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A combination of increased Rho kinase activity and N-terminal pro-B-type natriuretic peptide predicts worse cardiovascular outcome in patients with acute coronary syndrome

Ming Dong[a], **James K. Liao**[b], **Bryan Yan**[a], **Ruijie Li**[a], **Mang Zhang**[a], and **Cheuk-Man Yu**[a,*]
[a]Division of Cardiology, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, Peoples' Republic of China

[b]Vascular Medicine Research Unit, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States

Abstract

Background—Recent experimental evidence suggests that the Rho/Rho-kinase (ROCK) system may play an important role in the pathogenesis of acute coronary syndrome (ACS) but there are little clinical data. This study examined if ROCK activity is increased in patients with acute coronary syndrome and if ROCK activity predicts long-term cardiovascular event.

Method—Blood samples were collected from 188 patients within 12 h after admission for ACS (53% men; aged 70±13) and from 61 control subject. The main outcome measures were all cause mortality, readmission with ACS or congestive heart failure (CHF) from presentation within around 2 years (mean:14.4±7.2 months; range: 0.5 to 26 months).

Results—ROCK activity increased in ST elevation myocardial infarction (STEMI, n=90) (3.33±0.93), non-STEMI (NSTEMI, n=68) (3.37±1.04) and unstable angina (UA, n=30) (2.53±0.59) groups when compared with disease controls (n=31) (2.06±0.38, all p<0.001) and healthy controls (n=30) (1.54±0.43, all p<0.001). There were 24 deaths, 34 readmissions with ACS and 15 admissions with CHF within 2 years. Patients with a high N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high ROCK activity on admission had a five-fold risk of a cardiovascular event (RR: 5.156; 95% CI: 2.180–12.191) when compared to those with low NT-proBNP and low ROCK activity.

Conclusion—ROCK activity was increased in patients with ACS, particularly in those with myocardial infarction. The combined usage of both ROCK activity and NT-proBNP might identify a subset of ACS patients at particularly high risk.

Keywords

ROCK activity; Acute coronary syndrome; Rho kinase activity

*Corresponding author at: Li Ka Shing Institute of Health and Sciences, & Division of Cardiology, Heart Education and Research Training (HEART) Centre, SH Ho Cardiovascular and Stroke Centre, Department of Medicine and Therapeutics, Prince of Wales Hospital, & Institute of Vascular Medicine, The Chinese University of Hong Kong, Hong Kong. Tel: +852 2632 3127; fax: +852 2645 1699. cmyu@cuhk.edu.hk (C.-M. Yu).

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1. Introduction

The risk of death or new ischemic events varies widely in patients presenting with acute coronary syndromes (ACS) [1,2], probably because the term covers a wide range of patients with varying disease severity including ST elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NTSEMI) and unstable angina (UA). Several factors have been proposed to assess this heterogeneous risk profile among ACS patients including Global Registry of Acute Coronary Events (GRACE) risk score [3] or some cardiac biomarkers. The most widely studied biomarkers are C-reactive protein (CRP), an acute-phase reactant produced by hepatocytes in response to stimulation by inflammatory cytokines and N-terminal pro-B-type natriuretic peptide type (NT-proBNP) which is released in response to increased myocardial stretch secondary to ischemia induced left ventricular systolic and/or diastolic dysfunction [4].

Recently, evidence increasingly suggests that the Rho/Rho-kinase (ROCK) system may play an important role in the pathogenesis of ACS [5]. Preclinical studies showed that inhibition of Rho/ROCK pathway can limit early atherosclerotic plaque development [6], reduce the size of ischemic-reperfusion injury [7] and protect against vasopressin [8]. However, it is uncertain whether ROCK activity is elevated in patients during the acute phase of myocardial ischemia. Therefore, the objective of the present study was to investigate whether ROCK activity is elevated in ACS patients and determine the factors associated with increased ROCK activity in ACS. We also hypothesized that a multi-marker approach with the simultaneous assessment of CRP, NTpro-BNP and ROCK activity would provide complementary information to GRACE risk score in terms of prognosis in a population of patients with the diagnosis of ACS.

