CHAPTER III

RESULTS

1. Patient characteristics

There are 66 acute myocardial infarction patients (40 male and 26 female) and 21 normal controls (8 male and 13 female) in this study. The proportion of gender and the mean age of patient group were not significantly different from those of the normal control group (62.7 ± 11.3 vs. 63.8 ± 11.5 years for acute myocardial infarction patients and control, respectively). The LVEF, LVEDV, LVESV, LVWMI in controls were 76.08 ± 1.58%, 72.18 ± 5.65, 21.42 ± 1.77 and 1, respectively ($p < 0.05$ vs. acute myocardial infarction patients). The demographic features of participated patients are shown in table 3. After the follow-up data were recorded completely, 37 (56.1%) of the enrolled patients were still alive and free of cardiac adverse events, 9 (13.6%) had cardiac adverse events, 17 (25.8%) died and 3 (4.5%) was lost to follow-up. Median length of time from the onset to the endpoint of the study was 360 days with a range of 1- 489 days.

2. Urocortins levels in acute myocardial infarction patients and normal controls

The plasma urocortins levels observed in the normal control group were 100.09 ± 7.48 pmol/l (24.59 – 154.03 pmol/l, median 105.57 pmol/l), whereas the urocortins levels in acute myocardial infarction patients were 155.07 ± 10.73 pmol/l in day 0 (n = 59), 158.45 ± 8.16 pmol/l in day 1 (n = 60), 166.67 ± 13.76 pmol/l in day 3 (n = 50), 169.87 ± 9.84 pmol/l in day 5 (n = 37), 125.05 ± 10.57 pmol/l in month 1 (n = 39), 132.23 ± 10.78 pmol/l in month 3 (n = 29) and 132.76 ± 16.57 pmol/l in month 6

(n = 26). Plasma urocortins levels in acute myocardial infarction patients were significantly higher than those in normal controls when measured in day 0, 1, 3 and 5 after the onset, p = 0.004, 0.000, 0.000 and 0.000, respectively (figure 4). The plasma urocortins level was significantly rising continuously since the onset of infarction until day 5 and then decreased. The plasma urocortins levels in month-1 to month-6 follow up were not significantly different from those of the control.

The plasma urocortins level was not significantly different when compared between gender in normal control group (p = 0.210), but the plasma urocortins level was significantly higher in male patients comparing with female patients in month 6 after onset (male 159.29 ± 22.73 pmol/l vs. female 90.30 ± 16.77 pmol/l, p = 0.037) (figure 5). There were 51 STEMI and 15 NSTEMI acute myocardial infarction patients in this study. The plasma urocortins levels in STEMI group were significantly higher than those in normal controls in day 0, 1, 3 and 5 (p = 0.013, 0.002, 0.003 and 0.000, respectively) and the plasma urocortins levels in NSTEMI group were also significantly higher in day 0, 1, 3 and 5 (p = 0.008, 0.000, 0.000 and 0.001, respectively) (figure 6). The elevated plasma urocortins level measured in day 1 was significantly higher in NSTEMI than STEMI group (p = 0.022, 195.54 ± 18.62 pmol/l vs. 146.09 ± 8.27 pmol/l, respectively) but these differences were not found in other days (figure 6). Patients with NSTEMI were significantly correlated with more severity of infarction determined by Killip classes (r = 0.295, p = 0.021) than STEMI patients.

The plasma urocortins level was not significantly varied according to the severity of infarction. There was no significant difference in plasma urocortins levels among patients in various Killip classes (figure 7), at any experimental days. Plasma

urocortins and NT-proBNP levels were not significantly correlated with CK-MB mass and cardiac troponin T (CTnT) levels, which reflect the level of myocardial necrosis. However, plasma NT-proBNP level on the discharge day was significant higher in Killip class IV (p = 0.001 for the difference among groups). There were not significant difference of plasma NT-proBNP level on the discharge day between NTSEMI and STEMI groups (6907.52 ± 2898.53 pmol/l vs. 3005.10 ± 668.52 pmol/l, p = 0.114).

Table 3 Characteristics of acute myocardial infarction patients in the study. Values are mean ± SEM or number (percentage). $^{\#}p = 0.000$, significantly different from controls and $^{*}p < 0.05$, significantly different from controls, tested by Mann Whitney-*U* test.

	Controls	**Acute Myocardial Infarction patients**
Number	21	66
Male	8 (38.1%)	40 (60.6%)
Female	13 (61.9%)	26 (39.4%)
Age (years)	63.8 ± 11.5	62.7 ± 11.3
ST-elevation AMI	None	51 (77.3%)
Non- ST-elevation AMI	None	15 (22.7%)
Territory of culprit vessel	None	
LAD		22 (33.3%)
LCX		11 (16.7%)
RCA		28 (42.4%)
unknown		5 (7.6%)
Killip class on admission	None	
I		25 (39.7%)
II		10 (15.9%)
III		18 (28.6%)
IV		10 (15.9%)
Echocardiography data		
LVEF	76.08 ± 1.58	40.43 ± 1.74$^{\#}$
LVEDV	72.18 ± 5.65	94.31 ± 5.34*
LVESV	21.42 ± 1.77	60.53 ± 4.71$^{\#}$
LVWMI	1	1.69 ± 0.05$^{\#}$
BNP level		
Discharge day	-	3960.80 ± 885.03
Month 6	-	1459.41 ± 386.35
CK-MB (U/l)	-	213.73 ± 22.58
CTnT (ng/ml)	-	8.03 ± 1.07

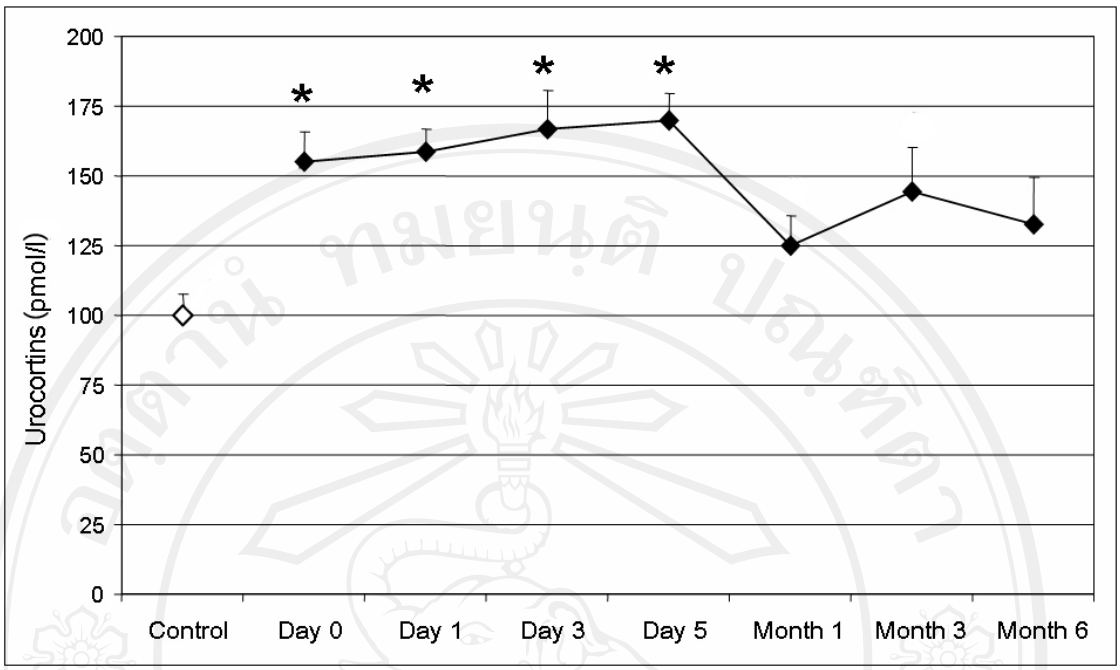


Figure 4 Plasma urocortins levels in normal controls and acute myocardial infraction patients during on admission and follow up. Values are mean ± SEM. *$p < 0.05$, significantly different from control, compared by Man Whitney-*U* test. The plasma urocortins levels in control group were 100.09 ± 7.48 pmol/l (n = 21), whereas the urocortins levels in acute myocardial infarction patients were 155.07 ± 10.73 pmol/l in day 0 (n = 59), 158.45 ± 8.16 pmol/l in day 1 (n = 60), 166.67 ± 13.76 pmol/l in day 3 (n = 50), 169.87 ± 9.84 pmol/l in day 5 (n = 37).

