twenty-one age-matched healthy persons with no cardiac abnormalities from echocardiogram were included as a control group. Written informed consent was obtained from each participant prior to study entry. All patients received standard treatment according to treatment guidelines.

Plasma sample collection and urocortin measurement

The patient's blood was collected at the same time of each experimental day. Blood samples were collected on the day of admission within 24 h of the onset of AMI (i.e. day 0) and on days 1, 3 and 5 after onset. Patients were called for a check-up and blood sample collection at 1, 3 and 6 months after discharge, and were followed up for 12 months after discharge to record the incidence of death and other adverse events including readmission to the hospital with adverse cardiac events. Blood samples were collected in EDTA venous blood collection tubes and centrifuged at 1600 g for 10 min at 4 °C within 30 min of collection. Samples were kept at −86 °C and stored for approximately 30 days before the urocortin assay. Plasma peptides were extracted by solid-phase extraction and an enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) was used to detect a specific peptide based on the competitive enzyme immunoassay principle.

For urocortin (or urocortin I) assay, the minimum detectable concentration (sensitivity) was 0.24 ng mL^{-1}. The intra–assay variation was < 5% and inter–assay variation was < 14%. Detectable range was 0–100 ng mL^{-1} (linear range 0.24–3.81 ng mL^{-1}). Plasma NT–proBNP level on the discharge day was also determined and used as a reference marker compared with urocortin.

Echocardiography

Complete transthoracic echocardiography was performed in all participants. AMI patients received echocardiography on the discharge day and again 6 months later. Left ventricular end diastolic volume (LVEDV), left ventricular end systolic volume (LVESV) and LVEF were derived from apical views using the Modified Simpsons' method. Left ventricular wall motion index (LVWMI) and area of infarction were also derived.

Statistical analyses

Statistical analyses were performed using the Mann–Whitney U–test or ANOVA in SPSS version 16 (SPSS Inc., Chicago, IL, USA) and data were reported as mean ± SD. The correlation between urocortin levels and other variables was examined by Spearman's correlations. Receiver–operating characteristic (ROC) curves were constructed to investigate the prognostic predictive value of urocortin and NT–proBNP. Kaplan–Meier survival curves were generated to visualize the relationship between urocortin and the endpoints. The independent prognostic significant of urocortin was analysed by Cox regression analysis. The accepted level of significance was $P < 0.05$.

Results

Patient characteristics

Demographic features of AMI patients are shown in Table 1. The mean age and gender were not different between AMI patients and the control group (62.7 ± 11.6 vs. 63.8 ± 11.5 years, $P > 0.05$). Among 51 ST elevation AMI (STEMI) patients, 43% underwent primary percutaneous intervention, 24% received thrombolytic therapy and 33% did not receive reperfusion therapy because of late presentation. Median duration from onset of symptoms to hospital presentation was 240 min (range 15–1540) and was longer in patients who did not receive any reperfusion therapy compared with the former two categories [420(IQR 240,735) vs. 240(IQR 120, 360) vs. 150(IQR 120, 228 min), $P > 0.05$]. Among 16 non–ST elevation AMI (NSTEMI) patients, 53% underwent coronary angiogram at index admission. The proportion of patients in Killip class I, II, III and IV were

36%, 19%, 28% and 16% respectively. Regarding medical treatment, 98% of the patients received aspirin, 45% received thienopyridine antagonists, 50% received angiotensin–converting enzyme inhibitors or angiotensin receptor blockers, 67% received b–blockers and 15% received aldosterone antagonist. After follow–up at 1 year, 38 (57.6%) patients were still alive and free of cardiac adverse events, nine (13.6%) had nonfatal adverse cardiac events, 17 (25.8%) died and two (3.0%) were lost to follow–up. Median follow–up time was 360 days (range 1–489 days). The LVEF, LVEDV, LVESV and LVWMI in the control subjects were 76.1 ± 6.3%, 72.2 ± 22.6 mL, 21.4 ± 7.1 mL and 1 respectively ($P < 0.05$ vs. AMI patients for all parameters).

Urocortin levels in AMI patients

Plasma urocortin level at onset (day 0) was significantly elevated compared with the control group (Fig. 1a), and continued until day 5. The levels of urocortin at months 1, 3 and 6 were decreased and were not different from those of the control group. The median plasma urocortin level was 124Æ1 (IQR 100.0, 181.1) pmol L^{-1}. Clinical characteristics of patients with high (i.e. >median) and low (i.e. <median) urocortin were not different except that the former group had higher peak cTnT level than in the latter (Table 2). The proportions of patients receiving b–blockers, angiotensin–converting enzyme inhibitors, aldosterone antagonists and statin were not different between patients with low and high urocortin level (all $P > 0.05$).

Plasma urocortin levels were not correlated with age, WBC, BUN, creatinine or albumin ($P > 0.05$ for all). Plasma NT–proBNP level at discharge was moderately correlated with age ($r = 0.44$, $P < 0.002$), BUN ($r = 0.54$, $P < 0.001$), creatinine ($r = 0.50$, $P < 0.001$) and albumin ($r = -0.59$, $P < 0.001$). Plasma urocortin levels were higher in NSTEMI than in STEMI on days 1, 3 and 5 but were not different on day 0. Plasma urocortin on day 0 was not correlated with the severity of myocardial necrosis measured by peak CK–MB mass or cTnT level ($r = 0.1$, $P = 0.462$ and $r = 0.08$, $P = 0.573$ for CK–MB mass and cTnT level, respectively). There was no difference in plasma urocortin levels among patients in different Killip classes on any of the experimental days. However, plasma NT–proBNP at discharge was higher in Killip class IV ($P < 0.001$ for the difference among groups) but was not different between NSTEMI and STEMI.

Relationship between plasma urocortin level and echocardiographic parameters

Echocardiographic parameters of AMI patients are shown in Table 1. Plasma urocortin levels were associated neither with echocardiographic parameters nor with NT–proBNP

level at onset. However, the NT–proBNP level at discharge was correlated with LVEF (r = –0.434, P = 0.002), LVESV (r = 0.42, P = 0.003), LVEDV (r = 0.34, P = 0.016) and LVWMI (r = 0.363, P = 0.01), and the level at 6–month follow–up was correlated with LVEF (r = –0.430, P = 0.011) and LVWMI (r = 0.508, P = 0.002) measured on the same day. NSTEMI patients had poorer echocardiographic parameters than STEMI patients (109.2 ± 46.5 vs. 87.6 ± 39.2 mL, P = 0.041; 29.1 ± 12.7 vs. 42.1 ± 14.3%, P = 0.002; for LVEDV and LVEF, respectively).

Factors associated with mortality

Factors associated with mortality are shown in Table 3. Patients who died were older, presented with Killip class IV at index event, had higher BUN, higher fasting blood sugar and lower LVEF. Serum creatinine at admission and before discharge and the changes in serum creatinine were not associated with mortality (P > 0.05 for both comparisons). Patients who presented a > 50% increase in serum creatinine during the hospital stay (RIFEL criteria of at least at risk of renal failure) [20] had significantly higher mortality rate (80 vs. 19.0%, P = 0.001). However, this association was not significant in Cox regression analysis. In STEMI patients, reperfusion therapy was associated neither with the mortality nor with the combined adverse cardiovascular events (P > 0.05). In NSTEMI patients, revascularization was not associated with the mortality of combined adverse cardiovascular events (P > 0.05). Medications at discharge were not different between patients who died and survived, except that the use of b–blocker was found to be higher in patients who survived (83 vs. 18%, P < 0.001).

Plasma urocortin level in the prediction of mortality

Plasma urocortin levels on day 0 and NT–proBNP levels at discharge day were higher in patients who died than in patients who survived (Table 3). They were also higher in patients with combined adverse cardiac events than in patients without adverse cardiac events.

At day 0, plasma urocortin levels were higher in patients who died or patients with combined adverse cardiac events than in patients who survived or patients without combined adverse cardiac events in multivariate analysis (P = 0.02 and 0.03, respectively). Plasma urocortin was also the only independent parameter associated with mortality in this group of patients (Table 3).

