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Original Article

Human recombinant-B-type natriuretic peptide protect ventricular function and structure in ST-elevation myocardial infarction

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Abstract: Background: ST-elevation myocardial infarction (STEMI) is the most serious clinical type of coronary artery disease (CAD), which will lead to a loss of contractile function asa result of adverse left ventricular (LV) remodeling. Post-myocardial infarction remodeling is detrimental to the left ventricular function, which is strongly related to clinical outcome,including heart failure and cardiac death. And our study was designed toassess the efficacy of 72-hour IV infusion of rh-BNP therapy in STEMI patients with or without successful primary PCI, in preventing adverse LV remodeling and preserving LV function. Methods: 100 patients diagnosed as STEMI combined with acute heart failure (Killip classification ≥2) were recorded. And they were dividedinto "rh-BNPtreatment group" (n=50) and "control group" (n=50). In addition to conventional heart failure therapy, patients in the rh-BNP group receivedrh-BNPinfusion for 72 hours. All patients were followed up at 3 month after discharge. Their medical history was taken, as well as the presence or absence of relevant symptoms. 6-minute walking test, as well as echocardiographic indexes were recorded to evaluate the improvement of cardiac function. Results: The data analysis about demographic comparison, including those related complicated diseases among groups showed no significant difference. After the follow-up, the indicators were all better than baseline among four subgroups (all P<0.001). Results showed that rh-BNP was able to significantly reduce the NT-pro BNP levels (P<0.001), decrease LVESD (P<0.01), and increase LVEF (P<0.05). The difference of 6WMT between two groups was significant (P<0.001). According to the classification of 6WMT, the multivariate Cox regression showed that the usage of rh-BNP was an independent predictor for 6WMT (OR 0.478, 95% CI, 0.290-0.787), while it may not for MACE (OR 1.762, 95% CI, 0.793-3.913). Conclusions: Although the use of rh-BNP was not an independent risk factor in prediction of MACE in our study, the current data still showed that rh-BNP is a useful prognosis factor of 6WMT in the STEMI patients. The protection of ventricular function and structure in STEMI patients is affirmative.

Keywords: Human recombinant BNP, left ventricular function, ST-elevation myocardial infarction, acute heart failure

Introduction

ST-elevation myocardial infarction (STEMI) is the most serious clinical type of coronary artery disease, which is characterized by ST-segment elevation in relevant leads on electrocardiogram. STEMI now has been a major public health problem for its high morbidity and mortality following the formation of the aging society in recent years [1]. Because of the formation and rupture of vulnerable coronary atherosclerotic plaques, the thrombosis and occlusion of coronary arteries occur. The occlusion will lead to a loss of contractile function asa result of adverse left ventricular (LV) remodeling whichincludes LV hypertrophy, dilatation, myocyte necrosis andapoptosis, collagen deposition, and fibrosiss [2, 3]. Post-myocardial infarction remodeling is detrimental to the left ventricular function, which is strongly related to clinical outcome,including heart failure and cardiac death [4]. With the development and popularity of primary percutaneous coronary intervention (PCI), the influence of remodeling has been weakened greatly. However, a significant percentage of STEMI patients still suffer from the decrease of left ventricular function. And the expected mortality and progression to heart

failure (HF) remains high. Considerable efforts have been made to reduce adverse remodeling with beneficialpharmacologic therapies, and some novel therapies like cells,devices, small molecule and peptides [5-7].

B-type natriuretic peptide (BNP) is a small endogenouscardiac peptide that possesses beneficial pleuripotent properties which may protect the heart from injury and preventunfavorable LV from remodeling after AMI [8]. Studies have shown that BNP generates the second messenger 3', 5'-cyclic guanosine monophosphateleading to a reduction inmyocardial oxygen consumption [9], enhancement of myocardial relaxation,retardation of adrenergic activation [10], induction of vascular regenerationand inhibition of cardiac fibroblast collagen synthesis and proliferation, with suppression of cardiomyocyte growth [11, 12]. In 2001, the United States Food and Drug Administration, respectively, approved human recombinant BNP (rh-BNP), for the management of acute decompensated HF.