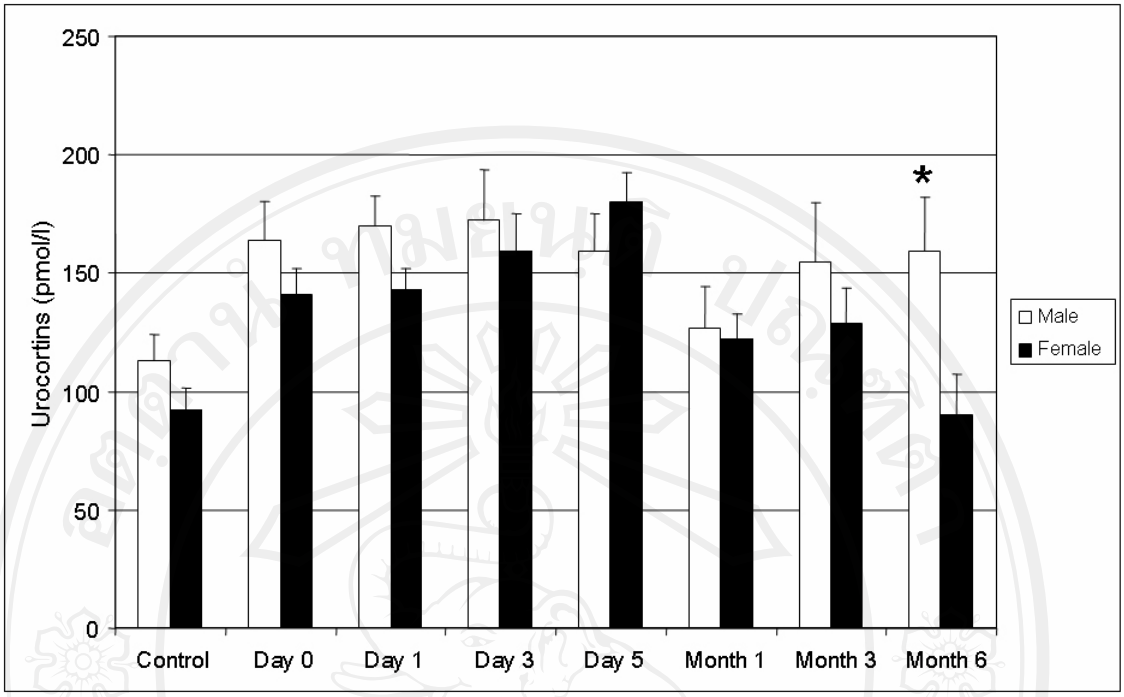


Figure 5 Comparison of the plasma urocortins levels between genders, both in controls and AMI groups. Values are mean ± SEM. There is significant difference between plasma urocortins level measured at month-6 follow up (male 159.29 ± 22.73 pmol/l vs. female 90.30 ± 16.77 pmol/l). *p = 0.037, significantly different from female.

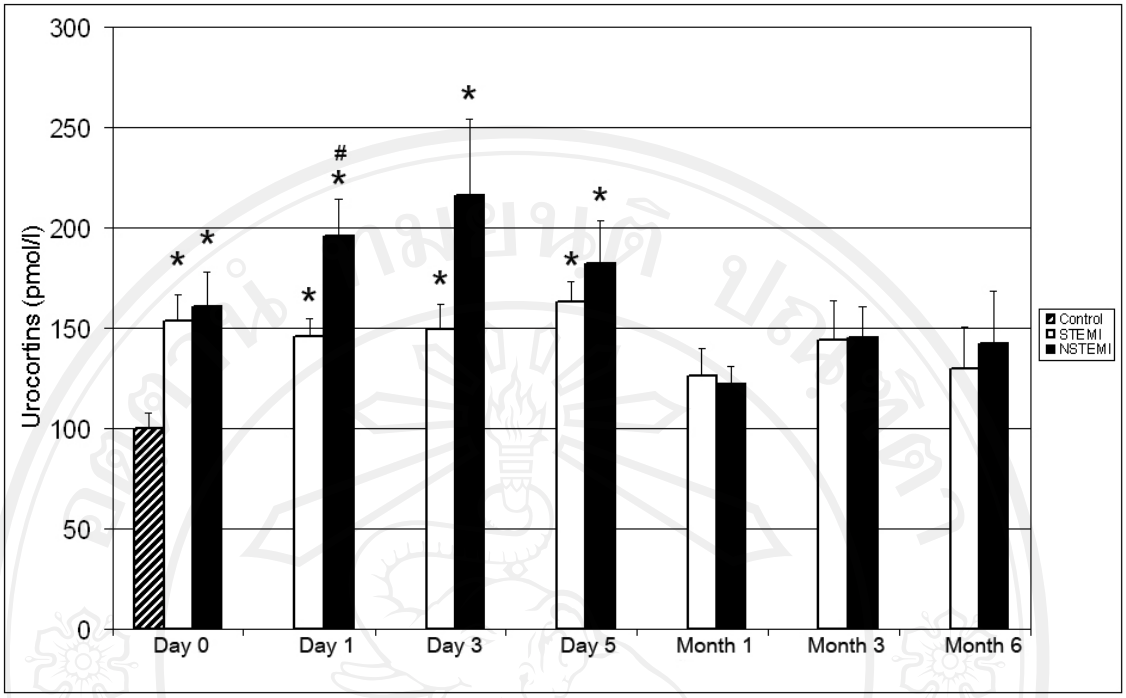


Figure 6 The plasma urocortins levels of STEMI and NSTEMI groups. Values are mean ± SEM. *$p < 0.05$, significantly different from controls. $^{\#}p < 0.05$, significantly different from STEMI. The elevated plasma urocortins level measured in day 1 was significantly higher in NSTEMI (n = 15) than STEMI (n = 51) group (195.54 ± 18.62 vs. 146.09 ± 8.27, respectively, $p = 0.022$) but these differences were not found in other days.

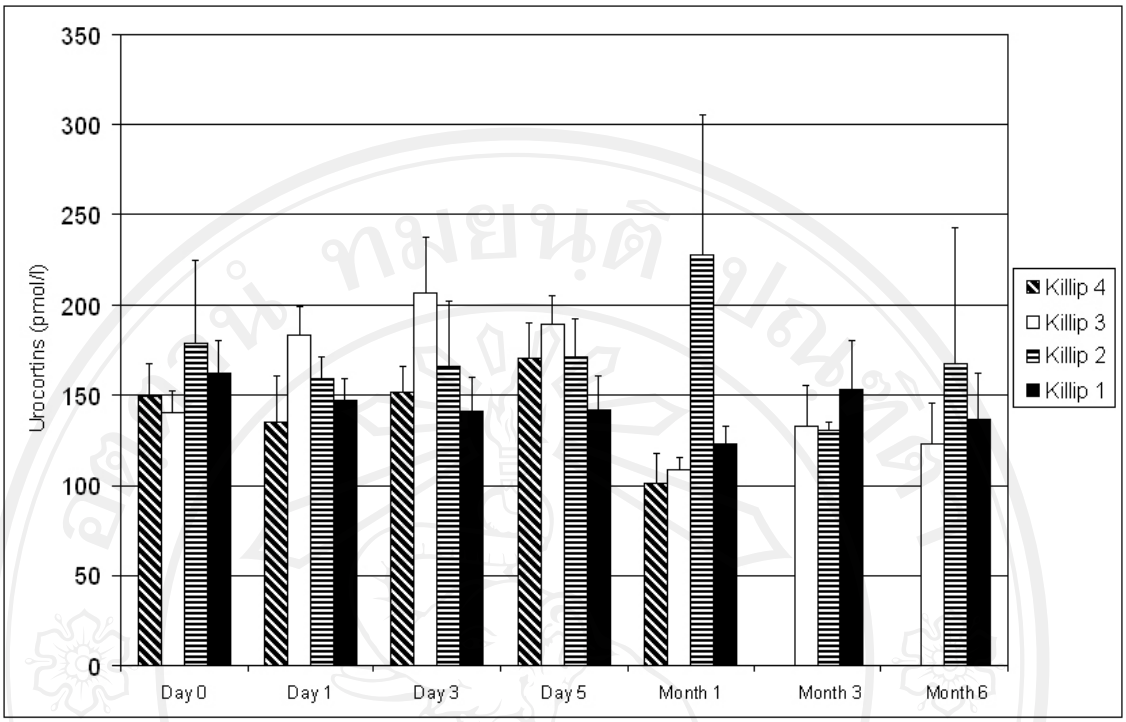


Figure 7 Plasma urocortins levels in acute myocardial infarction patients according to Killip class. Values are mean ± SEM. The plasma urocortins levels were not significantly different between the various Killip classes.

3. Relationship between plasma urocortins level and echocardiographic parameters.

Plasma urocortins level of normal control group was not significantly correlated with age, gender and any echocardiographic parameters. The plasma urocortins levels of patients were also not significantly correlated with echocardiographic parameters (i.e. LVEF, LVESV, LVEDV and WMI) and NT-proBNP level in either discharge-day or month-6 measurement. However, the level of NT-proBNP which measured at discharge day was significantly correlated with LVEF (p = 0.002, r = -0.439), LVESV (p = 0.003, r = 0.421), LVEDV (p = 0.016, r = 0.342), WMI (p = 0.01, r = 0.363) (figure 8-11, respectively). The plasma NT-proBNP level at 6-month follow up was also significantly correlated with LVEF (p = 0.011, r = -0.430) and WMI (p = 0.002, r = 0.508) which measured on the same day (figure 12-13, respectively). Moreover, the NT-proBNP levels, both measured on the discharge day and at 6-month follow up, were significantly correlated with age of patients (p = 0.016, r = 0.342 and p = 0.033, r = 0.366, respectively) (figure 14) and Killip classes (p = 0.000, r = 0.576 and p = 0.001, r = 0.562, respectively), which was not found in plasma urocortins level.