2. Methods

2.1. Patients

188 consecutive patients (53% men; aged 70±13 years) admitted to a university teaching hospital for ACS were enrolled between December 2007 and May 2009. ACS was diagnosed based on the ACC/AHA guideline [9]. Patients entered in the registry had to be ≥18 years old and alive at the time of hospital presentation, be admitted for ACS as a presumptive diagnosis, and had to have ≥1 of the following: electrocardiographic changes consistent with ACS, serial increases in serum biochemical markers of cardiac necrosis, and/or documentation of coronary artery disease. The qualifying ACS must not have been precipitated or accompanied by a significant comorbidity, trauma, or surgery. Patients were divided into ST elevation myocardial infarction (STEMI), non-STEMI (NSTEMI) and unstable angina (UA) groups. All the ACS subgroups matched for age, gender, and smoking status. Calculation of the GRACE score was based on clinical history, ECG and laboratory values upon first arrival to the CCU or the acute medical admissions unit [10]. All the patients were followed up for a mean of 14.4±7.2 months; range: 0.5 to 26 months, or until the occurrence of major events (death from any cause, readmission with ACS or admission with congestive heart failure).

2.2. Disease and normal controls

Sixty-one volunteers were subdivided into disease control group (n=31) (59% men; aged 69±8 years) and healthy control group (n=30) (67% men; aged 67±9 years) depending on the presence or absence of hypertension or smoking status which have been proved to influence ROCK activity [11,12]. The criteria for normal controls were: no history of cardiovascular or systemic illness, normal physical examination including blood pressure, as well as normal hemoglucostix and ECG, no echocardiographic evidence of structure or functional heart disease and no need for regular medications. All disease control subjects

proceeded coronary angiography but showed normal epicardial coronary arteries. Written informed consents were obtained from all subjects.

2.3. Analysis of ROCK1, ROCK2 and ROCK activity

Blood samples were collected in the first 12 h after admission. Fasting glucose and lipid were taken in the first morning after admittance. Leukocytes were isolated from 10 mL peripheral blood at the admission following a validated and standardized protocol. NIH 3 T3 cell lysates were used as a positive control and to standardize the results of Western blot analyses from several membranes. Samples were stored at −80 °C and tested together. To avoid overphosphorylation, 1 mM of hydroxyfasudil is added to the fixative solution [13]. The resulting samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and bound proteins were detected by immunoblotting. The samples were analyzed by the rabbit anti-MBS polyclonal antibody (Covance, Princeton, New Jersey), anti-ROCK1 monoclonal antibody, anti-ROCK2 monoclonal antibody (BD Biosciences, San Jose, California), anti-actin monoclonal antibody (Sigma), anti-phospho-specific Ser854-myosin binding subunit (MBS) [13] polyclonal antibody (kindly provided by Prof. Liao JK (Boston, Massachusetts)). Rho kinase activity was expressed as the ratio of phosphorylation levels of myosin binding subunit (pMBS) in each sample per pMBS in each positive control divided by MBS in each sample per MBS in each positive control(ROCK activity=[sample pMBS/positive control pMBS]/[sample total MBS/positive control total MBS]) [13].

2.4. Statistical analysis

All statistical analyses were conducted with the SPSS statistical package for Vista version 15.0 (SPSS Inc., Chicago, Illinois). One-way analysis of variance (ANOVA) was used for comparing of mean values of continuous variables among groups, and post-hoc analysis was performed by Scheffe's test to examine for inter-group differences. Dummy variable was used to adjusted age effect between different groups as healthy control subjects were inevitably younger than other diseased controls. Univariate linear regression models were used to assess the relation between parametric clinical variables and ROCK activity. Multivariate Cox regression analysis using the enter method was used to look at the independent predictors of clinical endpoint. Event rates for clinical outcomes were also determined using the Kaplan–Meier method and compared using the log rank test. Data were expressed as mean±SD and two-sided p value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics

The baseline clinical characteristics and laboratory parameters of the subjects in different groups were summarized in Table 1. There was no significant difference among the ACS groups, disease control and normal control groups for their body mass index, diastolic blood pressure and heart rate. All the ACS groups, the disease control and normal control groups were matched for age, gender, and smoking status. No significant difference of the medications was observed among ACS groups.

3.2. Expression of ROCK activity, ROCK1 and ROCK2 in ACS and control subjects

In all the 3 ACS groups, the ROCK activity (STEMI=3.33±0.93, NSTEMI=3.37±1.04 and UA=2.53±0.59) was significantly higher than the disease control and normal control groups (disease control=2.06±0.38 and normal control=1.54±0.43) (all $p<0.001$). Interestingly, there was no significant difference of ROCK activity between the STEMI and NSTEMI groups, though they were significantly higher than the UA group (both $p<0.001$) (Fig. 1). Similarly, protein levels of ROCK1 and ROCK2 of ACS subgroups were significantly

higher than that of disease control and normal control (all $p<0.001$), while no difference between disease and normal controls (Fig. 2).