Hillock and co-workers recently reported findings of a mini study defining the neurohumoral, renal and myocardial responses to 60 h of BNP infusion inhuman acute myocardial infarction with delayed or failed reperfusion and moderate left ventricular dysfunction [13]. This key study demonstrated that BNP used at conventional clinical doses was safe withtrends towards favourable ventricular remodeling. More and more evidences suggest that the use of rh-BNP has beneficial properties in humans with cardiorenal disease. And our study was designed toassess the efficacy of 72-hour IV infusion of rh-BNPtherapy in STEMI patients with or without successful primary PCI, in preventing adverse LV remodeling andpreserving LV function.

Methods

Data source and patient population

A total of 100 patients diagnosed as STEMI combined with acute heart failure (Killip classification ≥2) in our department were recorded in this study. According to the use of rh-BNP, two groups were divided: rh-BNPtreatment group (n=50) and control group (n=50). Then each group was divided into another two subgroups based on the time of PCI: emergency PCI and delayed PCI. Data on the level of N-terminal pro-B-type natriuretic peptide (NT-pro BNP), echocardiographic findings were collected, including left ventricular ejection fraction (LVEF), left ventricular end systolic diameter (LVESD), left ventricular end diastolic systolic diameter (LVEDD). At the end of follow-up, patients accepted the 6-minute walkingtest (6WMT) to evaluate their cardiac function. The demographics, including risk factors for CAD (age, gender, hypertension, smoking, diabetes mellitus) were documented. Medications including antiplatelet drugs, statins, calcium channel blocker (CCB), diuretics, especially β-blockers, angiotensin converting enzyme inhibitors (ACEI) and angiotensin II receptor antagonists (ARB) were also noted.

Patients who had a confirmed diagnosis of STEMI were eligible to participate in this study if 1) the age ranges from 18 to 80 years old; 2) the patients were able to understand the study content and provide consent; 3) the patients were willing to accept the necessary follow-up, therapy and laboratory examination. The exclusion criteria included 1) patients with cardiogenic shock: systolic blood pressure cessar Hg for more than 30 min with signs of low peripheral perfusion such as cyanosis or cold extremities; 2) patients with renal failure, severe liver disease; 3) patientswith a life expectancy of ≤12 months; 4) pregnant and lactating women; 5) the patients who were unable to understand the study content, or provide consent; 6) the patients who participate in other study program in the meantime. The diagnosis of STEMI was defined according to World Health Organization definition of myocardial infarction (2008-09 revision) [14]. The criteria includes: persistent chest pain of more than 30 minutes, ST elevation on two or more adjacent leadson body surface ECG and elevation of cardiac troponin T (cTnT) and creatine kinase (CK)-MB.

Treatments

In addition to conventional heart failure therapy, patients in the rh-BNP group receivedrh-BNP (marketed as Xinhuosu, Chengdu Rhodiola Pharmaceutical Holding Co., Chengdu, China) infusion for 72 hours. Rh-BNPwas administered as an intravenous bolus of 1.5 μg/kg followed by continuous infusion in doses of 0.0075-0.01 μg/kg/min according to each patient's clinicalstatus. According to their heart rates and blood

Table 1. The baseline characteristics

	Rh-BNP group		Control group	
	Emergency PCI (n=25)	Delayed PCI (n=25)	Emergency PCI (n=25)	Delayed PCI (n=25)
Sex (M/F)	12/13	14/11	13/12	14/11
Age (years)	61.68 years	67.44 yea 81	64.28 yea 81	64.08 yea 891
HBP (%, n)	68.0 (17)	64.0 (16)	60.0 (15)	48.0 (12)
Diabetes mellitus (%, n)	56.0 (14)	60.0 (15)	52.0 (13)	52.0 (13)
Smoking (%, n)	56.0 (14)	68.0 (17)	60.0 (15)	52.0 (13)
ACEI/ARB (%, n)	76.0 (19)	80.0 (20)	68.0 (17)	72.0 (18)
β-blocker (%, n)	76.0 (19)	72.0 (18)	80.0 (20)	76.0 (19)