NSTEMI patients had significant poor LVEF than STEMI patients (31.11± 3.40 vs. 42.91 ± 1.81, p = 0.005), but had significant higher LVESV (83.28 ± 11.17 vs.53.83 ± 4.55, p = 0.005).

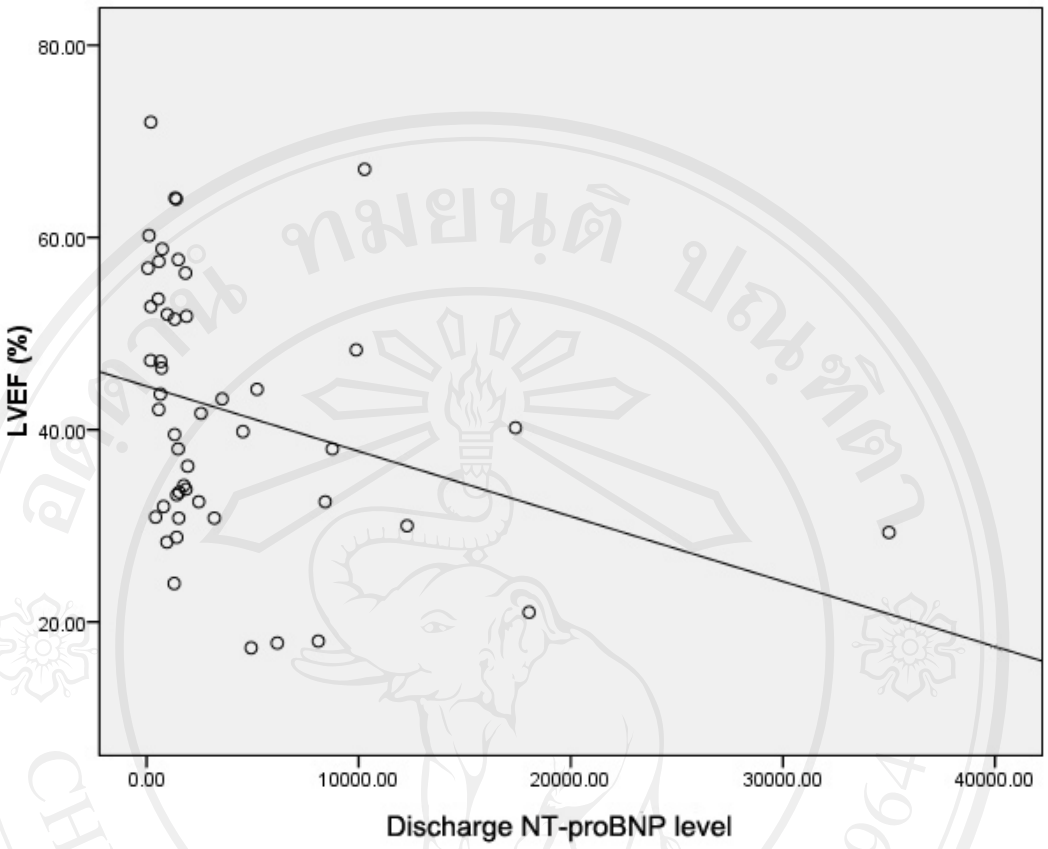


Figure 8 The relationship between the plasma NT-proBNP level and LVEF, measured on the discharge day, in the acute myocardial infarction patients, p = 0.002 and r = -0.439.

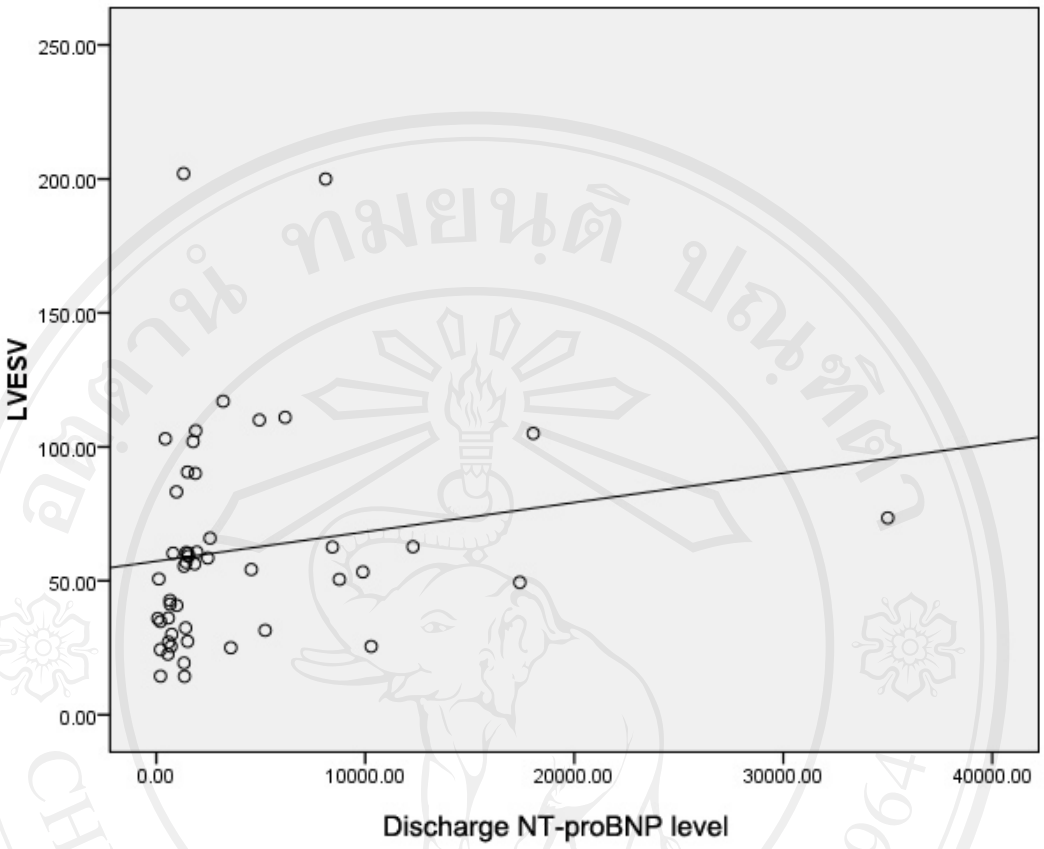


Figure 9 Relationship of NT-proBNP level with LVESV, measured on the discharge day, in the acute myocardial infarction patients, $p = 0.003$ and $r = 0.421$.

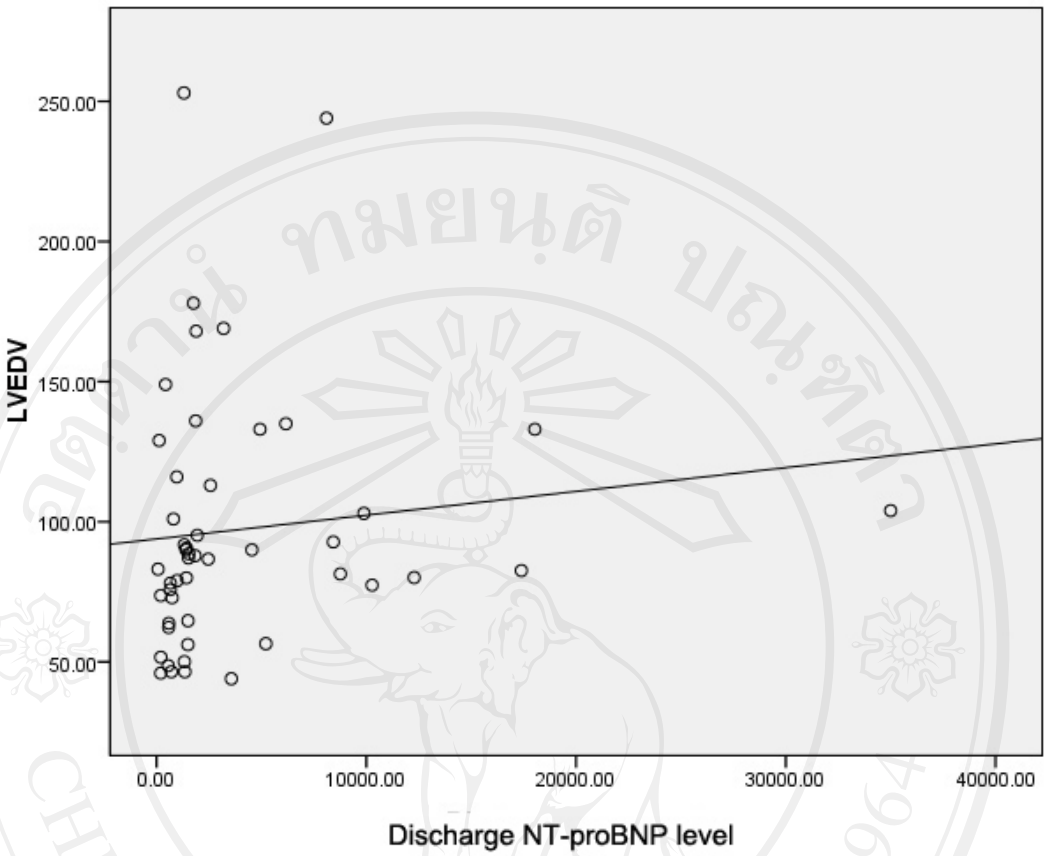


Figure 10 Relationship of NT-proBNP level with LVEDV, measured on the discharge day, in the acute myocardial infarction patients, $p = 0.016$ and $r = 0.342$.