Receiver–operating characteristic curves, constructed to study the prognostic accuracy of using plasma urocortin and NT–proBNP levels to predict mortality, demonstrated that

plasma urocortin levels on day 0 and NT–proBNP level at discharge had a significant predictive value for mortality (Fig. 1b). Area under curve of day 0 urocortin was 0.750 (95%CI, 0.619–0.881; P = 0.004), whereas that of NT–proBNP was 0.857 (95%CI, 0.722–0.992; P = 0.003). The best cut–off value for mortality prediction by urocortin was 124 pmol L^{-1} (sensitivity = 0.857 and specificity = 0.647) and by NT–proBNP was 1901 pmol mL^{-1} (sensitivity = 0.857 and specificity = 0.735). Unlike urocortin and NT–proBNP, cTnT was not significantly different between patients who died and those who survived (Table 3), and did not have significant prognostic performance in predicting mortality in the present study (ROC 0.671, 95%CI 0.412, 0.930, P = 0.183).

Prognostic probability analysis, using the Kaplan–Meier survival model, demonstrated that day 0 urocortin with levels higher than the cut–off value (124 pmol L^{-1}) was associated with mortality (P = 0.026, Fig. 2a) with a relative risk of 3.5 and a 95% CI of 1.1–10.6. NT–proBNP levels, measured on the day of discharge that were higher than the cut–off value (1901 pmol L^{-1}), were also associated with mortality (P = 0.002, Fig. 2b) with a relative risk of 9.7 and a 95% CI of 1.2–72.9. Using Cox regression analysis adjusting for age, Killip class, LVEF, NT–proBNP and changes in serum creatinine during admission, the level of urocortin at day 0 was associated with mortality (P = 0.03) but not for combined adverse cardiovascular events (P = 0.07).

The additive benefit of combined cardiac markers on prognosis was studied by dividing patients into four groups based on the cut–off value of urocortin and NT–proBNP derived from ROC curves (Fig. 2c). The mortality rate was higher in patients with elevated levels of plasma urocortin and / or NT–proBNP than in patients with low levels of both urocortin and NT–proBNP. The combination of urocortin and NT–proBNP showed good sensitivity and specificity in predicting the mortality in AMI (Table 4).

Discussion

This is the first study investigating the plasma urocortin levels in AMI patients in the acute phase at 1–year follow–up. Its level in AMI patients was significantly higher than the normal control group from days 0–5 of AMI. Plasma urocortin level on AMI day 0 demonstrated a significant and independent predictive value for mortality and adverse cardiac events. Patients with plasma urocortin levels > 124 pmol L^{-1} had a higher mortality and adverse cardiac events similar to patients with plasma NT–proBNP levels > 1901 pmol L^{-1}. More importantly, we demonstrated for the first time that plasma urocortin can be another beneficial marker to add to the prognostic performance of NT–proBNP.

Urocortin in STEMI and NSTEMI

Plasma urocortin levels were significantly elevated in both NSTEMI and STEMI patients. Previous studies have demonstrated that urocortin has a marked coronary vasodilator effect [21–23] and could increase coronary blood flow and myocardial function through the release of nitric oxide [21]. As myocardial ischaemia and reperfusion cause marked endothelial dysfunction and reduction in coronary relaxation because of reduced nitric oxide release [24], increased levels of urocortin after AMI could be a part of the cardioprotective response to ischaemic / reperfusion injury in these patients. In the present study, plasma urocortin levels in NSTEMI patients were higher than in STEMI patients. This may be correlated with the severity of obstructive coronary artery disease. Although it causes less fatal acute cardiac damage, NSTEMI is more often associated with multi–vessel disease and repeated myocardial ischaemia [25,26], and could be responsible for a high level of plasma urocortin.

In the present study, the high proportion of patients without reperfusion therapy was mainly due to prolonged onset of chest pain prior to hospital presentation. The median time was 420 min (IQR 240, 720 min). The proportion of patients receiving reperfusion therapy in the present study was similar to the proportion reported from the Global Registry of Acute Coronary Events (GRACE registry) [27,28] (67% vs. 62%), and was slightly better than that reported in the Thai ACS Registry (52.6%) [29]. For NSTEMI patients, the rate of coronary angiography in the present study was similar to that in the GRACE registry (53% vs. 47.5%), and ours was slightly better than that reported in the Thai ACS registry (43.9%).

In an animal study, a short period of ischaemia could cause a significant increase in urocortin mRNA and protein concentrations in plasma, without expression of myocyte apoptosis or necrosis [6]. However, long period of ischaemia (at least 30 min) significantly reduced urocortin levels with an expression of apoptosis and necrosis of cardiomyocytes [6]. These findings suggest that urocortin expression and release are mainly sustained by ischaemic–triggered but still viable myocytes [6]. In the present study, it is possible that STEMI, which reflects transmural myocardial injury and necrosis [30], could have post–triggered viable myocytes in a lesser amount and with lower functional recovery, resulting in lower plasma urocortin levels than in NSTEMI.

In contrast to plasma NT–proBNP levels, plasma urocortin levels in our AMI patients were not correlated with LVEF. It is possible that the triggers for the release of these cardiac

markers are different, whereby plasma NT–proBNP is released mainly in response to increased ventricular wall stress from left ventricular dysfunction induced by myocardial injury or ischaemia [31], whereas urocortin in AMI patients is released in direct response to myocardial ischaemia [6].

Prognostic value of plasma urocortin

When measured within 24 h after the onset of AMI, the plasma urocortin level was significantly higher in patients incurring an endpoint of death than patients who had adverse events and who survived. Although previous studies demonstrated a cardioprotective effect of urocortin [12–15], our study demonstrated that high levels of urocortin within 24 h after the onset are associated with poor outcome. It is known that urocortin can be released when myocardium is ischaemic [12]. However, it is possible that high levels of plasma urocortin could indicate severe myocardial damage [6], and may explain their association with poor outcome as found in the present study. This scenario has likewise been reported with NT–proBNP levels [32]. A previous study reported that plasma NT–proBNP measured 2–4 days after myocardial infarction independently predicted left ventricular function and 2–year survival, and that high plasma NT–proBNP levels above the median significantly correlated with a lower survival rate [32].

In the present study, the prognostic probabilities of plasma urocortin and NT–proBNP levels were examined. Plasma urocortin levels within 24 h of the onset were significantly predictive for mortality. Furthermore, plasma urocortin and NT–proBNP levels above the cut–off value were significantly associated with poor survival rates, a finding consistent with a previous report [32]. Unlike urocortin, the other measured parameters such as plasma cTnT, creatinine, changes in serum creatinine, revascularization and medications were not independently associated with mortality in this group of patients.

The pathophysiology of AMI is complex, involving myocardial ischaemia, myocardial necrosis and myocardial stretch. The multi–marker approach for the prediction of adverse cardiac events in acute coronary syndrome patients would be beneficial [4,33]. In the present study, we demonstrated increased sensitivity to predict mortality when using both urocortin and NT–proBNP levels. Our findings indicate that the combination of the two markers should be used for risk stratification in AMI patients.

Conclusion

Plasma urocortin level is elevated in AMI patients starting from the onset and remains at high levels for 5 days. Despite its proposed cardioprotective actions, the high level of

plasma urocortin within 24 h of onset is associated with high mortality in AMI patients. Plasma urocortin level has a significant prognostic value in AMI patients and the combination with NT–proBNP could enhance prognostic performance.

Study limitations

There were a small number of enrolled patients and control subjects. Although the association between urocortin levels and Killip classes was not found in the present study, it is still possible that their relationship exists but could not be detected because of small number of enrolled patients. The numbers at risk in each group of patients (as shown in Fig. 2c) were low and therefore the statistical conclusions were limited. Future studies with large numbers of AMI patients and longer periods of follow–up are required to warrant its clinical significance in prognostic prediction.

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Table 1 Characteristics of AMI patients

Characteristics	AMI patients
Number	66
Male	40 (60.6%)
Female	26 (39.4%)
Age (years)	62.7 ± 11.6
Type of AMI	
STEMI	51 (77.3%)
NSTEMI	15 (22.7%)
Territory of culprit vessel	
LAD	22 (33.3%)
LCX	11 (16.7%)
RCA	28 (42.4%)
Unknown	5 (7.6%)
Killip class on admission	
I	25 (39.7%)
II	10 (15.9%)
III	18 (28.6%)
IV	10 (15.9%)
Echocardiography data	
LVEF (%)	40.43 ± 13.6
LVEDV (mL)	94.31 ± 41.96
LVESV (mL)	60.53 ± 37.36
LVWMI	1.69 ± 0.39
NT-proBNP level (pmol L^{-1})	
Discharge day	3960.80 ± 6302.2
Month 6	1459.41 ± 2285.1
CK-MB (U L^{-1})	213.73 ± 184.2
CTnT (ng mL^{-1})	8.03 ± 8.6
Serum Cr (mg dL^{-1})	1.8 ± 1.4
Estimated CrCl (mL min^{-1}, n = 52)	44.4 ± 26.8

Values are mean ± SD or number (per cent). AMI, acute myocardial infarction;CK-MB, creatinine kinase-MB; LVEDV, Left ventricular end diastolicvolume; LVESV, left ventricular end systolic volume; LVWMI, Left ventricular wall motion index; NSTEMI, non-ST elevation AMI.