Abbreviations: HBP: hypertension; ACEI: Angiotensin-Converting Enzyme Inhibitors; ARB: Angiotensin Receptor Blocker.

Table 2. The effect of rh-BNP

		Rh-BNP group		Control group	
		Emergency PCI	Delayed PCI	Emergency PCI	Delayed PCI
Baseline	Classification (II/III/IV)	9/7/9	9/9/7	8/10/7	7/13/5
	LVEF (%)	39.04±5.53	39.00±6.23	38.88±6.02	39.68±6.15
	LVESD (mm)	29.52±4.68	29.84±4.51	30.72±4.29	30.64±3.71
	LVEDD (mm)	46.48±4.67	48.08±4.71	47.40±4.32	46.20±4.82
	NT-pro BNP (pg/ml)	4839±656	5060±602	4988±625	5013±613
3 month	Classification (I/II/III)	17/7/1	12/11/2	11/9/5	7/9/9*
	LVEF (%)	48.88±4.26	46.40±5.25*	46.08±5.05$^{*,\#}$	44.80±5.25$^{*,\#}$
	LVESD (mm)	25.08±4.73	26.40±4.41**	27.96±3.92$^{**,\#\#\#}$	29.36±3.71$^{**,\#\#\#}$
	LVEDD (mm)	41.40±4.56	44.44±3.95*	44.12±4.32*	44.92±5.06*
	NT-pro BNP (pg/ml)	1332±144	1578±140***	1758±147$^{***,\#\#\#}$	1877±137$^{***,\#\#\#}$
	6MWT (meters)	511.6±23.2	489.4±21.3***	470.3±26.7$^{***,\#\#\#}$	460.0±34.4$^{***,\#\#\#}$
	MACE (n)	(4.0%) 1	(16.0%) 4	(12.0) 3	(16.0%) 4

*: P<0.05; **: P<0.01; ***: P≤0.01; ***: effect of rmergency PCI subgroup in rh-BNP group #: P<0.05; ##: P<0.01; ###: P≤0.001; compared with rh-BNP group. Abbreviations: LVEF: left ventricular ejection fraction; LVESD: Left Ventricular End Systolic Diameter; LVEDD: Left Ventricular End Diastolic Diameter; 6WMT: 6-minute walk test; MACE: Major Adverse Cardiovascular Events.

pressures, patients in all the 4 subgroups received ACEI/ARBs and β-blockers to suppress myocardial remodeling and reduced myocardial oxygen consumption.

Follow-up and data source

All patients were followed up at 3 month after discharge. Their medical history was taken, as well as the presence or absence of relevant symptoms. 6-minute walking test, as well as echocardiographic indexes were recorded to evaluate the improvement of cardiac function. At the same time, major adverse cardiac events (MACE) were recorded. The MACE consists of a composite of: 1) cardiac death; 2) a recurrent nonfatal myocardial infarction; 3) acute left ventricular failure.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) for continuous variables and frequencies for categorical variables. Differences among groups were examined by nonparametric test and chi-square test for continuous and categorical variables respectively. The effect of rh-BNP on ventricular function was assessed with the use of a multivariate Cox proportional hazards model. Other variables that were significantly associated with outcome entered into the model in a stepwise procedure. An alpha value of 0.05, corresponding to a p value<0.05, served as criterion for establishing statistical significance. The 95% confidence intervals of the hazard ratio were reported for all of the significant risk factors. Analysis was performed