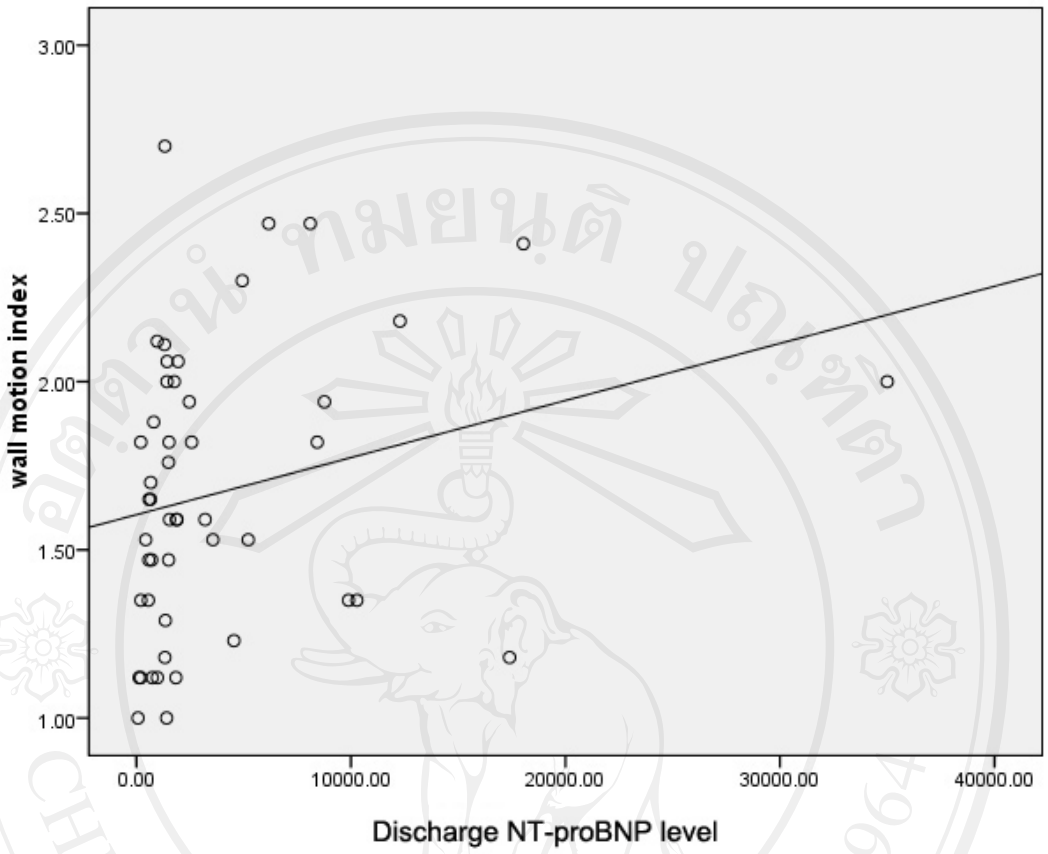


Figure 11 Relationship of NT-proBNP level with wall motion index, measured on the discharge day, in the acute myocardial infarction patients, $p = 0.01$ and $r = 0.363$.

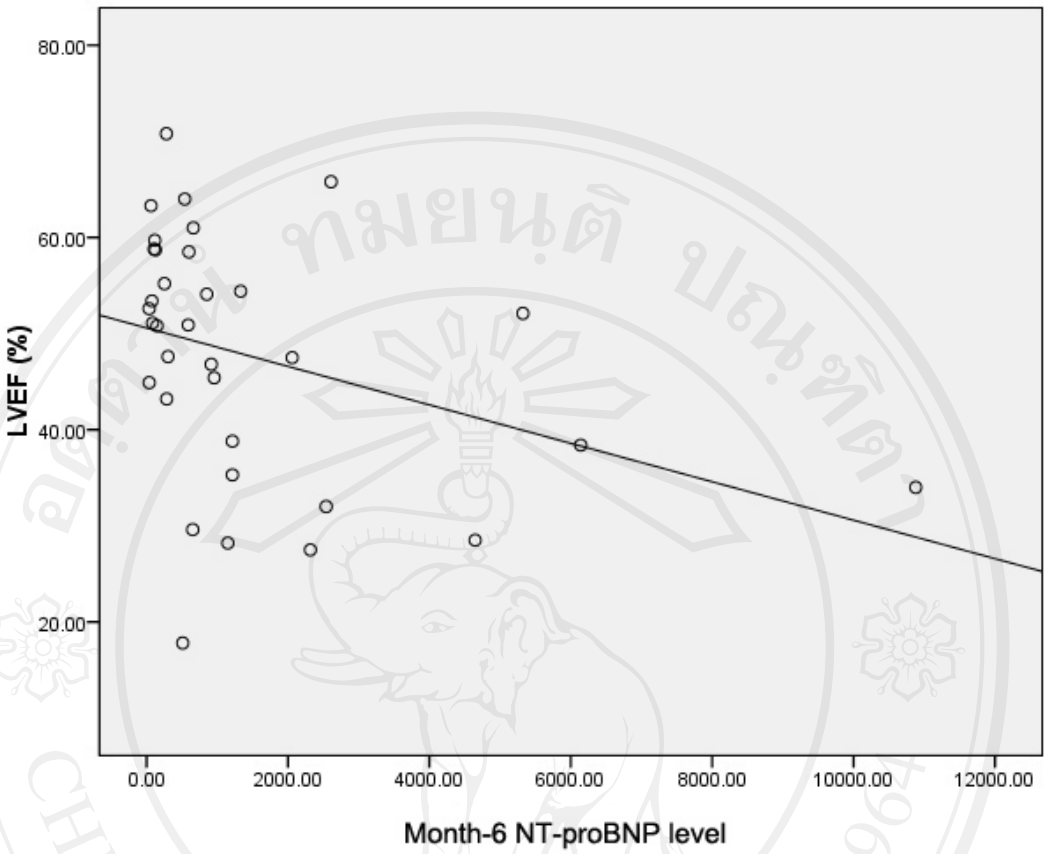


Figure 12 Relationship of NT-proBNP level with LVEF, measured at month-6 follow up, in the acute myocardial infarction patients, $p = 0.011$ and $r = -0.430$.