3.3. Independent predictors of ROCK activity in ACS subjects

On multivariate analysis, heart failure symptom on presentation ($p=0.002$), LDL-C level ($p=0.001$), and number of diseased coronary vessels ($p=0.048$) were independent predictors of the ROCK activity in ACS patients (Table 2).

3.4. Univariate and multivariate predictors of clinical endpoint

Totally, there were 24 deaths, 34 readmissions with ACS and 15 admissions with CHF within 2 years. Table 3 shows the univariate correlation between different clinical variables and long-term cardiovascular endpoint including the medications on ACS patients at discharge. Variables with the correlation of clinical endpoint less than 0.05 were chosen into the multivariate model. PCI treatment in admission (HR: 0.312; 95% CI: 0.121–0.554), ROCK activity (HR: 1.402; 95%CI: 1.010–1.908), NT-proBNP (per 200 pg/ml increased) (HR: 1.003; 95%CI: 1.001–1.005) and statins usage after discharge (HR: 0.483; 95%CI: 0.211–0.846) were independent predictors for long-term clinical endpoint (Table 4).

3.5. ROCK activity and NT-proBNP as a composite measure

1,986 pg/ml and 3.03 (best cut-off value for long-term event) was used as a cut-off value of NT-proBNP and ROCK activity in our study population. As demonstrated by the Kaplan–Meier survival curves (Fig. 3), patients with a high NT-proBNP-high ROCK activity on admission were approximately five times more likely to experience a cardio-vascular event at around two years (RR: 5.156; 95% CI: 2.180–12.191) compared to those with low NT-proBNP and low ROCK activity. In addition, patients with high NT-proBNP-high ROCK activity were also more likely to die or experience a cardiovascular event at two years compared to those with high NT-proBNP-low ROCK activity (RR: 2.624; 95% CI: 1.035–6.651) (Fig. 3). From the incremental statistics results, combination of ROCK activity and NT-proBNP (Log rank $X^2=40.3$) significantly predicted long-term outcome more accurately when comparing with NT-proBNP alone (Log rank $X^2=36.1$) ($p=0.022$).

4. Discussion

Our study demonstrated that peripheral leukocyte ROCK activity was increased in ACS compared to normal or at-risk subjects, especially in those with elevated cTnT ≥0.1 μg/l. In addition, heart failure symptom on admission, LDL-C level and number of diseased coronary vessels were independent predictors for increased ROCK activity in ACS. We also defined the utility of the combined measurement of baseline ROCK activity and NT-proBNP as biomarkers to predict adverse events.

4.1. ROCK activity across spectrum of coronary artery disease

Elevated ROCK activity has been shown to be involved in many cardiovascular diseases [5]. In addition, Feska demonstrated that ROCK activity was also increased in acute stroke patients, suggesting its pathogenesis role in acute ischemia [14]. Our findings corroborate and extend the above by demonstrating that there is a gradation of ROCK activity from normal in the healthy control, mildly elevated in at-risk group without ACS to those with unstable angina and myocardial infarction. Interestingly, there was no significant difference in ROCK activity between STEMI and NSTEMI according to differing peak TnT levels which represents the degree of myocardial injury. A previous study has found that ROCK-2 phosphororylated the Tn complex, most likely at cTnT, which means that the Rho/ROCK pathway is involved during the whole process of myocardial injury [15]. We could not demonstrate a difference of ROCK activity between STEMI and NSTEMI, probably

because ROCK activation is nonspecific, and occurs in many thrombotic, inflammatory, and malignant neoplastic disorders, including acute stroke [14]. ROCK activity increase might be due to the atherosclerosis burden and degrees of inflammation.

One of our novel findings was that LDL-cholesterol and severity of coronary artery disease were two independent predictors for ROCK activity in the ACS patients. Atherosclerosis is a progressive disease characterized by the accumulation of lipid, such as the increase of LDL-cholesterol. Inflammation plays a key role during this whole pathological process. In this study, triple-vessel disease may represent more severe underlying atherosclerosis and inflammation. Evidence indicates that ROCK-mediated pathway is involved at all stages of the inflammatory process. Activated ROCK down-regulates eNOS [16], whereas ROCK inhibition by hydroxylfasudil rapidly increases endothelial eNOS activity [17]. Nitric oxide itself antagonizes the vasoconstrictor effect of ROCK through activation of myosin phosphatase [18]. ROCK activation leads to endothelial hyperpermeability and hence enhances atherosclerosis [19]. Furthermore, ROCK1 plays a key role in macrophage chemotaxis, cholesterol uptake and foam cell formation, all of which are hallmark events in the pathogenesis of atherosclerosis [20]. ROCK1 also mediates neointimal proliferation via recruitment of circulating leukocytes and infiltration of inflammatory cells into the vessel wall [21]. In a low-density lipoprotein (LCL) receptor knockout mice model, activation of the transcription factor NF-κB via Rho/ROCK pathway was enhanced after a high-fat cholate-free diet while inhibition of ROCK significantly was associated with suppression of early atherosclerotic plaque development [6]. In this study, triple-vessel disease may represent more severe underlying atherosclerosis and inflammation. Furthermore, long-term inhibition of ROCK has been shown to cause marked regression of coronary arteriosclerosis in a pig model [22]. Rho/ROCK signaling inhibition by HMG-CoA reductase inhibitors (statins) offers a potential mechanism for some of the pleotropic effects of these agents [23]. Importantly, Nohira et al. (2008) were first to demonstrate that statins inhibit ROCKs activity and improve endothelial function in patients with stable atherosclerosis [24].