Table 2 Clinical characteristics of patients with high and low urocortin levels

	High urocortin (n = 36)	Low urocortin (n = 28)	P value
Age (years)	63 ± 12	62 ± 10	0.52
Gender (M:F)	21 : 15	14 : 14	0.51
Type of AMI, n (%)			
STEMI	25(78)	19 (77)	0.66
NSTEMI	7 (22)	9 (27)	
Time from onset to admission	307 ± 306	323 ± 312	0.89
Killip class, n (%)			0.99
I	13 (36)	10 (37)	
II	7 (19)	5 (18)	
III	10 (28)	8 (30)	
IV	6 (17)	4 (15)	
CK–MB (U L^{-1})	225 ± 173	220 ± 200	0.61
cTnT (ng mL^{-1})	10.2 ± 9.2	5.9 ± 7.3	0.049
Hct (%)	37.2 ± 7.5	38.7 ± 5.8	0.27
WBC	13 280 ± 3985	11 782 ± 4839	0.09
BUN (mg dL^{-1})	25.6 ± 23.4	19.6 ± 20.0	0.35
Creatinine	1.7 ± 1.9	2.0 ± 1.8	0.55
LVEF (%)	41.1 ± 13.6	49.7 ± 13.4	0.73
FBS (mg day^{-1})	155 ± 75	139 ± 50	0.62
Urocortin day 0 (pmol L^{-1})	178.9 ± 53.6	95.9 ± 23.1	<0.001
NT–proBNP at discharge (pmol L^{-1})	5238 ± 8117	2959 ± 3210	0.49
Death, n (%)	13 (36)	3 (11)	0.02
CV events, n (%)	19 (53)	8 (29)	0.05

Values are mean ± SD or number (per cent). AMI, acute myocardial infarction; CK–MB, creatinine kinase–MB; cTnT, cardiac troponin T; NSTEMI, non–ST elevation AMI.

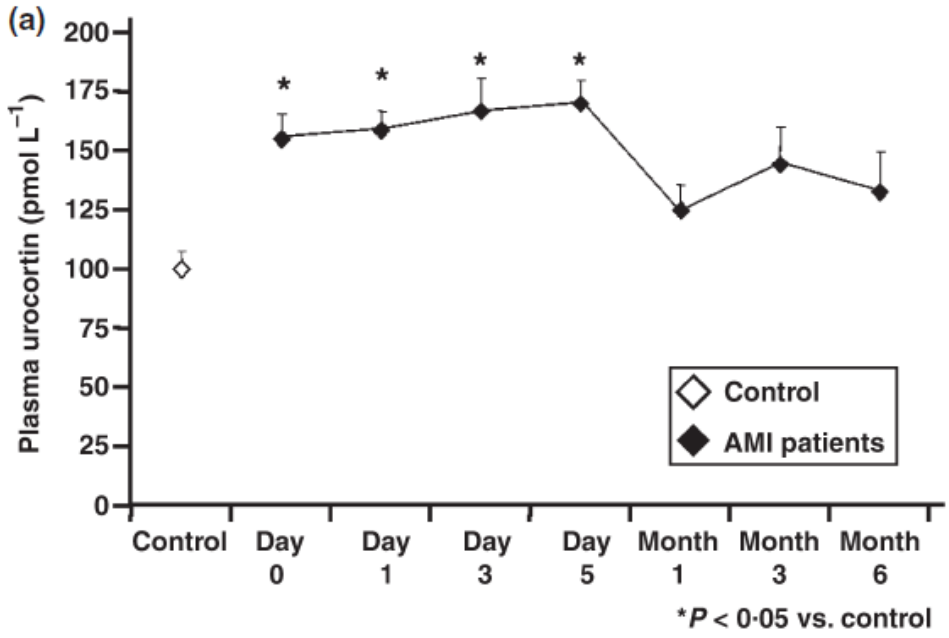
Table 3 Factors associated with mortality

	Death (n = 17)	Survival (n = 47)	P value (univariate)	P value (multivariate)
Age (years)	68 ± 11	60 ± 11	0.02	0.57
Gender (M:F)	9 : 8	28 : 19	0.64	
Type of AMI, n (%)				
STEMI	12 (70.6)	38 (80.9)	0.39	
NSTEMI	5 (29.4)	9 (19.1)		
Time from onset to admission	445 ± 408	267 ± 197	0.14	
Killip class, n (%)			0.001	0.58
I	3 (17.6)	20 (45.5)		
II	3 (17.6)	7 (15.9)		
III	3 (17.6)	15 (34.1)		
IV	8 (47.2)	2 (4.5)		
CK–MB (U L^{-1})	261 ± 196	193 ± 178	0.19	
CTnT (ng mL^{-1})	10.9 ± 9.9	7.0 ± 7.9	0.14	
Hct (%)	35.2 ± 7.2	39.2 ± 6.3	0.06	
WBC	13232 ± 9050	12397 ± 4196	0.51	
BUN (mg dL^{-1})	31.0 ± 29.8	20.2 ± 18.1	0.194	
Creatinine (mg dL^{-1})	1.8 ± 1.3	1.8 ± 1.4	0.49	
>50% increased Cr, n (%)	4 (80)	1 (20)	0.013	0.99
LVEF (%)	32.6 ± 14.1	41.7 ± 12.8	0.03	0.95
FBS (mg day^{-1})	181 ± 89	134 ± 51	0.06	0.87
b–blocker use, n (%)	3 (19)	40 (83)	< 0.001	0.16
Urocortin day 0 (pmol L^{-1})	175.0 ± 57.8	132.4 ± 55.0	0.004	0.02
NT–proBNP at discharge (pmol L^{-1})	11895 ± 11064	2704 ± 3637	0.003	0.17

Values are mean ± SD or number (per cent). Multivariate model adjusting with age, Killip class, LVEF, renal injury, FBS and NT–proBNP. AMI, acute myocardial infarction; CK–MB, creatinine kinase–MB; NSTEMI, non–ST elevation AMI.

Table 4 Predictive values of mortality with plasma urocortin and NT-proBNP levels

Performance	Urocortin	NT-proBNP	Urocortin and NT-proBNP
Sensitivity (95% CI)	0.81 (0.54–0.95)	0.86 (0.42–0.99)	1.0 (0.56–1.0)
Specificity (95% CI)	0.54 (0.48–0.69)	0.70 (0.53–0.99)	0.41 (0.25–0.59)
Positive predictive value (95%CI)	0.41 (0.24–0.59)	0.33 (0.24–0.59)	0.26 (0.12–0.47)
Negative predictive value (95%CI)	0.89 (0.68–0.97)	0.97 (0.80–0.99)	1.0 (0.73–1.0)