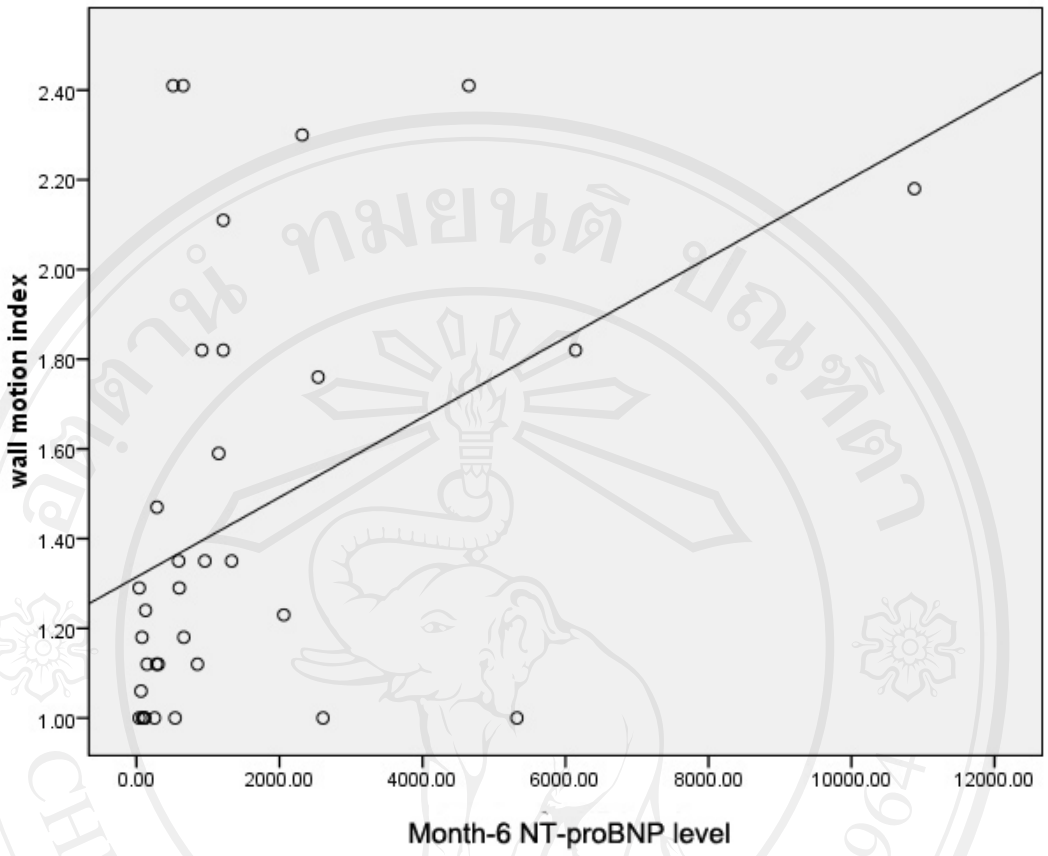
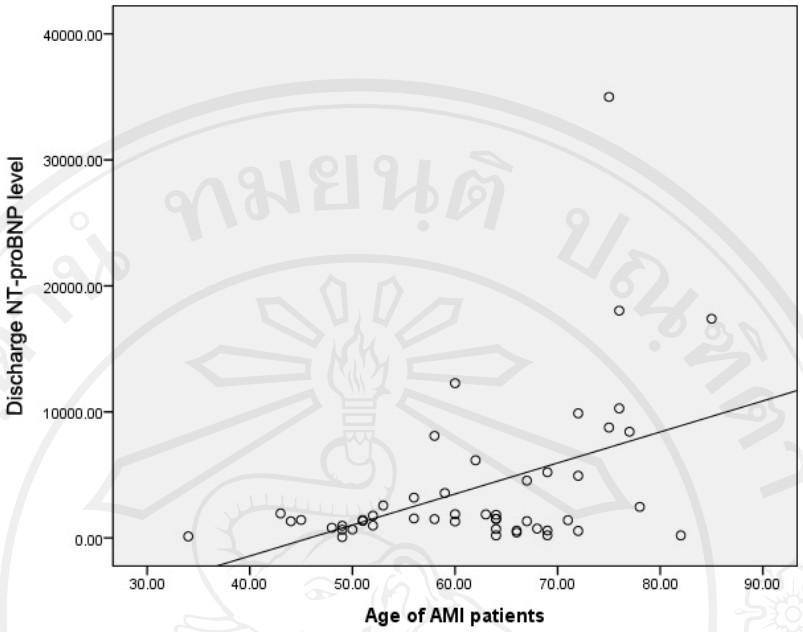


Figure 13 Relationship of NT-proBNP level with wall motion index, measured at month-6 follow up, in the acute myocardial infarction patients, p = 0.002 and r = 0.508.

A



B

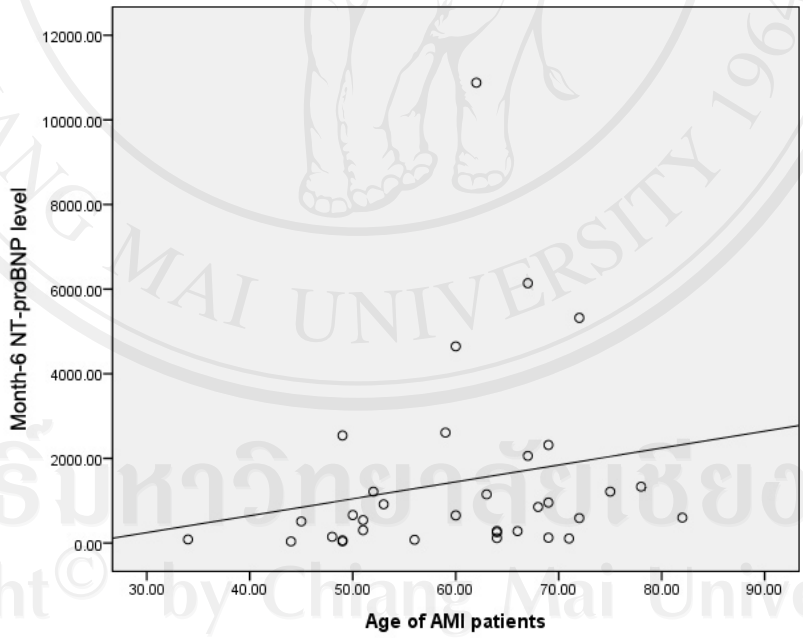


Figure 14 Relationship of age and NT-proBNP level, measured on the discharge day (A) and at month-6 follow-up (B), in the acute myocardial infarction patients, p = 0.016, r = 0.342 and p = 0.033, r = 0.366, respectively.

4. Plasma urocortins levels and the adverse events and death.

After the 1-year follow up, data were recorded completely. Median length of time from the onset to the endpoint of the study was 360 days with a range of 1- 489 days. Thirty-seven (56.1%) of the enrolled patients were still alive and free of cardiac adverse events, 9 (13.6%) had cardiac adverse events, 17 (25.8%) died and 3 (4.5%) was lost during follow-up. Factors associated with mortality were shown in table 4. Dead patients were older (68.3 ± 2.6 vs. 60.9 ± 1.6 years, $p = 0.025$), presented with Killip class IV (8 vs. 2, $p = 0.003$), had higher WMI and LVESV (1.92 ± 0.09 vs. 1.63 ± 0.05, p = 0.015 and 78.05 ± 11.58 vs. 53.97 ± 4.98, $p = 0.024$, respectively), higher BUN (31.00 ± 7.44 vs. 20.32 ± 2.70, $p = 0.038$), higher fasting blood sugar (181.00 ± 22.99 vs. 133.09 ± 7.72, $p = 0.022$) and lower LVEF (32.59 ± 3.77 vs. 42.31 ± 1.93, $p = 0.024$).

The plasma urocortins profiles are shown in figure 15. Plasma urocortins level on day 0 of patients who were dead was significantly higher than patients who are event-free alive (175.02 ± 14.47 vs. 145.19 ± 15.25 pmol/l, $p = 0.010$). However, there was no significant difference between plasma urocortins level of patients who are alive and patients with incurring adverse events (145.19 ± 15.25 vs. 146.36 ± 21.79 pmol/l, $p = 0.789$). No significant difference was found between patients who had incurring adverse events compare to those who were dead ($p = 0.180$).

Plasma NT-proBNP levels of patients who were dead, with incurring adverse event and event-free alive were 11,895.57 ± 4,385.98, 3,983.43 ± 1,453.15 and 2,352.64 ± 629.21 pmol/l, respectively. Plasma level of NT-proBNP in patients who were dead was also significantly higher than those of patients who were event-free alive ($p = 0.002$), however there were no significant difference between plasma NT-

proBNP level of patients who were dead and incurring adverse event (p = 0.081), either between patients who had incurring adverse event and event-free alive (p = 0.286).

Plasma urocortins level measured on day 0 and NT-proBNP level measured on the discharge day had a significant predictive accuracy for mortality, examined by using ROC curves model (figure 16). Area under curve of day 0 urocortins was 0.786 (95% Confidence Interval 0.626-0.945, p = 0.018), where those of NT-proBNP was 0.878 (95% Confidence Interval 0.760-0.997, p = 0.002). Both area under curve for urocortins and NT-proBNP were significantly higher than the diagonal area of 0.5, reflecting that the deaths were associated with the higher levels of these peptides. From ROC curve, the best cutoff value for mortality prediction of urocortins was 149.99 pmol/l (sensitivity = 0.714 and specificity = 0.735) and the cutoff value of NT-proBNP was 1,901.50 pmol/ml (sensitivity = 0.857 and specificity = 0.735). The sensitivity and specificity values of each cutoff values were shown in table 5. However, both plasma levels of urocortins and NT-proBNP were not significantly predictive for adverse event outcome.

The prognosis probability analysis, using Kaplan-Meier survival model, demonstrated that day 0 urocortins level which was higher than cutoff value was significantly associated with death (log rank 8.525, mean survival time 249.4 days, 95%Confidence Interval 162.58-336.417 days, p = 0.004) (figure 17), but not the adverse events outcome (log rank 0.358, mean survival time 381.6 days, 95%Confidence Interval 308.75-454.47 days, p = 0.550) (figure 18). NT-proBNP level, measured on the discharge day, higher than the cutoff value was also significantly associated with death, but not associated with adverse events outcome

(log rank 11.424, mean survival time 286.1 days, 95%Confidence Interval 187.075-385.30 days, p = 0.001 and log rank 3.205, mean survival time 317.8 days, 95%Confidence Interval 214.62-421-13 days, p = 0.073, respectively) (figure 19, 20). This suggested that both plasma urocortins and BNP level were both beneficial for mortality prediction but may not be suitable for adverse events prediction.