In the study, protein levels of ROCK1 and ROCK2 were significantly higher in ACS groups than that of disease and normal controls. Similar observations were found in ischemia/reperfusion injury animal model [25]. However, no obvious increase of ROCK1 and ROCK2 was found between disease and normal control, which was similar in previous studies [26,27]. Thus the increase of ROCK activity might be due to the elevated ROCK itself as well as the rise of protein level. Severe inflammation during atherosclerosis will cause many leukocytes recruitment and endothelial cell injury. But it is relatively mild and stable in subjects with only smoking habit or hypertension. The increase of ROCK protein level and ROCK activity are probably more from the results although current study could not clarify it.

Interestingly in this study, ACS patients with heart failure symptom had higher ROCK activity than those without heart failure. Recently, many cellular and molecular biology studies have proved an involvement of RhoA/ROCK signaling pathway in many aspects of cardiovascular functions such as cardiac hypertrophy and ventricular remodeling after myocardial infarction [28]. Sauzeau et al. reported that human urotensin II-induced VSMC proliferation was inhibited by a ROCK inhibitor, suggesting that RhoA and ROCK mediate the stimulation of VSMC growth [29]. In the adult rat myocardium, pressure overload induces a rapid activation of ROCK, suggesting that it could play a critical role in the coordination of initial mechanisms and adaptive changes triggered by mechanical stress in cardiac myocytes [30]. Recent genetic studies by Wei L's laboratory and others support the concept that ROCK1 and ROCK2 have distinct non-redundant functions in cardiac hypertrophy and remodeling [31]. ROCK1 deletion did not impair compensatory hypertrophic response, but significantly reduced cardiomyocyte apoptosis and fibrosis in

response to pressure overload induced by transverse aortic constriction [32]. A latest study provided the long-term beneficial effects of ROCK1 deficiency in hypertrophic decompensation and suggested that ROCK1 may be an attractive therapeutic target to limit heart failure progression [33].

4.2. Combination of NT-proBNP and ROCK activity can identify a subset of ACS patients at particularly high risk

In this study, the GRACE score and CRP levels were also examined. The GRACE score significantly correlated with long-term events but it was not an independent predictor. This might be because our sample size was small and the follow-up period was more than 2 years. In our cohort, in-hospital PCI was also independent predictors and strongly correlated with the future outcome. It appears to be better than the GRACE score in predicting long-term outcomes. In this study, clopidogrel was routinely used for with PCI. On the other hand, among these ACS patients, the usage of statins after discharge had a greater protection against death or major cardiovascular events, which is consistent with previous studies [34]. The more severely ill ACS subjects often get better treatment in hospital. This might be why in this study, PCI treatment and statins usage were independent predictors. However, the predictive value of GRACE score may be increased when combined with other biomarkers, such as NT-proBNP [35]. It is probable that biomarkers might be more useful than the GRACE score alone for predicting long-term outcome. Similarly, no correlation between CRP and long-term outcome was found. This might also due to the small sample size as previously this kind of acute response marker has been shown to be a strong independent predictor of future cardiovascular events [36]. CRP also correlates with the number of angiographically complex coronary artery stenoses. Similar results were found in this study between ROCK activity and the severity of coronary arterial disease. Probably, this indicates that ROCK activity might be a stronger predictor than CRP for future cardiovascular events.