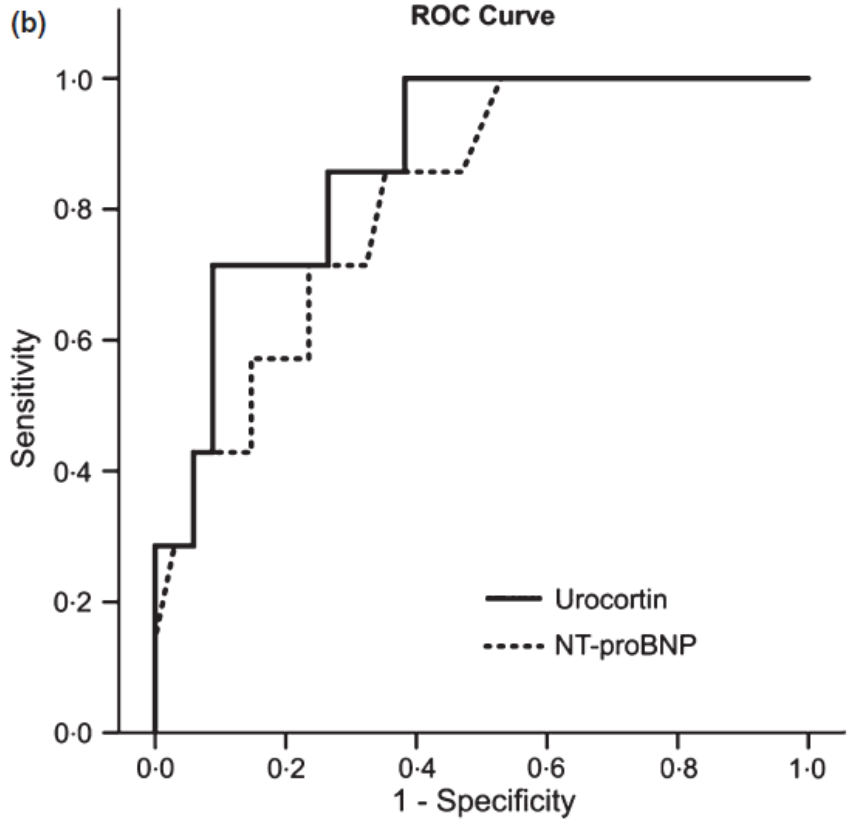
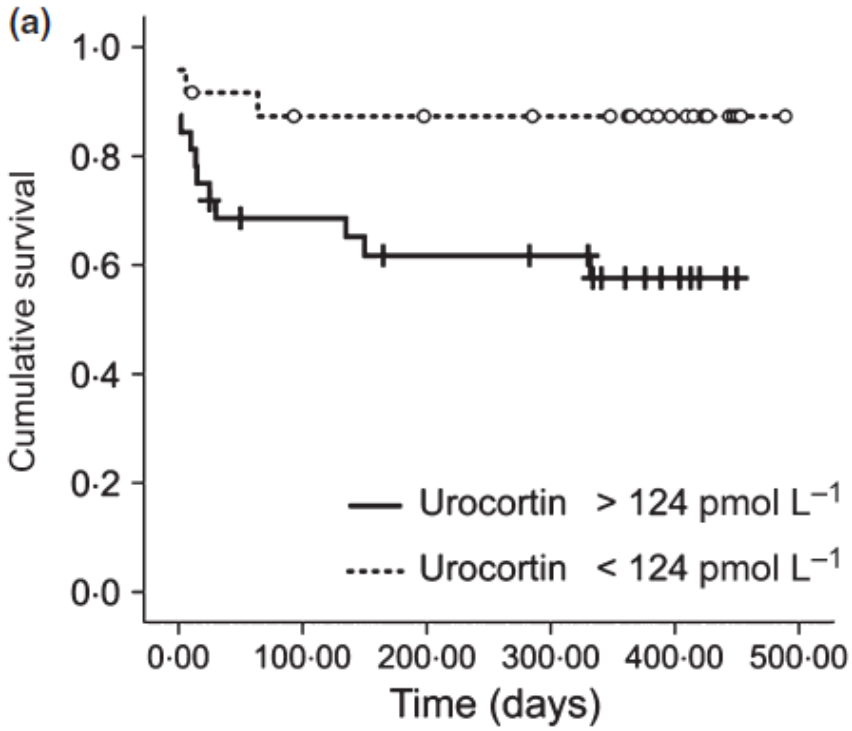
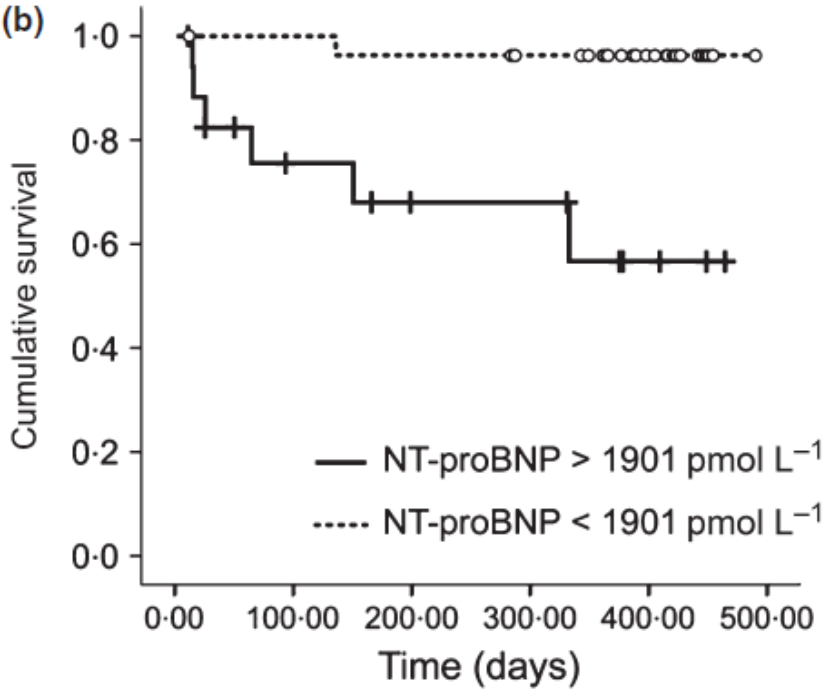


Figure 1 (a) Plasma profile of urocortin levels in control subjects and acute myocardial infarction (AMI) patients. Values are mean ± SE. (b) Receiver–operating characteristic curve of allcause mortality at 1 year after AMI onset for levels of plasma urocortin on day 0 and NT–proBNP at discharge.



Number at risk					
Low urocortin	10	20	21	22	26
High urocortin	8	18	20	23	35



Number at risk					
Low NT-proBNP	12	24	26	26	28
High NT-proBNP	2	7	8	11	18

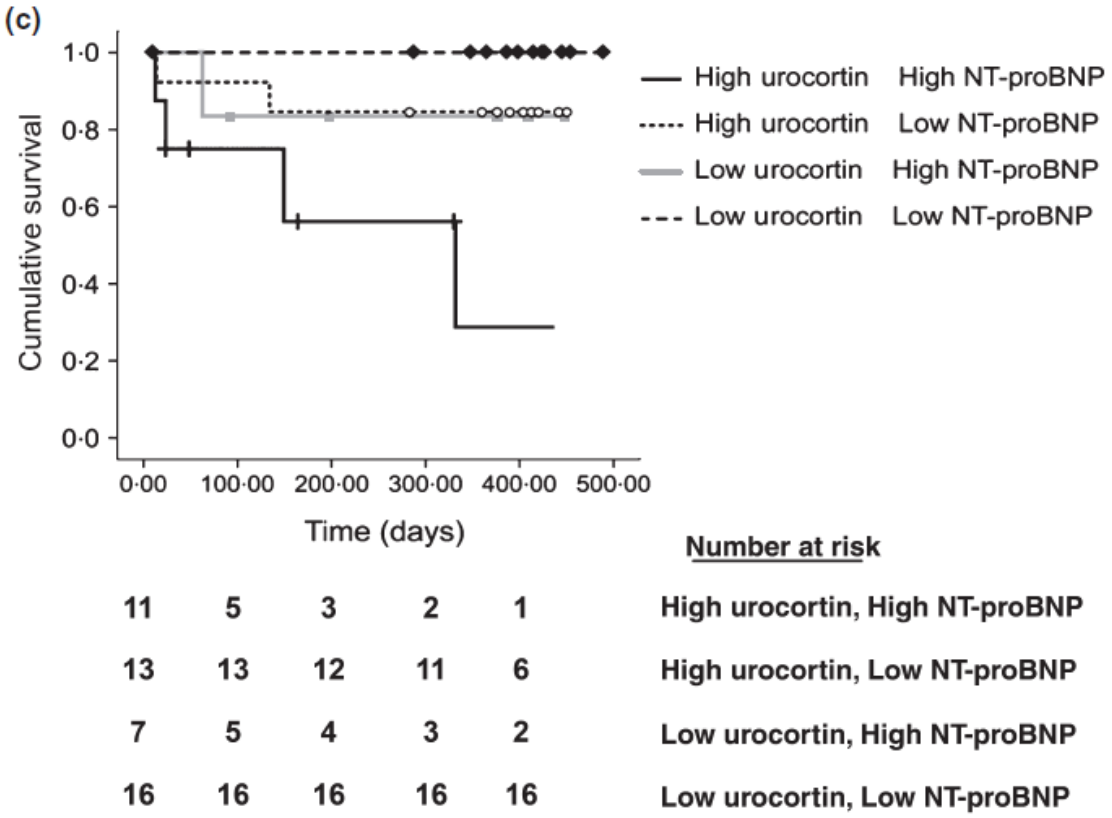


Figure 2 (a) Kaplan–Meier curves showing cumulative mortality during 1 year, according to the level of plasma urocortin. P = 0.026 for the difference between groups. (b) Kaplan–Meier curves showing cumulative mortality during 1 year, according to the level of plasma NT–proBNP. P = 0.002 for the difference between groups. (c) Kaplan–Meier curves showing cumulative mortality during 1 year, according to the levels of both plasma urocortin and NT–proBNP. P = 0.001 for the difference among groups.