Moreover, Kaplan-Meier survival model was used for analyses the prognosis probability of combined markers. There were 29 patients who have low levels of both urocortins and NT-proBNP, 11 patients who have high level of only NT-proBNP, 15 patients who have high level of only urocortins and 7 patients who have high level of both urocortins and NT-proBNP by using the cutoff values for dividing into subgroups. The less survival rate was significantly correlated with the high levels of both urocortins and NT-proBNP (log rank 10.91, mean survival time 199.5 days, 95% Confidence Interval 73.62-325.42 days, p = 0.012) (figure 21). The mortality rate was higher for patients with elevated both plasma urocortins and NT-proBNP, patients with elevated only plasma urocortins, patients with elevated only plasma NT-proBNP and patients with low both plasma urocortins and NT-proBNP, respectively. The sensitivity and specificity in predicting mortality of urocortins, NT-proBNP and combined urocortins and NT-proBNP were shown in table 6.

Table 4 Factors associated with mortality in acute myocardial infarction patients. Values are mean ± S.E.M., number (%).

Factors	Death (n = 17)	Alive (n = 46)	*p* value
Age (years)	68.3 ± 2.6	60.9 ± 1.6	0. 025
Killip class (n (%))			0.003
• I	3 (17.6 %)	20 (45.5 %)	
• II	3 (17.6 %)	7 (15.9 %)	
• III	3 (17.6 %)	15 (34.1 %)	
• IV	8 (17.6 %)	2 (4.5 %)	
WMI	1.92 ± 0.09	1.63 ± 0.05	0.015
LVEF (%)	32.59 ± 3.77	42.31 ± 1.93	0.024
LVESV	78.05 ± 11.58	53.97 ± 4.98	0.013
BUN (mg/ml)	31.00 ± 7.44	20.32 ± 2.70	0.038
FBS (mg/d)	181.00 ± 22.99	133.09 ± 7.72	0.022
NT-proBNP (pmol/l)	11,895.57 ± 4385.98	2,728.98 ± 589.43	0.003
Urocortins day 0 (pmol/l)	175.02 ± 14.47	145.39 ± 13.05	0.010

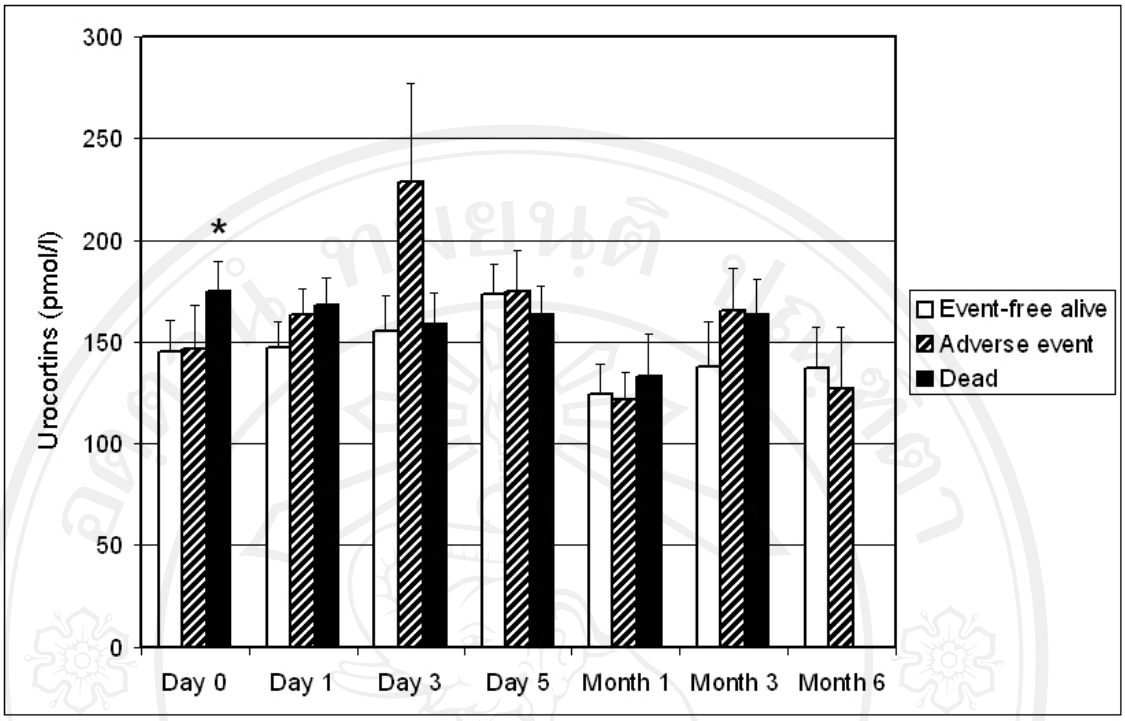


Figure 15 The plasma urocortins levels comparing between the events free, incurring adverse events and dead group. Values are mean ± S.E.M. Plasma urocortins level on day 0 of patients who were dead was significantly higher than patients who are event-free alive (175.02 ± 14.47 vs. 145.19 ± 15.25 pmol/l). $^{*}p = 0.010$ vs. event-free alive patients.

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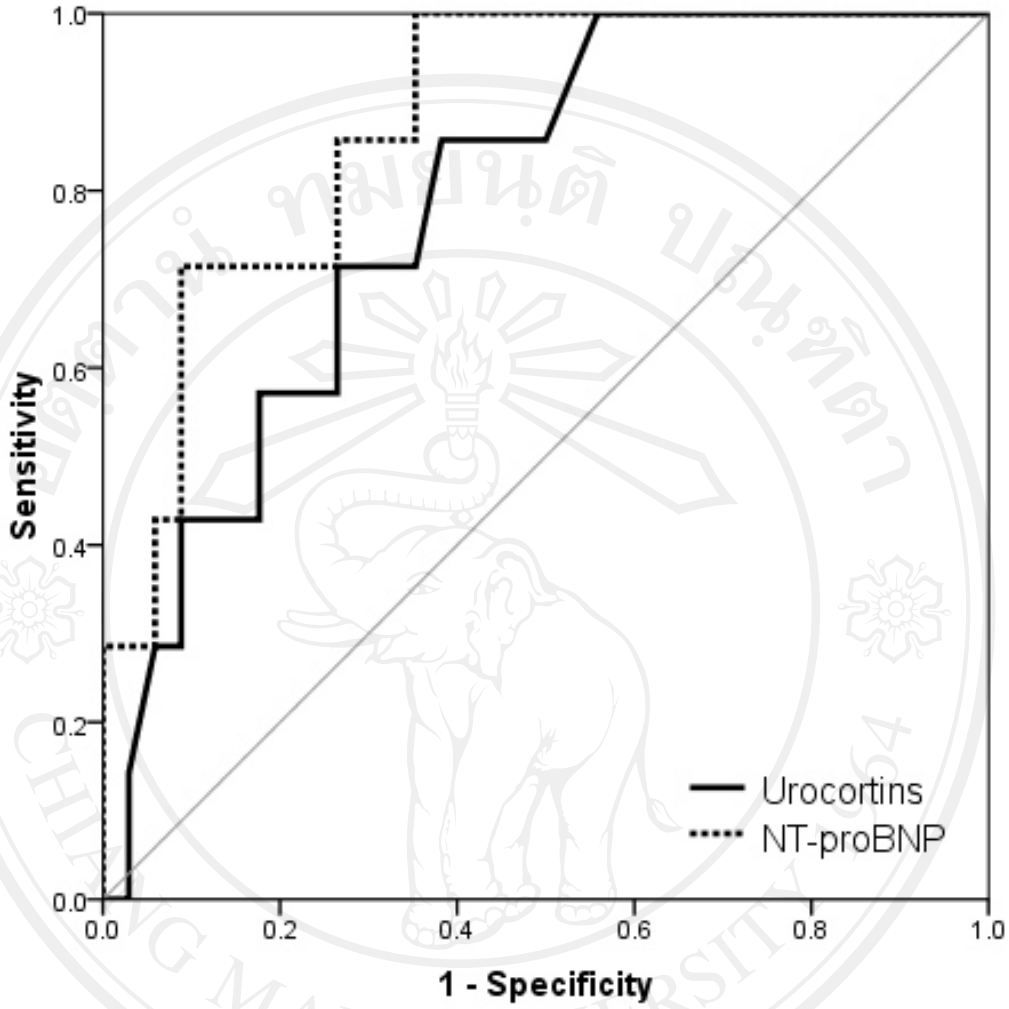

Figure 16 Receiver Operating Curve comparing day-0 plasma urocortins and discharge NT-proBNP to predict the probabilities of death. Area under curve of day 0 urocortins was 0.786 and p= 0.018, where those of NT-proBNP was 0.878 and p = 0.002.

Table 5 Specificities and Sensitivities of plasma urocortins and NT-proBNP level in the prognosis of mortality in acute myocardial infarction patients.