Table 3. Multivariate Cox regression analysis

	MACE		6MWT	
Characteristics	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Sex	0.324 (0.077-1.364)	0.111	2.422 (0.663-8.850)	0.174
HBP	0.843 (0.679-0.980)	0.035	0.817 (0.174-3.824)	0.797
DM	1.097 (0.916-1.313)	0.315	0.633 (0.111-3.603)	0.605
Smoking	0.491 (0.092-2.630)	0.415	1.851 (0.405-8.453)	0.426
Rh-BNP	1.762 (0.793-3.913)	0.164	0.478 (0.290-0.787)	0.014
Emergency PCI	0.201 (0.036-1.031)	0.046	1.778 (0.501-6.316)	0.370
β-blocker	1.970 (0.753-5.153)	0.754	1.970 (0.123-4.626)	0.760
ACEI/ARB	0.202 (0.040-1.020)	0.040	0.396 (0.041-3.809)	0.421

using SPSS for Windows (SPSS Inc., Version 19.0, Chicago, Illinois) and STATA (Version 12.0).

Results

Baseline characteristics

All the 100 patients completed the treatment and received a period of follow-up. The mean age of finally enrolled 100 patients was 64.37±9.35. Male patients enrolled were only 53, corresponding to 47 female patients were enrolled in this study. According to the use of rh-BNP and the time of PCI, four subgroups composed of 25 patients were founded. The data analysis about demographic comparison, including those related complicated diseases among groups showed no significant difference (**Table 1**). Because of β-blockers and ACEI/ARBs' effects on the protection of cardiac structure and function, we also recorded the service condition for further analysis. The differences of β-blockers and ACEI/ARBs' usage were not significant (P=0.984; P=0.996).

Echocardiographic findings, including LVEF, LVESD and LVEDD were collected. Importantly, these indicators were all similar among the four subgroups at baseline (all P>0.05). Furthermore, basal level of NT-pro BNP andcardiac functional classification were all similar, too (P=0.628).

Functional improvements, as assessed by NYHA class, were observed so as to evaluate the therapeutic efficacyof the treatments. The proportion of patients showing a functional improvement ofat least 1 grade in NYHA class was greaterin the rh-BNP group (96.0%, 48/50) than that in the control group (72.0%, 36/50) (P>0.05). However, the difference between "rh-BNP + emergency PCI" group and "control + delayed PCI" group was significant (P=0.048). Three months after discharge, all patients were required to review echocardiography for the comparison with baseline. In the same way, 6WMT was taken to evaluate their cardiac function. There was no doubt that the indicators were all better than baseline among four subgroups (all P<0.001). In the rh-BNP group, especially the "rh-BNP + emergency PCI" subgroup, results showed that rh-BNP was able to significantly reduce the NT-pro BNP levels (P<0.001), decrease LVESD (P<0.01), and increase LVEF (P<0.05). Although the decrease of LVEDD was significant different in "rh-BNP + emergency PCI" subgroup from the other three subgroups (P<0.05), the decrease in rh-BNP group and control group was similar. Patients could walk 500.52±24.73 meters in average in rh-BNP group, while 465.16±30.92 meters in control group. The difference was significant (P<0.001). The same thing happened in the comparison between "rh-BNP + emergency PCI" subgroup and the other three subgroups (all P<0.001). According to our definition of MACE, the incidence in four subgroups were similar (P>0.05), but one case occurred cardiac death in the fourth subgroup. All the analysis results were summarized in **Table 2**.

To demonstrate the effectiveness of rh-BNP in the protection of ventricular function and structure, we did the multivariate Cox regression to exclude other factors' influence. We defined the 6WMT distance (over 450 meters) as another important endpoint. Then the result showed a significant association between rh-BNP and 6WMT after adjusting for other relevant factors: sex, hypertension, diabetes mellitus, tobacco use, emergency PCI and the use of β-blockers and ACEI/ARB. All other factors were considered to haveno significant influence on 6WMT. Analysis for MACE showed that the use of rh-BNP may not be an independent factor to predict MACE after adjusting for other risk factors. However, hypertension and the use of ACEI/ARB might be independent predictors (**Table 3**).