Our study showed that both ROCK activity and NT-proBNP are independent predictors for future cardiovascular events but not elevated cardiac biomarkers (cTnT and CPK). Congestive heart failure (CHF) is a common complication in patients with an acute coronary syndrome [37]. Patients with unstable angina who present with CHF have 4-fold higher hospital death rates than those without CHF at admission [38]. NT-proBNP level could be used to detect the presence of heart failure on admission. Clinical studies have clearly demonstrated that NT-proBNP levels are increased after episodes of ischemia: elevated NT-proBNP level has been observed in patients with unstable angina [39] and during and after percutaneous coronary intervention. Thus, NT-proBNP reflects ischemia as well as haemodynamics and the magnitude and duration of the increase in plasma concentrations of NT-proBNP after ACS are proportional to myocardial infarct size and the degree of left ventricular dysfunction (although of course ischemia impairs LV function). Additionally, elevated ROCK activation is involved in the pathogenesis of not only atherosclerosis but also inflammation and the contraction of the smooth muscle cells, which plays an important role in the pathogenesis of ACS. Thus, ROCK activity could represent the severity of ACS, as well as number of actively diseased coronary arteries. Knowing more about how ROCK is involved in the disease processes of ACS may help to understand the underlying mechanism and guide the treatment and improve the prognosis of ACS.

Finally, in the current study, we demonstrated that ROCK activity increased in ACS patients and proved that ROCK activity might be a good biomarker to predict long-term event. ROCK activity plus NT-proBNP can identify risk better than NT-proBNP alone in the majority of ACS patients. This is especially true for the ACS patients who have high NT-proBNP but low ROCK activity.

5. Limitations

The main limitation of this study is the relatively small number of patients and a moderately short duration of follow-up. Usually for hazard ratios of 2, a much sample size of patients and events should be needed. Comparison with other biomarkers such as myeloperoxidase and lipocalin would also be interesting.

6. Conclusions

ACS is associated with increased ROCK activity. In addition, both ROCK activity and NT-proBNP can be used synergistically to identify a subset of ACS patients who are at a particularly high risk of future cardiovascular events.

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Abbreviations

ACS	Acute coronary syndrome
cTnT	Cardiac troponin T
ANOVA	One-way analysis of variance
BH	Body height
BMI	Body mass index
BW	Body weight
CHD	Coronary heart disease
CHF	Congestive heart failure
CIHD	Coronary ischemic heart disease
CPK	Creatine phosphokinase
CRP	C-reactive protein
CVA	Cardio vascular accidents
DBP	Diastolic blood pressure
DM	Diabetes mellitus
eGFR	Estimated Glomerular Filtration Rate
GRACE	Global Registry of Acute Coronary Events
HDL-C	High-density lipoprotein cholesterol
HR	Heart rate
LDL-C	Low-density lipoprotein cholesterol
LVEF	Left ventricular ejection fraction

NSTEMI	Non-ST elevation myocardial infarction
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PCI	Percutaneous coronary intervention
ROCK	Rho kinase
SBP	Systolic blood pressure
STEMI	ST elevation myocardial infarction
TC	Total cholesterol
TG	Triglycerides
WBC	White blood cells
UA	Unstable angina

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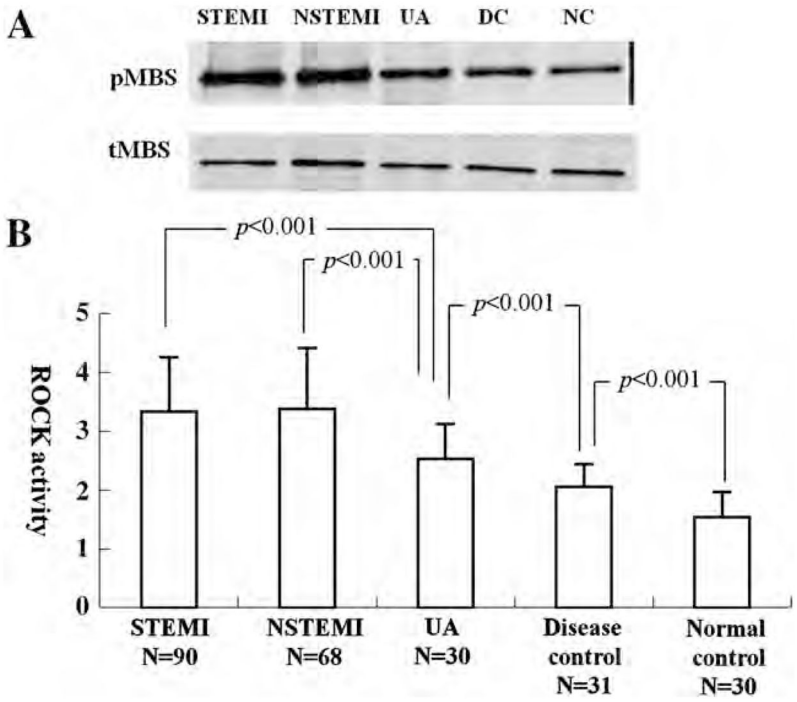

Fig. 1.
Comparison of ROCK activity in different ACS groups, disease and normal control groups.