Cutoff value	Specificity	1-Sensitivity
Urocortins (pmol/l)		
120.860835	.857	.471
124.118712	.857	.382
127.568211	.714	.353
134.648200	.714	.324
142.111535	.714	.294
149.990099	**.714**	**.265**
158.305137	.571	.265
167.077973	.571	.235
176.340550	.571	.176
186.124800	.429	.176
191.150037	.429	.147
196.452087	.429	.118
213.241864	.429	.088
NT-proBNP (pmol/ml)		
1324.0000	1.000	.500
1336.5000	1.000	.471
1374.5000	1.000	.441
1411.5000	1.000	.412
1422.0000	1.000	.382
1457.5000	1.000	.353
1517.0000	.857	.353
1688.0000	.857	.324
1848.5000	.857	.294
1901.5000	**.857**	**.265**
2200.5000	.714	.265
2515.0000	.714	.235
2882.0000	.714	.206
3379.0000	.714	.176
4247.0000	.714	.147

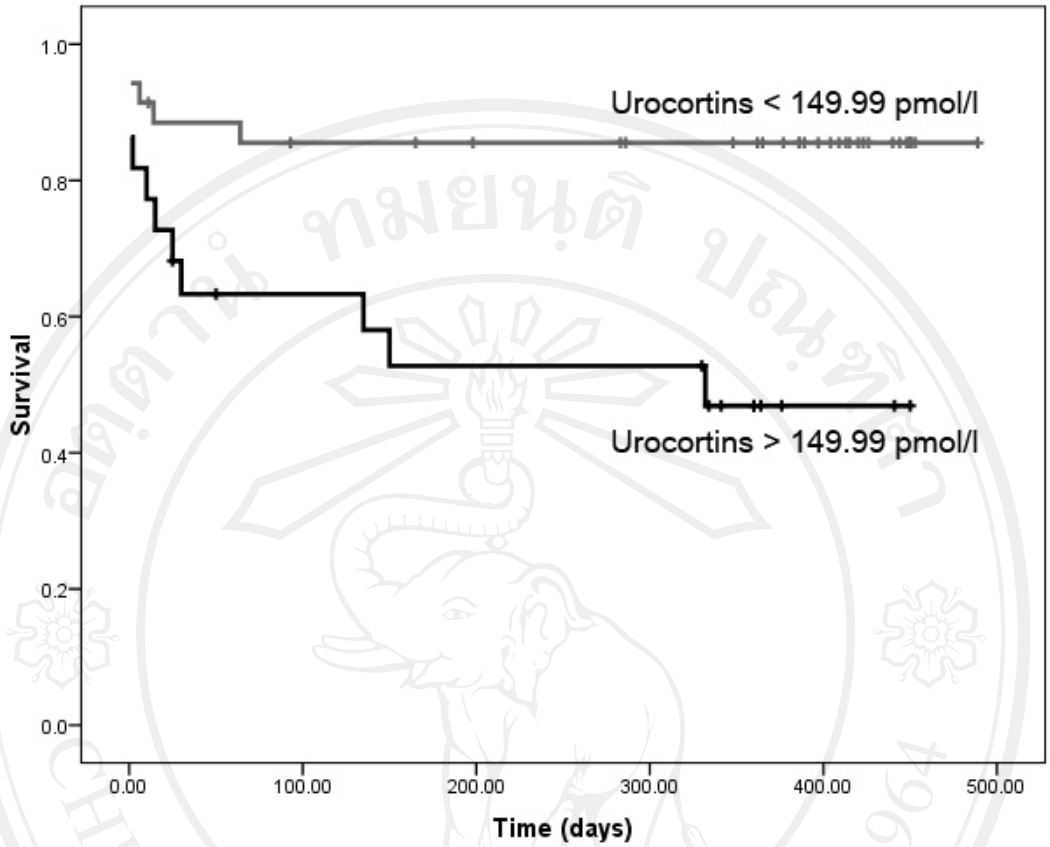

Figure 17 Kaplan-Meier Curve: Time to death related to the cutoff value of plasma urocortins level. Plasma urocortins level, measure on the onset day, at above the cutoff value was significantly correlated with lesser survival rate (log rank 8.525, mean survival time 249.4 days, 95%Confidence Interval 162.58-336.417 days , p = 0.004).

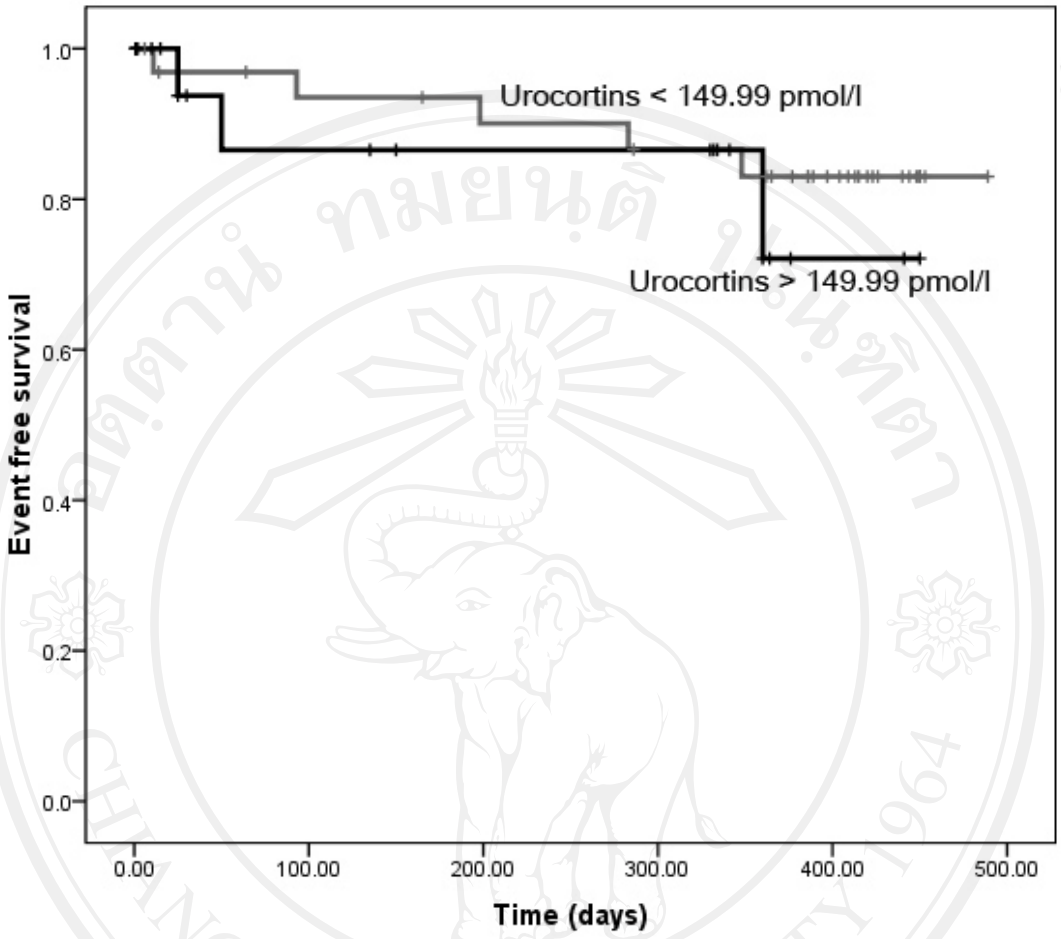

Figure 18 Kaplan-Meier Curve: Time to adverse events related to the cutoff value of plasma urocortins level. Plasma urocortins level, measured on the onset day, which higher than cutoff value was not significantly correlated with the adverse events outcome (log rank 0.358, mean survival time 381.6 days, 95%Confidence Interval 308.75-454.47 days, $p = 0.550$).