Discussion

In our study, 72 h of intravenousinfusion of rh-BNP not only corrected cardiac dysfunction obviously and decreased serum level of NT-pro BNP in the course of infusion, but also reduced cardiac dilatation and improvedleft ventricular ejection fraction 3 month later in comparison with baseline and control group. These results support the potential efficacy of rh-BNP therapy in patients with STEMI to prevent adverse LV fromremodeling and improve LV function.

STEMI is a serious cardiovascular disorder with high morbidity and mortality. The late effects of STEMI are usually characterized by progressive dilation andfibrosis of the LV myocardium, leading to heart failure. Therefore, it is necessary to protect the heart from excessivehypertrophy, fibrosis, dilatation and loss of contractile function. Antagonism ofthe fibrosis-inducing steroid hormone aldosterone proved to be efficacious after AMI whenHF was present [15]. Cell therapy, especially in humans, has followed a similar paradigm thatemploys intra-cardiac delivery after AMI [16]. Rh-BNP is a recombinant B-type, natriuretic peptide that is structurally identical to the endogenous hormone produced by the ventricle in response to increased wall stress, hypertrophy, and volume overload. The main pharmacological actions of rh-BNP are vasodilatation and natriuresis. It also reduces pulmonary capillary wedge pressure and systemic vascular resistance, and indirectly increases stroke volume and cardiac output.

The demographic results showed great homogeneity among four subgroups. At the same time, we can easily find that, 3 month later, there are significant differences of serum level of NT-pro BNP and echocardiograph resultsbetween it and baseline in all groups (P<0.001). It means that not only rh-BNP but also other anti-remodeling drugs and timely and effectively revascularization can bring benefits for the recovery of cardiac function. However, we still couldn't exclude other factors effects on the protection of cardiac function. So it becomes necessary for us to make the comparison among subgroups. The proportion of functional improvement in NYHA class was greaterin the rh-BNP group (96.0%, 48/50) than in the control group (72.0%, 36/50) (P>0.05), while the difference between "rh-BNP + emergency PCI" group and "control + delayed PCI" group was significant (P=0.048). Compared with the other three subgroups, the echocardiograph improvements (LVEF and LVEDD) in "rh-BNP + emergency PCI" group were relatively obvious (P<0.05), but the level of LVESD was significantly different (P<0.001). Besides these serous or structural indicators, we still did the 6 minutes' walking test to evaluate their real time cardiac function. The result was gratifying and excepted that patients in "rh-BNP + emergency PCI" group walked longer (511.6±23.2 meters) than other three groups (all P<0.001) and rh-BNP users walked longer (500.52±24.7 meters) than control group (P<0.001), too. It proved that patients in rh-BNP group had better exercise tolerance. These significant differences in different aspects demonstrate the positive effects of rh-BNP in cardiac functional protection which is the most important contribution to our study.

Multivariate Cox regression analysis showed a significant association among HBP, emergency PCI, usage of ACEI/ARB and major adverse cardiac events after adjusting for other relevant factors: gender, diabetes mellitus, tobacco use and usage of β-blockers (**Table 3**). The adhibition of rh-BNP may not be an independent factor to predict MACE after adjusting for other risk factors (OR 1.762, 95% CI, 0.793-3.913). According to the classification of 6WMT, the multivariate Cox regression was analyzed once again. The usage of rh-BNP was an independent predictor for 6WMT (OR 0.478, 95% CI, 0.290-0.787), while other factors were not. This is another important conclusion of our study in result.