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

1. Plasma urocortin in acute myocardial infarction patients Arintaya Phrommintikul, Sivaporn Sivasinprasasn, Narissara Lailerd, Siriporn Chattipakorn,Srun Kuanprasert, and Nipon Chattipakorn Eur J Clin Invest 2010; 40 (10): 874–882

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ORIGINAL ARTICLE

Plasma urocortin in acute myocardial infarction patients

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ABSTRACT

Background Despite its proposed cardioprotective effect, the role of plasma urocortin in acute myocardial infarction (AMI) remains unknown. We investigated plasma profile of urocortin in AMI patients and evaluated its long-term prognostic performance.

Material and methods Sixty-six AMI patients and 21 healthy subjects were included in this study. Blood samples for urocortin were collected on days 0 (onset), 1, 3 and 5 and at 3 and 6 months. Primary endpoint was mortality within 1 year of follow-up. Secondary endpoint was combined death and nonfatal adverse cardiac events (i.e. myocardial reinfarction, urgent revascularization or hospitalization due to heart failure) within 1 year.

Results During follow-up at 1 year, 38 (57·6%) patients were alive without cardiac events, nine (13·6%) had nonfatal cardiac events and 17 (25·8%) died. Plasma urocortin in AMI patients were increased on days 0, 1, 3 and 5 ($P < 0·05$ vs. control). The receiver-operating characteristic curve showed an area under curve (AUC) of day 0 urocortin to be 0·750 with 95% confidence interval (CI) of 0·619–0·881 ($P = 0·004$), whereas AUC of NT-proBNP was 0·857 (95% CI, 0·722–0·992; $P = 0·003$). Sensitivity values for predicting the mortality of urocortin NT-proBNP and a combined urocortin and NT-proBNP were 0·81 (95% CI, 0·54–0·95), 0·86 (95% CI, 0·42–0·99) and 1·0 (95% CI, 0·56–1·0), respectively.

Conclusions Plasma urocortin level is elevated in AMI patients for 5 days from onset. High plasma urocortin within 24 h after the onset is associated with increased mortality. Combined urocortin and NT-proBNP enhance prognostic performance in AMI patients.

Keywords Acute myocardial infarction, cardiac function, cardiac markers, urocortin.

Eur J Clin Invest 2010; 40 (10): 874–882

Introduction

Biochemical markers play a pivotal role in the diagnosis and management of patients with acute coronary syndromes [1–3]. The multi-marker approach for prognostic and risk stratification has been indicated for its benefits in predicting the future adverse cardiac events in these patients [4]. In the past decades, advanced techniques in biomarker investigation have led investigators to the discovery of several novel bioactive peptides that could be potentially useful in prognostic evaluation.

Urocortin is a peptide belonging to the corticotrophin-releasing factor peptide family. Urocortin is released when the heart is under stress (such as ischaemia or heart failure) and has been demonstrated in both animal and human studies [5,6]. It has been shown to cause coronary vasodilatation, positive inotropic effect, and also has an anti-apoptotic effect in the myocardium that has undergone ischaemia–reperfusion injury [7]. In clinical situations, urocortin levels have been shown to elevate in patients with systolic heart failure (left ventricular ejection fraction, LVEF, ≤ 45%), especially in the New York Heart Association (NYHA) functional classes I–II [5].

Growing evidence suggests that urocortin may have cardioprotective effects and could play an important role in both normal and diseased hearts [8–11]. In experimental sheep models, urocortin has profound and sustained haemodynamic, hormonal and renal effects, and its level was increased in sheep with systolic heart failure [5,11]. During ischaemia/reperfusion, the cardioprotective effects of urocortin were reflected in increased cell survival [12], reduced infarct size [13,14], improved contractile function [15], reduced arrhythmic

incidences [16], and reduced creatine kinase (CK) and lactate dehydrogenase (LDH) levels [17]. In rat cardiomyocytes, urocortin mRNA expression was increased by ischaemia, rapidly translated to urocortin protein and then released into circulation, which exerted cardioprotective effects through its receptor [6,12].

Plasma urocortin levels are significantly correlated with LVEF in systolic heart failure patients and negatively correlated with increasing age and disease severity [5]. However, plasma urocortin levels in acute myocardial infarction (AMI) patients have never been studied. In the present study, we investigated the plasma profile of urocortin in AMI patients and assessed whether its plasma level could be used as a prognostic marker in those patients. We tested the hypothesis that plasma urocortin level is increased in AMI patients and that high levels of plasma urocortin are associated with poor prognosis in this group of patients.

Methods

This study was approved by an institutional ethical review committee at the Faculty of Medicine, Chiang Mai University, and reporting of the study conforms to STROBE [18]. Sixty-six AMI patients admitted to Maharaj Nakorn Chiang Mai Hospital from June 2006 to September 2006 were enrolled. The AMI patients were defined by the presence of typical prolonged chest pain (> 30 min) accompanied by electrocardiogram changes indicating new ischaemia (new ST-T changes or new left bundle branch block) and significant (> 99th percentile of the upper reference limit) increase in myocardial damage markers [i.e. creatinine kinase-MB mass (CK-MB mass) or cardiac troponin T (cTnT)] [19]. Patients undergoing chronic dialysis, and those with decompensated liver disease and cancer were excluded from the study. Twenty-one age-matched healthy persons with no cardiac abnormalities from echocardiogram were included as a control group. Written informed consent was obtained from each participant prior to study entry. All patients received standard treatment according to treatment guidelines.

Plasma sample collection and urocortin measurement

The patient's blood was collected at the same time of each experimental day. Blood samples were collected on the day of admission within 24 h of the onset of AMI (i.e. day 0) and on days 1, 3 and 5 after onset. Patients were called for a check-up and blood sample collection at 1, 3 and 6 months after discharge, and were followed up for 12 months after discharge to record the incidence of death and other adverse events including readmission to the hospital with adverse cardiac events.

Blood samples were collected in EDTA venous blood collection tubes and centrifuged at 1600 *g* for 10 min at 4 °C within 30 min of collection. Samples were kept at −86 °C and stored for approximately 30 days before the urocortin assay.

Plasma peptides were extracted by solid-phase extraction and an enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) was used to detect a specific peptide based on the competitive enzyme immunoassay principle. For urocortin (or urocortin I) assay, the minimum detectable concentration (sensitivity) was 0·24 ng mL^{-1}. The intra-assay variation was < 5% and inter-assay variation was < 14%. Detectable range was 0–100 ng mL^{-1} (linear range 0·24–3·81 ng mL^{-1}). Plasma NT-proBNP level on the discharge day was also determined and used as a reference marker compared with urocortin.

Echocardiography

Complete transthoracic echocardiography was performed in all participants. AMI patients received echocardiography on the discharge day and again 6 months later. Left ventricular end diastolic volume (LVEDV), left ventricular end systolic volume (LVESV) and LVEF were derived from apical views using the Modified Simpsons' method. Left ventricular wall motion index (LVWMI) and area of infarction were also derived.

Endpoint

Primary endpoint was mortality within 1 year of follow-up. Secondary endpoint was combined major cardiovascular events including death, nonfatal myocardial reinfarction, urgent revascularization and hospitalization due to heart failure within 1 year.

Statistical analyses

Statistical analyses were performed using the Mann–Whitney *U*-test or ANOVA in SPSS version 16 (SPSS Inc., Chicago, IL, USA) and data were reported as mean ± SD. The correlation between urocortin levels and other variables was examined by Spearman's correlations. Receiver-operating characteristic (ROC) curves were constructed to investigate the prognostic predictive value of urocortin and NT-proBNP. Kaplan–Meier survival curves were generated to visualize the relationship between urocortin and the endpoints. The independent prognostic significant of urocortin was analysed by Cox regression analysis. The accepted level of significance was $P < 0{\cdot}05$.