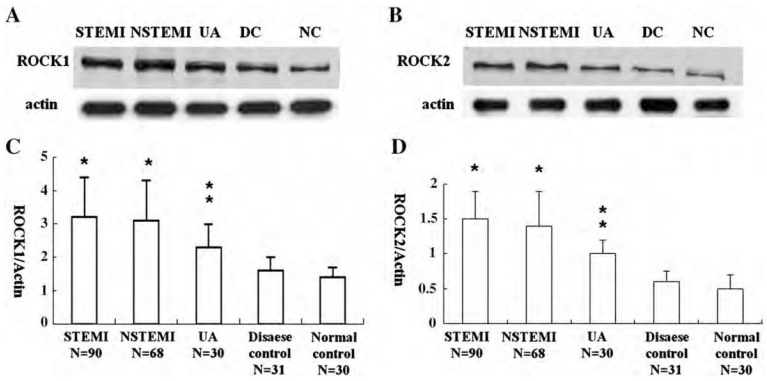

Fig. 2.

Expression of ROCK1 and ROCK2 protein levels in different groups. ROCK1/actin and ROCK2/actin in STEMI and NSTEMI were significantly higher than that in UA, disease and normal controls (all $p<0.001$). ROCK1/actin and ROCK2/actin in UA were significantly higher than that in disease and normal controls (all $p<0.001$). However, no significant difference of ROCK1 and ROCK2 between STEMI and NSTEMI, similarly between disease and normal controls.

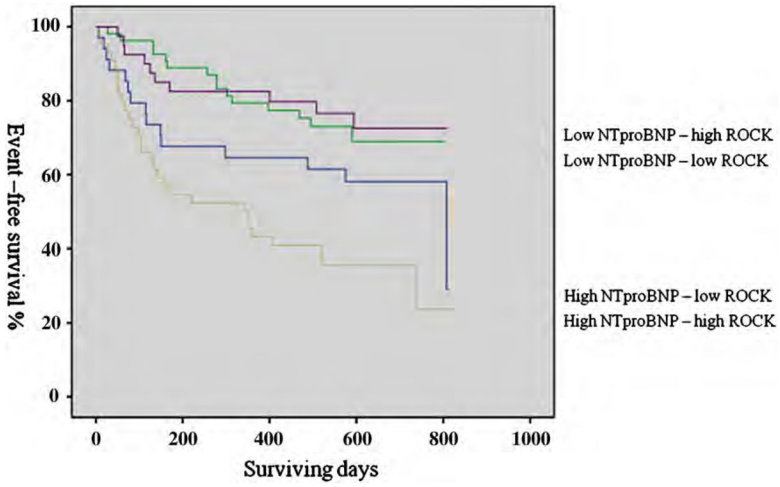

Fig. 3.

Kaplan–Meier survival curves for cardiovascular events. High NT-proBNP defined as 1986 pg/ml (sensitivity is 63.8% and specificity is 67.3%) and high ROCK activity defined as 3.03 (sensitivity is 56.5% and specificity is 56.7%). High NT-proBNP-high ROCK activity group has higher risk (RR: 5.156; 95% CI: 2.180 to 12.191) to low NT-proBNP-low ROCK activity group. Similarly, high NT-proBNP-high ROCK activity group has higher risk (RR: 2.624; 95% CI: 1.035 to 6.651) to high NT-proBNP-low ROCK activity group. Log rank=20, $p<0.001$.

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Table 1

Baseline Characteristics of acute coronary syndrome and control subjects.