All the findings in our study were not only a great help for us to find a refined risk stratification for prognosis of STEMI patients, but also an encouragement in the treatment of myocardial remodeling, including ACEI/ARB, β-blockers and rh-BNP in consideration. The definite curative efficacy of rh-BNP preformed not only in the timely improvement in acute heart failure, but also the long-term protection of left ventricular function. At the same time, indiscriminate occurrence of MACE showed a good security of rh-BNP.

Several limitations to this study couldn't be ignored. The sample scale of our study was small and the follow-up time was relatively short, which may have an influence on the eval-

uation of relationship between rh-BNP and prognosis of STEMI. Because of rh-BNP's contraindication in cardiogenic shock and whose systolic pressure ≤90 mmHg, we excluded a certain amount of HF patients. Can we use rh-BNP in those patients on condition that systolic pressure has been improved by pressor agent? At last, we focused on the protection of left ventricular function and structure, but not on the correction of acute heart failure. That's what we need to analyze next time or in the future. Despite of these limitations, rh-BNP's protection of ventricular function and structure in STEMI patients is affirmative.

Conclusions

Although the use of rh-BNP was not an independent risk factor in prediction of MACE in our study, the current data still showed that rh-BNP is a useful prognosis factor of 6WMT in the STEMI patients. The protection of ventricular function and structure in STEMI patients is affirmative.

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Disclosure of conflict of interest

None.

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References

[1] American College of Emergency Physicians; Society for Cardiovascular Angiography and Interventions, O'Gara PT, Kushner FG, Ascheim DD, Casey DE Jr, Chung MK, de Lemos JA, Ettinger SM, Fang JC, Fesmire FM, Franklin BA, Granger CB, Krumholz HM, Linderbaum JA, Morrow DA, Newby LK, Ornato JP, Ou N, Radford MJ, Tamis-Holland JE, Tommaso CL, Tracy CM, Woo YJ, Zhao DX, Anderson JL, Jacobs AK, Halperin JL, Albert NM, Brindis RG, Creager MA, DeMets D, Guyton RA, Hochman JS, Kovacs RJ, Kushner FG, Ohman EM, Stevenson WG and Yancy CW. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2013; 61: 485-510.

[2] Sutton MG and Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. Circulation 2000; 101: 2981-2988.

[3] Jugdutt BI. Ventricular remodeling after infarction and the extracellular collagen matrix: when is enough enough? Circulation 2003; 108: 1395-1403.

[4] Tapanainen JM, Lindgren KS, Makikallio TH, Vuolteenaho O, Leppaluoto J and Huikuri HV. Natriuretic peptides as predictors of non-sudden and sudden cardiac death after acute myocardial infarction in the beta-blocking era. J Am Coll Cardiol 2004; 43: 757-763.

[5] Yousef M, Schannwell CM, Kostering M, Zeus T, Brehm M and Strauer BE. The BALANCE Study: clinical benefit and long-term outcome after intracoronary autologous bone marrow cell transplantation in patients with acute myocardial infarction. J Am Coll Cardiol 2009; 53: 2262-2269.

[6] Chung ES, Dan D, Solomon SD, Bank AJ, Pastore J, Iyer A, Berger RD, Franklin JO, Jones G, Machado C and Stolen CM. Effect of peri-infarct pacing early after myocardial infarction: results of the prevention of myocardial enlargement and dilatation post myocardial infarction study. Circ Heart Fail 2010; 3: 650-658.

[7] Timmers L, Henriques JP, de Kleijn DP, Devries JH, Kemperman H, Steendijk P, Verlaan CW, Kerver M, Piek JJ, Doevendans PA, Pasterkamp G and Hoefer IE. Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. J Am Coll Cardiol 2009; 53: 501-510.

[8] Chen HH, Martin FL, Gibbons RJ, Schirger JA, Wright RS, Schears RM, Redfield MM, Simari RD, Lerman A, Cataliotti A and Burnett JC Jr. Low-dose nesiritide in human anterior myocardial infarction suppresses aldosterone and preserves ventricular function and structure: a proof of concept study. Heart 2009; 95: 1315-1319.