Results

Patient characteristics

Demographic features of AMI patients are shown in Table 1. The mean age and gender were not different between AMI patients and the control group (62·7 ± 11·6 vs. 63·8 ± 11·5 years, $P > 0{\cdot}05$). Among 51 ST elevation AMI (STEMI) patients, 43% underwent primary percutaneous intervention, 24% received thrombolytic therapy and 33% did not receive reperfusion therapy because of late presentation. Median duration from onset

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Serum Cr (mg dL^{-1})	1·8 ± 1·4
Estimated CrCl (mL min^{-1}, n = 52)	44·4 ± 26·8

Values are mean ± SD or number (per cent). AMI, acute myocardial infarction; CK-MB, creatinine kinase-MB; LVEDV, Left ventricular end diastolic volume; LVESV, left ventricular end systolic volume; LVWMI, Left ventricular wall motion index; NSTEMI, non-ST elevation AMI.

of symptoms to hospital presentation was 240 min (range 15–1540) and was longer in patients who did not receive any reperfusion therapy compared with the former two categories [420(IQR 240,735) vs. 240(IQR 120, 360) vs. 150(IQR 120, 228 min), $P > 0·05$]. Among 16 non-ST elevation AMI (NSTEMI) patients, 53% underwent coronary angiogram at index admission. The proportion of patients in Killip class I, II, III and IV were 36%, 19%, 28% and 16% respectively. Regarding medical treatment, 98% of the patients received aspirin, 45% received thienopyridine antagonists, 50% received angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, 67% received β-blockers and 15% received aldosterone antagonist. After follow-up at 1 year, 38 (57·6%) patients were still alive and free of cardiac adverse events, nine (13·6%) had nonfatal adverse cardiac events, 17 (25·8%) died and two (3·0%) were lost to follow-up. Median follow-up time was 360 days (range 1–489 days). The LVEF, LVEDV, LVESV and LVWMI in the control subjects were 76·1 ± 6·3%, 72·2 ± 22·6 mL, 21·4 ± 7·1 mL and 1 respectively ($P < 0·05$ vs. AMI patients for all parameters).

Urocortin levels in AMI patients

Plasma urocortin level at onset (day 0) was significantly elevated compared with the control group (Fig. 1a), and continued until day 5. The levels of urocortin at months 1, 3 and 6 were decreased and were not different from those of the control group. The median plasma urocortin level was 124·1 (IQR 100·0, 181·1) pmol L^{-1}. Clinical characteristics of patients with high (i.e. >median) and low (i.e. <median) urocortin were not different except that the former group had higher peak cTnT level than in the latter (Table 2). The proportions of patients receiving β-blockers, angiotensin-converting enzyme inhibitors, aldosterone antagonists and statin were not different between patients with low and high urocortin level (all $P > 0·05$).

Plasma urocortin levels were not correlated with age, WBC, BUN, creatinine or albumin ($P > 0·05$ for all). Plasma NT-proBNP level at discharge was moderately correlated with age ($r = 0·44$, $P < 0·002$), BUN ($r = 0·54$, $P < 0·001$), creatinine ($r = 0·50$, $P < 0·001$) and albumin ($r = -0·59$, $P < 0·001$). Plasma urocortin levels were higher in NSTEMI than in STEMI on days 1, 3 and 5 but were not different on day 0. Plasma urocortin on day 0 was not correlated with the severity of myocardial necrosis measured by peak CK-MB mass or cTnT level ($r = 0·1$, $P = 0·462$ and $r = 0·08$, $P = 0·573$ for CK-MB mass and cTnT level, respectively). There was no difference in plasma urocortin levels among patients in different Killip classes on any of the experimental days. However, plasma NT-proBNP at discharge was higher in Killip class IV ($P < 0·001$ for the difference among groups) but was not different between NSTEMI and STEMI.

Relationship between plasma urocortin level and echocardiographic parameters

Echocardiographic parameters of AMI patients are shown in Table 1. Plasma urocortin levels were associated neither with echocardiographic parameters nor with NT-proBNP level

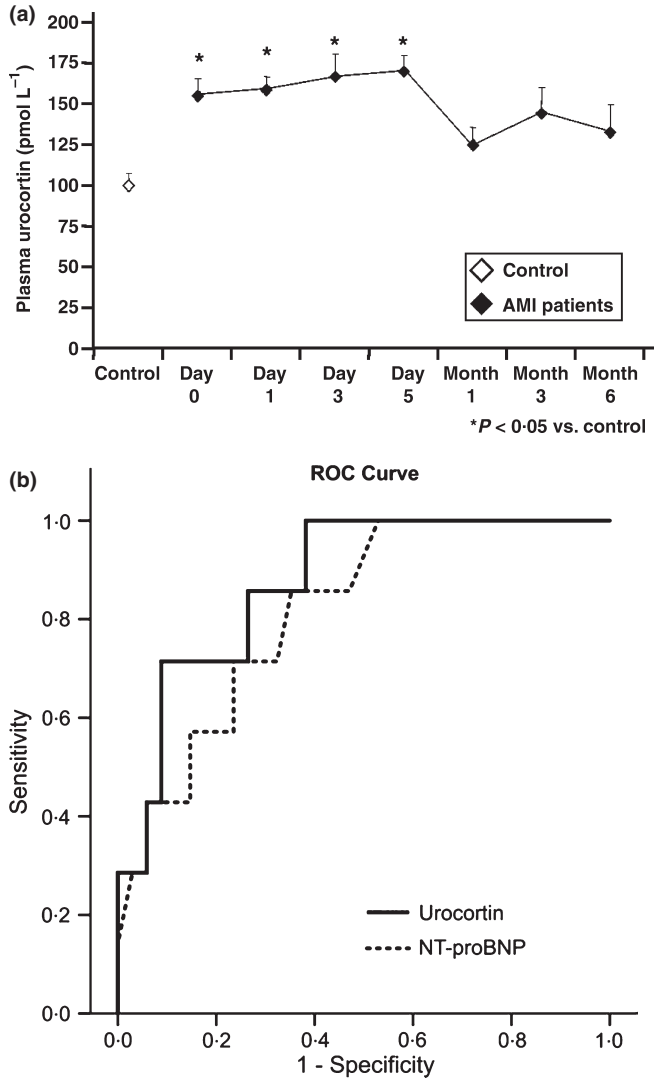

Figure 1 (a) Plasma profile of urocortin levels in control subjects and acute myocardial infarction (AMI) patients. Values are mean ± SE. (b) Receiver-operating characteristic curve of all-cause mortality at 1 year after AMI onset for levels of plasma urocortin on day 0 and NT-proBNP at discharge.

Table 2 Clinical characteristics of patients with high and low urocortin levels

	High urocortin (n = 36)	Low urocortin (n = 28)	P value
Age (years)	63 ± 12	62 ± 10	0·52
Gender (M:F)	21 : 15	14 : 14	0·51
Type of AMI, n (%)			
STEMI	25 (78)	19 (77)	0·66
NSTEMI	7 (22)	9 (27)	
Time from onset to admission	307 ± 306	323 ± 312	0·89
Killip class, n (%)			0·99
I	13 (36)	10 (37)	
II	7 (19)	5 (18)	
III	10 (28)	8 (30)	
IV	6 (17)	4 (15)	
CK-MB (U L^{-1})	225 ± 173	220 ± 200	0·61
cTnT (ng mL^{-1})	10·2 ± 9·2	5·9 ± 7·3	0·049
Hct (%)	37·2 ± 7·5	38·7 ± 5·8	0·27
WBC	13 280 ± 3985	11 782 ± 4839	0·09
BUN (mg dL^{-1})	25·6 ± 23·4	19·6 ± 20·0	0·35
Creatinine	1·7 ± 1·9	2·0 ± 1·8	0·55
LVEF (%)	41·1 ± 13·6	49·7 ± 13·4	0·73
FBS (mg day^{-1})	155 ± 75	139 ± 50	0·62
Urocortin day 0 (pmol L^{-1})	178·9 ± 53·6	95·9 ± 23·1	< 0·001
NT-proBNP at discharge (pmol L^{-1})	5238 ± 8117	2959 ± 3210	0·49
Death, n (%)	13 (36)	3 (11)	0·02
CV events, n (%)	19 (53)	8 (29)	0·05

Values are mean ± SD or number (per cent). AMI, acute myocardial infarction; CK-MB, creatinine kinase-MB; cTnT, cardiac troponin T; NSTEMI, non-ST elevation AMI.

at onset. However, the NT-proBNP level at discharge was correlated with LVEF (r = −0·434, P = 0·002), LVESV (r = 0·42, P = 0·003), LVEDV (r = 0·34, P = 0·016) and LVWMI (r = 0·363, P = 0·01), and the level at 6-month follow-up was correlated with LVEF (r = −0·430, P = 0·011) and LVWMI (r = 0·508, P = 0·002) measured on the same day. NSTEMI patients had poorer echocardiogrpahic parameters than STEMI patients (109·2 ± 46·5 vs. 87·6 ± 39·2 mL, P = 0·041; 29·1 ± 12·7 vs. 42·1 ± 14·3%, P = 0·002; for LVEDV and LVEF, respectively).