Baseline characteristic	STEMI (*n*=90)	NSTEMI (*n*=68)	UA (*n*=30)	Disease control (*n*=31)	Normal control (*n*=30)	ANOVA *p*
Age(yrs)	69±13	72±10	72±10	69±8	67±9	NA
Gender (male)	46(51%)	38 (56%)	15 (50%)	16 (52%)	20 (67%)	NA
Current smoker	18 (20%)	9 (11%)	5 (17%)	4 (13%)	0	NA
Medications						
Hypertension	36 (40%)	39 (48%)	16 (53%)	17 (81%)	0	NA
DM	19 (21%)	20 (25%)	9 (30%)	0	0	NA
Hyperlipidemia	12 (13%)	10 (12%)	6 (22%)	0	0	NA
CVA	6 (7%)	3 (4%)	3 (10%)	0	0	NA
Chronic renal failure	7 (8%)	3 (4%)	2 (7%)	0	0	NA
LVEF (%)	46.5±9.6§	50.2±12.0	58.0±10.0	64.2±4.1	68.5±3.5	<0.001
SBP (mmHg)	135±29	147±29	151±30	143±20	129±22$^{//}$	0.007
DBP (mmHg)	76±18	77±18	77±15	82±10	77±13	0.857
HR (/minute)	80±21	87±29	78±19	69±13	87±11	0.080
BMI (kg/m^2)	23.9±3.4	24.2±3.6	25.8±2.9	25.3±8.5	23.6±3.2	0.629
Heart failure symptom on presentation	11 (14%)	21 (46%)	4 (15%)	NA	NA	NA
Laboratory test						
Fasting glucose	7.6±1.9§	6.6±1.7	5.8±0.9	5.2±0.8	5.2±0.3	<0.001
TC (mmol/l)	4.9±1.1	4.7±1.2	4.2±0.9	4.8±0.8	5.1±0.5	0.050
LDL-C (mmol/l)	2.8±1.0	2.9±1.0	2.4±0.8	2.9±1.0	3.0±0.3	0.119
TG (mmol/l)	1.7±1.3	1.6±1.0	1.6±0.8	1.7±1.9	1.3±0.2	0.317
HDL-C (mmol/l)	1.3±0.5	1.2±0.4	1.2±0.3	1.4±0.4	1.5±0.6	0.051
WBC (10^9/l)	13.1±3.8$^{\#}$	11.5±4.4§	8.6±3.3	8.4±3.7	5.5±1.8	<0.001
Creatinine (μmol/l)	154±187	155±97	183±184	91±13**	71±19**	0.031
eGFR	67.2±31.3	57.4±26.5	53.4±25.3	79.8±2.5**	82.8±4.1**	0.023
Peak cTnT (μg/l)	3.80±5.77†	0.76±1.12	0.023±0.03	NA	NA	<0.001
Peak CPK (U/l)	2392.7±2026.0*	658.0±1397.0	110.7±80.1	127.3±62.9	NA	<0.001

*p<0.05 vs NSTEMI, UA & Disease Control.

† $p<0.05$ vs NSTEMI & UA.

‡ $p<0.05$ vs ETEMI & Disease Control.

§ $p<0.05$ vs UA, Disease Control & Normal Control.

// $p<0.05$ vs NSTEMI.

# $p<0.05$ vs NSTEMI, UA, Disease control & Normal Control.

** $p<0.05$ vs STEMI, NSTEMI & UA.

All results are presented as mean±SD.

Table 2

Prediction of ROCK activity in univariate and multivariate regression models.

Variables	Univariate		Multivariate (stepwise)	
	Coefficient	*p*	â	*p*
Age (yrs)	0.285	<0.001	–	–
Gender (male)	0.056	0.410	–	–
Current smoker	0.143	0.050	–	–
Heart failure symptom on presentation	0.211	0.010	0.287	0.002
LVEF (%)	−0.387	<0.001	–	–
cTnT (μg/l) (peak)	0.403	<0.001	–	–
CPK (U/l) (peak)	0.301	<0.001	–	–
Creatinine (μmol/l)	0.260	<0.001	–	–
eGFR (60 ml/min/1.73 m^2)	−0.209	0.010	–	–
Fasting Glucose	0.164	0.040	–	–
WBC ($\times 10^9$/l)	0.375	<0.001	–	–
LDL-C (mmol/L)	0.270	0.001	0.315	0.001
TC (mmol/l)	0.082	0.250	–	–
CRP	0.277	<0.001	–	–
NT-proBNP	0.296	<0.001	–	–
Number of diseased coronary vessels	0.383	<0.001	0.227	0.048
Current usage of Statin	−0.118	0.137	–	–

Table 3

Baseline clinical characteristics (including medications at discharge) between no event and event groups.