[9] Michaels AD, Klein A, Madden JA and Chatterjee K. Effects of intravenous nesiritide on human coronary vasomotor regulation and myocardial oxygen uptake. Circulation 2003; 107: 2697-2701.

[10] Brunner-La Rocca HP, Kaye DM, Woods RL, Hastings J and Esler MD. Effects of intravenous brain natriuretic peptide on regional sympathetic activity in patients with chronic heart failure as compared with healthy control subjects. J Am Coll Cardiol 2001; 37: 1221-1227.

[11] Kapoun AM, Liang F, O'Young G, Damm DL, Quon D, White RT, Munson K, Lam A, Schreiner GF and Protter AA. B-type natriuretic peptide exerts broad functional opposition to transforming growth factor-beta in primary human cardiac fibroblasts: fibrosis, myofibroblast conversion, proliferation, and inflammation. Circ Res 2004; 94: 453-461.

[12] Tsukada T, Yokoyama K, Arai T, Takemoto F, Hara S, Yamada A, Kawaguchi Y, Hosoya T and Igari J. Evidence of association of the ecNOS gene polymorphism with plasma NO metabolite levels in humans. Biochem Biophys Res Commun 1998; 245: 190-193.

[13] Hillock RJ, Frampton CM, Yandle TG, Troughton RW, Lainchbury JG and Richards AM. B-type natriuretic peptide infusions in acute myocardial infarction. Heart 2008; 94: 617-622.

[14] Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, Joint ESCAAHAWHF-TFfUDoMI, Authors/Task Force Members C, Thygesen K, Alpert JS, White HD, Biomarker S, Jaffe AS, Katus HA, Apple FS, Lindahl B, Morrow DA, Subcommittee ECG, Chaitman BR, Clemmensen PM, Johanson P, Hod H, Imaging S, Underwood R, Bax JJ, Bonow JJ, Pinto F, Gibbons RJ, Classification S, Fox KA, Atar D, Newby LK, Galvani M, Hamm CW, Intervention S, Uretsky BF, Steg PG, Wijns W, Bassand JP, Menasche P, Ravkilde J, Trials, Registries S, Ohman EM, Antman EM, Wallentin LC, Armstrong PW, Simoons ML, Trials, Registries S, Januzzi JL, Nieminen MS, Gheorghiade M, Filippatos G, Trials, Registries S, Luepker RV, Fortmann SP, Rosamond WD, Levy D, Wood D, Trials, Registries S, Smith SC, Hu D, Lopez-Sendon JL, Robertson RM, Weaver D, Tendera M, Bove AA, Parkhomenko AN, Vasilieva EJ, Mendis S, Guidelines ESCCfP, Bax JJ, Baumgartner H, Ceconi C, Dean V, Deaton C, Fagard R, Funck-Brentano C, Hasdai D, Hoes A, Kirchhof P, Knuuti J, Kolh P, McDonagh T, Moulin C, Popescu BA, Reiner Z, Sechtem U, Sirnes PA, Tendera M, Torbicki A, Vahanian A, Windecker S, Document R, Morais J, Aguiar C, Almahmeed W, Arnar DO, Barili F, Bloch KD, Bolger AF, Botker HE, Bozkurt B, Bugiardini R, Cannon C, de Lemos J, Eberli FR, Escobar E, Hlatky M, James S, Kern KB, Moliterno DJ, Mueller C, Neskovic AN, Pieske BM, Schulman SP, Storey RF, Taubert KA, Vranckx P and Wagner DR. Third universal definition of myocardial infarction. J Am Coll Cardiol 2012; 60: 1581-1598.

[15] Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M, Eplerenone Post-Acute Myocardial Infarction Heart Failure E and Survival Study I. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med 2003; 348: 1309-1321.

[16] Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S and Zeiher AM. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation 2002; 106: 3009-3017.