Factors associated with mortality

Factors associated with mortality are shown in Table 3. Patients who died were older, presented with Killip class IV at index event, had higher BUN, higher fasting blood sugar and lower LVEF. Serum creatinine at admission and before discharge and the changes in serum creatinine were not associated with mortality (P > 0·05 for both comparisons).

Table 3 Factors associated with mortality

	Death (n = 17)	Survival (n = 47)	P value (univariate)	P value (multivariate)
Age (years)	68 ± 11	60 ± 11	0·02	0·57
Gender (M:F)	9 : 8	28 : 19	0·64	
Type of AMI, n (%)				
STEMI	12 (70·6)	38 (80·9)	0·39	
NSTEMI	5 (29·4)	9 (19·1)		
Time from onset to admission	445 ± 408	267 ± 197	0·14	
Killip class, n (%)			0·001	0·58
I	3 (17·6)	20 (45·5)		
II	3 (17·6)	7 (15·9)		
III	3 (17·6)	15 (34·1)		
IV	8 (47·2)	2 (4·5)		
CK-MB (U L^{-1})	261 ± 196	193 ± 178	0·19	
CTnT (ng mL^{-1})	10·9 ± 9·9	7·0 ± 7·9	0·14	
Hct (%)	35·2 ± 7·2	39·2 ± 6·3	0·06	
WBC	13232 ± 9050	12397 ± 4196	0·51	
BUN (mg dL^{-1})	31·0 ± 29·8	20·2 ± 18·1	0·194	
Creatinine (mg dL^{-1})	1·8 ± 1·3	1·8 ± 1·4	0·49	
>50% increased Cr, n (%)	4 (80)	1 (20)	0·013	0·99
LVEF (%)	32·6 ± 14·1	41·7 ± 12·8	0·03	0·95
FBS (mg day^{-1})	181 ± 89	134 ± 51	0·06	0·87
β-blocker use, n (%)	3 (19)	40 (83)	< 0·001	0·16
Urocortin day 0 (pmol L^{-1})	175·0 ± 57·8	132·4 ± 55·0	0·004	0·02
NT-proBNP at discharge (pmol l^{-1})	11895 ± 11064	2704 ± 3637	0·003	0·17

Values are mean ± SD or number (per cent). Multivariate model adjusting with age, Killip class, LVEF, renal injury, FBS and NT-proBNP. AMI, acute myocardial infarction; CK-MB, creatinine kinase-MB; NSTEMI, non-ST elevation AMI.

Patients who presented a > 50% increase in serum creatinine during the hospital stay (RIFEL criteria of at least at risk of renal failure) [20] had significantly higher mortality rate (80 vs. 19·0%, P = 0·001). However, this association was not significant in Cox regression analysis.

In STEMI patients, reperfusion therapy was associated neither with the mortality nor with the combined adverse cardiovascular events (P > 0·05). In NSTEMI patients, revascularization was not associated with the mortality of combined adverse cardiovascular events (P > 0·05). Medications at discharge were not different between patients who died and survived, except that the use of β-blocker was found to be higher in patients who survived (83 vs. 18%, P < 0·001).

Plasma urocortin level in the prediction of mortality

Plasma urocortin levels on day 0 and NT-proBNP levels at discharge day were higher in patients who died than in patients who survived (Table 3). They were also higher in patients with combined adverse cardiac events than in patients without adverse cardiac events.

At day 0, plasma urocortin levels were higher in patients who died or patients with combined adverse cardiac events than in patients who survived or patients without combined adverse cardiac events in multivariate analysis (P = 0·02 and 0·03, respectively). Plasma urocortin was also the only independent parameter associated with mortality in this group of patients (Table 3).

Receiver-operating characteristic curves, constructed to study the prognostic accuracy of using plasma urocortin and NT-proBNP levels to predict mortality, demonstrated that plasma urocortin levels on day 0 and NT-proBNP level at discharge had a significant predictive value for mortality (Fig. 1b). Area under curve of day 0 urocortin was 0·750 (95%CI, 0·619–0·881; P = 0·004), whereas that of NT-proBNP was 0·857 (95%CI, 0·722–0·992; P = 0·003). The best cut-off value for mortality prediction by urocortin was 124 pmol L^{-1} (sensitivity = 0·857 and specificity = 0·647) and by NT-proBNP was 1901 pmol mL^{-1} (sensitivity = 0·857 and specificity = 0·735). Unlike urocortin and NT-proBNP, cTnT was not significantly different between patients who died and those who survived (Table 3), and did not have significant prognostic performance in predicting mortality in the present study (ROC 0·671, 95%CI 0·412, 0·930, P = 0·183).

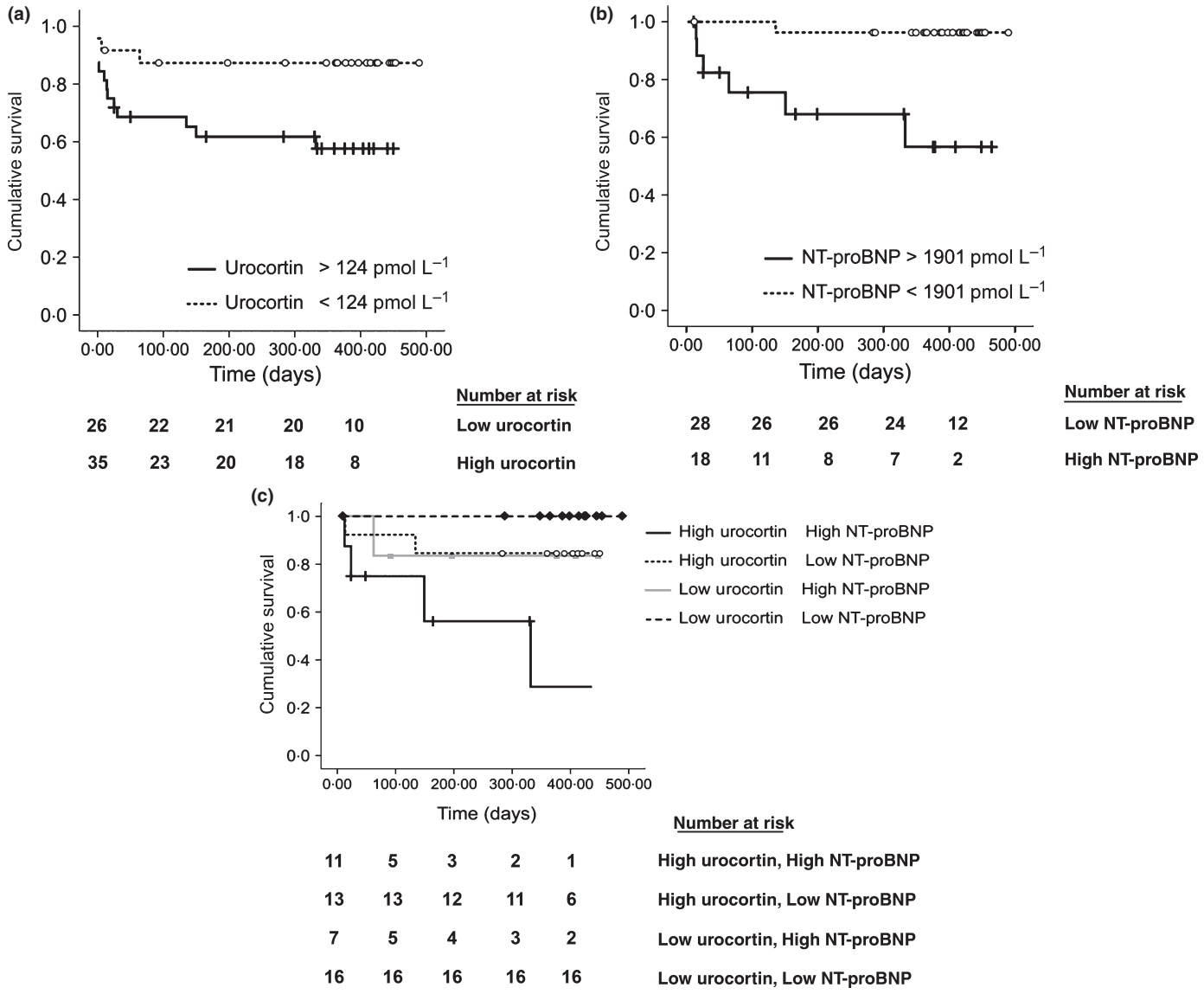

Figure 2 (a) Kaplan–Meier curves showing cumulative mortality during 1 year, according to the level of plasma urocortin. P = 0·026 for the difference between groups. (b) Kaplan–Meier curves showing cumulative mortality during 1 year, according to the level of plasma NT-proBNP. P = 0·002 for the difference between groups. (c) Kaplan–Meier curves showing cumulative mortality during 1 year, according to the levels of both plasma urocortin and NT-proBNP. P = 0·001 for the difference among groups.