Characteristic	No event (*n*=104)	Event (*n*=69)	ANOVA *p*
Age(yrs)	65±12	73±13	<0.001
Gender(male)	73 (78%)	37 (54%)	0.020
Current Smoker	24 (23%)	12 (17%)	0.406
Heart failure on admission	8 (8%)	27 (39%)	<0.001
PCI done during hospitalization	75 (72%)	20 (29%)	<0.001
Medical history			
Hypertension	59 (57%)	38 (55%)	0.578
DM	20 (19%)	29 (42%)	0.001
Hyperlipidemia	17 (16%)	13 (19%)	0.393
CIHD	14 (13%)	20 (29%)	0.011
Clinic Test			
LVEF (%)	51±10	47±13	0.028
SBP (mmHg)	145±27	142±32	0.868
DBP (mmHg)	80±18	75±17	0.045
HR (/minute)	83±24	82±24	0.864
BMI	24±3	24±4	0.737
Laboratory test			
Fasting glucose (mmol/l)	6.8±1.7	6.8±2.0	0.893
TC (mmol/l)	4.8±1.2	4.5±1.1	0.018
LDL-C (mmol/l)	3.0±1.0	2.5±0.9	0.004
TG (mmol/l)	1.7±1.2	1.7±1.0	0.998
HDL-C (mmol/l)	1.3±0.4	1.2±0.4	0.493
WBC (109/l)	11.8±4.3	11.6±4.2	0.768
Creatinine (umol/l)	111±46	218±202	<0.001
eGFR (ml/min/1.73 m^2)	61±21	46±32	<0.001
TnT (peak) (μg/l)	1.7±2.9	2.5±5.6	0.440
Creatine phoshokinase (peak) (U/l)	1721±2049	830±1485	0.002
ROCK activity	3.07±0.83	3.49±1.17	0.011
NT-proBNP	3169±7479	15749±31329	<0.001
CRP	26.2±36.7	37.9±49.2	0.074
GRACE score 6 month predictor	0.29±0.15	0.34±0.16	0.026
Medications at discharge			
Aspirin	100 (96.7%)	62 (90.4%)	0.110
Clopidogrel	70 (67.4%)	15 (21.2%)	<0.001
Statins	88 (84.6%)	40 (57.7%)	<0.001
Beta blockers	68 (65.2%)	36 (51.9%)	0.117
Calcium channel blockers	7 (6.5%)	19 (26.9%)	0.001
ACEI	56 (54.3%)	29 (42.3%)	0.165

Characteristic	No event (*n*=104)	Event (*n*=69)	ANOVA *p*
Diuretics	11 (10.4)	24 (34.7%)	0.001
Regular Nitrates	16 (15.2%)	27 (38.5%)	0.002

Table 4

Univariate and multivariate analyses of clinical endpoints.

	Variables		Multivariate (enter)		
	Coefficient	***p***	**HR**	**95%CI**	***p* value**
Age(yrs)	0.385	<0.001	–	–	–
Gender (male)	−0.169	0.020	–	–	–
Current Smoker	0.437	0.406	–	–	–
Heart failure on admission	0.478	<0.001	–	–	–
Medications					
Hypertension	0.381	0.578	–	–	–
DM	0.248	0.001	–	–	–
Hyperlipidemia	0.318	0.393	–	–	–
CVA	0.192	0.154	–	–	–
Chronic renal failure	0.512	0.010	–	–	–
CIHD past medical history	0.191	0.011	–	–	–
LVEF (%)	−0.177	0.028	–	–	–
SBP (mmHg)	0.142	0.868	–	–	–
DBP (mmHg)	0.213	0.045	–	–	–
HR (/minute)	0.261	0.864	–	–	–
BMI (kg/m^2)	0.314	0.737	–	–	–
Laboratory test					
Fasting glucose	0.131	0.893	–	–	–
TC (mmol/l)	0.251	0.018	–	–	–
LDL-C (mmol/l)	0.352	0.004	–	–	–
TG (mmol/l)	0.106	0.998	–	–	–
HDL-C (mmol/l)	−0.267	0.493	–	–	–
WBC (109/l)	0.412	0.768	–	–	–
Creatinine (μmol/l)	0.576	<0.001	–	–	–
eGFR (ml/min/1.73 m^2)	0.561	<0.001	–	–	–
Peak cTnT (μg/l)	0.302	0.44	–	–	–
Peak CPK (U/l)	0.471	0.002	–	–	–

	Variables		Multivariate (enter)		
	Coefficient	***p***	**HR**	**95%CI**	***p* value**
CRP	0.361	0.074	–	–	–
Baseline ROCK activity	0.208	0.011	1.402	1.010–1.908	0.039
NT-proBNP (per 200 pg/ml increased)	0.312	<0.001	1.003	1.001–1.005	0.032
PCI treatment in admission	−0.42	<0.001	0.312	0.121–0.554	0.001
GRACE score	0.19	0.026	–	–	–
Medications at discharge					
Aspirin	−0.114	0.110	–	–	–
Clopidogrel	0.215	<0.001	–	–	–
Statins	−0.321	<0.001	0.483	0.211–0.846	0.017
Beta blockers	−0.387	0.117	–	–	–
Calcium channel blockers	−0.219	0.001	–	–	–
ACEI	0.107	0.165	–	–	–
Diuretics	−0.351	0.001	–	–	–
Regular Nitrates	0.241	0.002	–	–	–