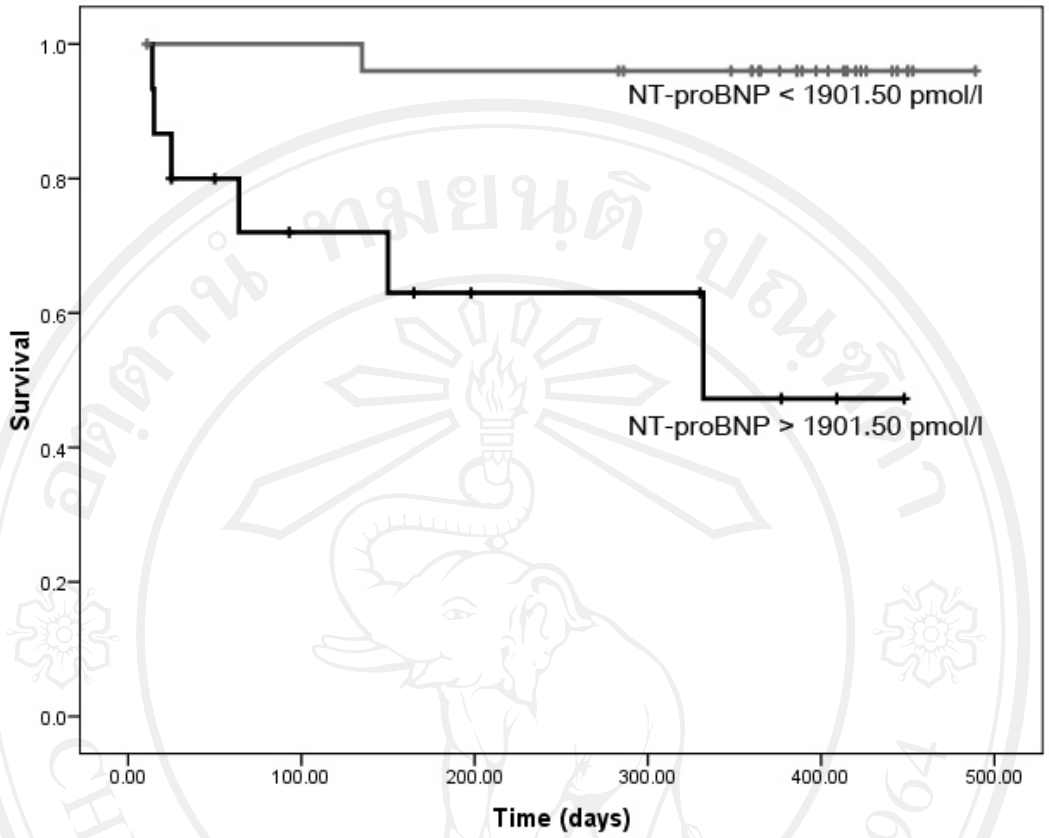

Figure 19 Kaplan-Meier Curve: Time to death related to the cutoff value of NT-proBNP level. NT-proBNP level, measured on the discharge day, higher than the cutoff value was significantly correlated with lesser survival rate (log rank 11.424, mean survival time 286.1 days, 95%Confidence Interval 187.075-385.30 days, p = 0.001).

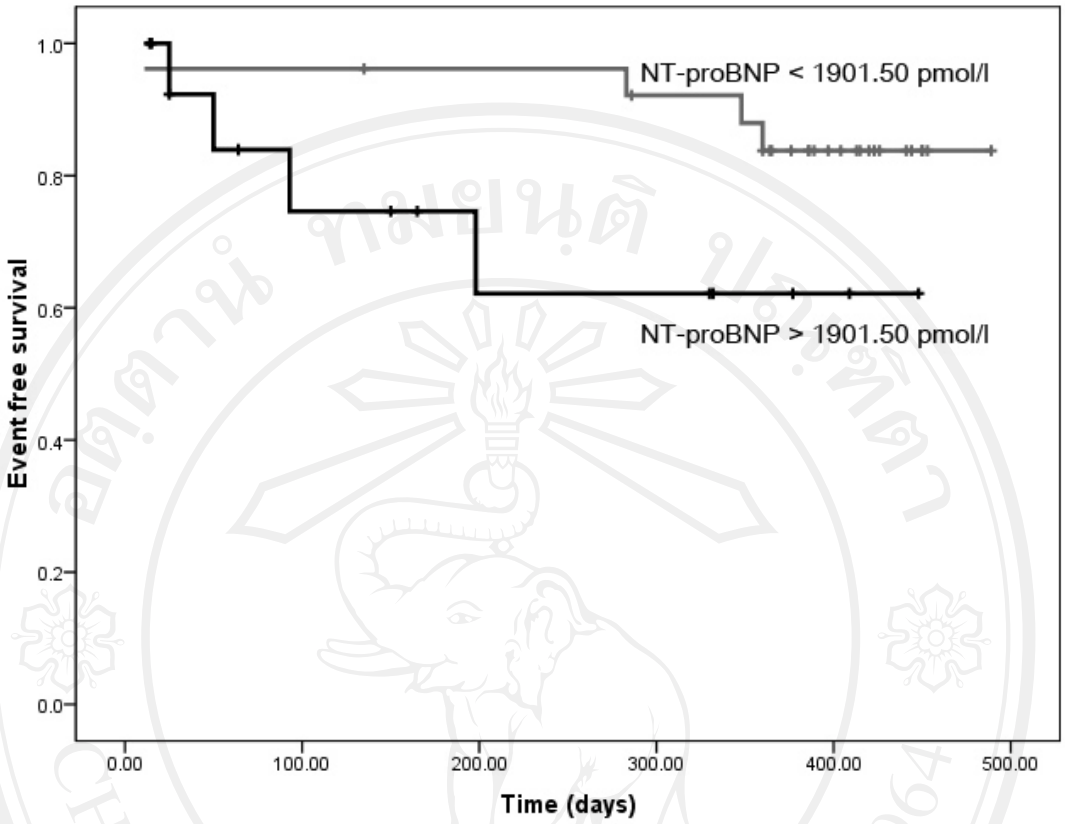

Figure 20 Kaplan-Meier Curve: Time to adverse events related to the cutoff value of NT-proBNP level. NT-proBNP level, measured on the discharge day, higher than the cutoff value was not significantly correlated with adverse events outcome (log rank 3.205, mean survival time 317.8 days, 95%Confidence Interval 214.62-421-13 days, $p = 0.073$).

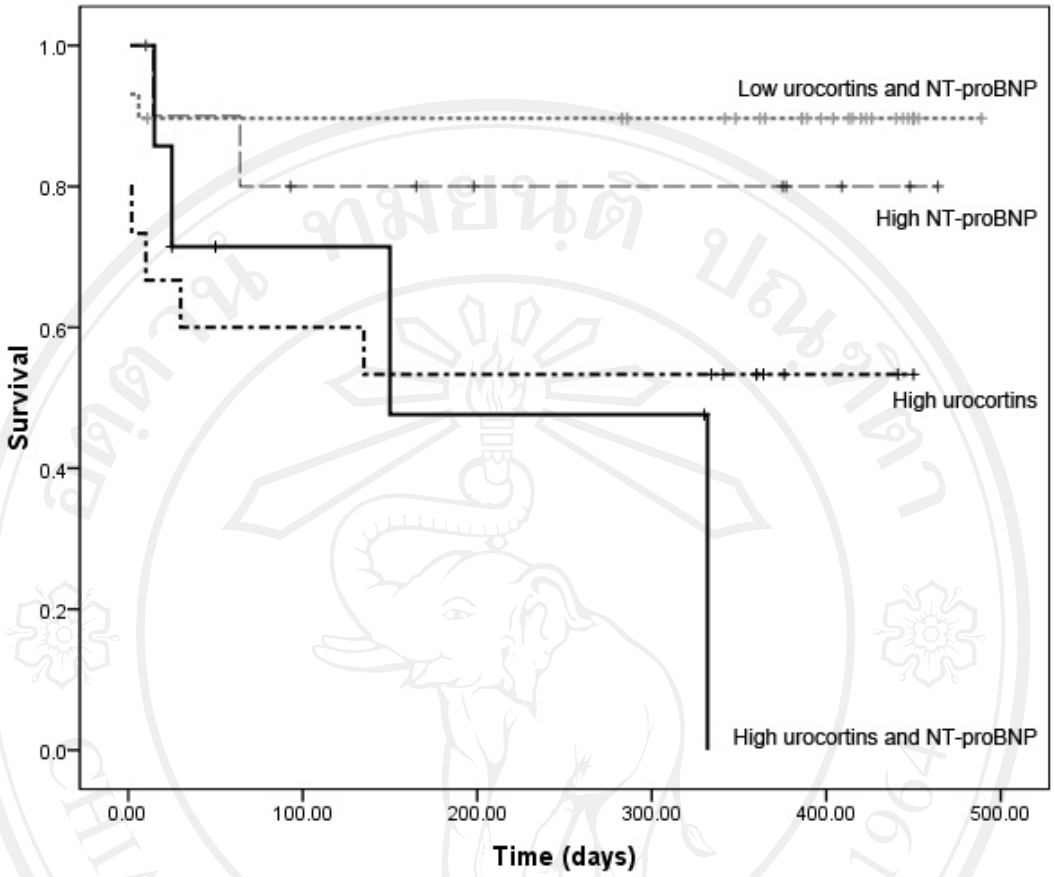

Figure 21 Kaplan-Meier Curve: Time to death related to the cutoff value of urocortins and NT-proBNP level. The less survival rate was significantly correlated with the high levels of both urocortins and NT-proBNP (log rank 10.91, mean survival time 199.5 days, 95% Confidence Interval 73.62-325.42 days, $p = 0.012$).

Table 6 Prediction of mortality with plasma urocortins and NT-proBNP levels.

Performance	**Urocortins**	**NT-proBNP**	**Combined urocortins and NT-proBNP**
Sensitivity	68.75 %	85.71 %	81.25 %
Specificity	73.17 %	73.52 %	56.52 %
Positive predictive value	50.00 %	40.00 %	39.39 %
Negative predictive value	85.70 %	96.15 %	89.65 %