Table 4 Predictive values of mortality with plasma urocortin and NT-proBNP levels

Performance	Urocortin	NT-proBNP	Urocortin and NT-proBNP
Sensitivity (95% CI)	0·81 (0·54–0·95)	0·86 (0·42–0·99)	1·0 (0·56–1·0)
Specificity (95% CI)	0·54 (0·48–0·69)	0·70 (0·53–0·99)	0·41 (0·25–0·59)
Positive predictive value (95%CI)	0·41 (0·24–0·59)	0·33 (0·24–0·59)	0·26 (0·12–0·47)
Negative predictive value (95%CI)	0·89 (0·68–0·97)	0·97 (0·80–0·99)	1·0 (0·73–1·0)

Prognostic probability analysis, using the Kaplan–Meier survival model, demonstrated that day 0 urocortin with levels higher than the cut-off value (124 pmol L^{-1}) was associated with mortality (P = 0·026, Fig. 2a) with a relative risk of 3·5 and a 95% CI of 1·1–10·6. NT-proBNP levels, measured on the day of discharge that were higher than the cut-off value (1901 pmol L^{-1}), were also associated with mortality (P = 0·002, Fig. 2b) with a relative risk of 9·7 and a 95% CI of 1·2–72·9. Using Cox regression analysis adjusting for age, Killip class, LVEF, NT-proBNP and changes in serum creatinine during admission, the level of urocortin at day 0 was associated with mortality (P = 0·03) but not for combined adverse cardiovascular events (P = 0·07).

The additive benefit of combined cardiac markers on prognosis was studied by dividing patients into four groups based on the cut-off value of urocortin and NT-proBNP derived from ROC curves (Fig. 2c). The mortality rate was higher in patients with elevated levels of plasma urocortin and/or NT-proBNP than in patients with low levels of both urocortin and NT-proBNP. The combination of urocortin and NT-proBNP showed good sensitivity and specificity in predicting the mortality in AMI (Table 4).

Discussion

This is the first study investigating the plasma urocortin levels in AMI patients in the acute phase at 1-year follow-up. Its level in AMI patients was significantly higher than the normal control group from days 0–5 of AMI. Plasma urocortin level on AMI day 0 demonstrated a significant and independent predictive value for mortality and adverse cardiac events. Patients with plasma urocortin levels > 124 pmol L^{-1} had a higher mortality and adverse cardiac events similar to patients with plasma NT-proBNP levels > 1901 pmol L^{-1}. More importantly, we demonstrated for the first time that plasma urocortin can be another beneficial marker to add to the prognostic performance of NT-proBNP.

Urocortin in STEMI and NSTEMI

Plasma urocortin levels were significantly elevated in both NSTEMI and STEMI patients. Previous studies have demonstrated that urocortin has a marked coronary vasodilator effect [21–23] and could increase coronary blood flow and myocardial function through the release of nitric oxide [21]. As myocardial ischaemia and reperfusion cause marked endothelial dysfunction and reduction in coronary relaxation because of reduced nitric oxide release [24], increased levels of urocortin after AMI could be a part of the cardioprotective response to ischaemic/reperfusion injury in these patients. In the present study, plasma urocortin levels in NSTEMI patients were higher than in STEMI patients. This may be correlated with the severity of obstructive coronary artery disease. Although it causes less fatal acute cardiac damage, NSTEMI is more often associated with multi-vessel disease and repeated myocardial ischaemia [25,26], and could be responsible for a high level of plasma urocortin.

In the present study, the high proportion of patients without reperfusion therapy was mainly due to prolonged onset of chest pain prior to hospital presentation. The median time was 420 min (IQR 240, 720 min). The proportion of patients receiving reperfusion therapy in the present study was similar to the proportion reported from the Global Registry of Acute Coronary Events (GRACE registry) [27,28] (67% vs. 62%), and was slightly better than that reported in the Thai ACS Registry (52·6%) [29]. For NSTEMI patients, the rate of coronary angiography in the present study was similar to that in the GRACE registry (53% vs. 47·5%), and ours was slightly better than that reported in the Thai ACS registry (43·9%).

In an animal study, a short period of ischaemia could cause a significant increase in urocortin mRNA and protein concentrations in plasma, without expression of myocyte apoptosis or necrosis [6]. However, long period of ischaemia (at least 30 min) significantly reduced urocortin levels with an expression of apoptosis and necrosis of cardiomyocytes [6]. These findings suggest that urocortin expression and release are mainly sustained by ischaemic-triggered but still viable myocytes [6]. In the present study, it is possible that STEMI, which reflects transmural myocardial injury and necrosis [30], could have post-triggered viable myocytes in a lesser amount and with lower functional recovery, resulting in lower plasma urocortin levels than in NSTEMI.

In contrast to plasma NT-proBNP levels, plasma urocortin levels in our AMI patients were not correlated with LVEF. It is possible that the triggers for the release of these cardiac

markers are different, whereby plasma NT-proBNP is released mainly in response to increased ventricular wall stress from left ventricular dysfunction induced by myocardial injury or ischaemia [31], whereas urocortin in AMI patients is released in direct response to myocardial ischaemia [6].

Prognostic value of plasma urocortin

When measured within 24 h after the onset of AMI, the plasma urocortin level was significantly higher in patients incurring an endpoint of death than patients who had adverse events and who survived. Although previous studies demonstrated a cardioprotective effect of urocortin [12–15], our study demonstrated that high levels of urocortin within 24 h after the onset are associated with poor outcome. It is known that urocortin can be released when myocardium is ischaemic [12]. However, it is possible that high levels of plasma urocortin could indicate severe myocardial damage [6], and may explain their association with poor outcome as found in the present study. This scenario has likewise been reported with NT-proBNP levels [32]. A previous study reported that plasma NT-proBNP measured 2–4 days after myocardial infarction independently predicted left ventricular function and 2-year survival, and that high plasma NT-proBNP levels above the median significantly correlated with a lower survival rate [32].

In the present study, the prognostic probabilities of plasma urocortin and NT-proBNP levels were examined. Plasma urocortin levels within 24 h of the onset were significantly predictive for mortality. Furthermore, plasma urocortin and NT-proBNP levels above the cut-off value were significantly associated with poor survival rates, a finding consistent with a previous report [32]. Unlike urocortin, the other measured parameters such as plasma cTnT, creatinine, changes in serum creatinine, revascularization and medications were not independently associated with mortality in this group of patients.

The pathophysiology of AMI is complex, involving myocardial ischaemia, myocardial necrosis and myocardial stretch. The multi-marker approach for the prediction of adverse cardiac events in acute coronary syndrome patients would be beneficial [4,33]. In the present study, we demonstrated increased sensitivity to predict mortality when using both urocortin and NT-proBNP levels. Our findings indicate that the combination of the two markers should be used for risk stratification in AMI patients.

Conclusion

Plasma urocortin level is elevated in AMI patients starting from the onset and remains at high levels for 5 days. Despite its proposed cardioprotective actions, the high level of plasma urocortin within 24 h of onset is associated with high mortality in AMI patients. Plasma urocortin level has a significant prognostic value in AMI patients and the combination with NT-proBNP could enhance prognostic performance.

Study limitations

There were a small number of enrolled patients and control subjects. Although the association between urocortin levels and Killip classes was not found in the present study, it is still possible that their relationship exists but could not be detected because of small number of enrolled patients. The numbers at risk in each group of patients (as shown in Fig. 2c) were low and therefore the statistical conclusions were limited. Future studies with large numbers of AMI patients and longer periods of follow-up are required to warrant its clinical significance in prognostic prediction.

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