BMJ Open

Predictive value of apelin-12 in ST-elevation myocardial infarction patients with different renal function

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-018595
Article Type:	Research
Date Submitted by the Author:	10-Jul-2017
Complete List of Authors:	Yang, Lingchang Zheng, Ting Wu, Haopeng Xin, Wenwei Mou, Xiongneng Lin, Hui Chen, Yide Wu, Xiaoyu
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Diagnostics, Emergency medicine
Keywords:	Coronary heart disease < CARDIOLOGY, Coronary intervention < CARDIOLOGY, Adult cardiology < CARDIOLOGY

Predictive value of apelin-12 in ST-elevation myocardial infarction patients with different renal function

Running head: apelin-12 predict MACEs

Lingchang Yang MD, Ting Zheng MD, Haopeng Wu MD, Wenwei Xin MD, Xiongneng Mou MD, Hui Lin MD, Yide Chen MD, Xiaoyu Wu MD

Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China

Correspondence: Xiaoyu Wu Email: acmilancaca@126.com

the word count: 2569

indexing words: MACEs; ACS; renal function; adipokines; cardiac death; cardiogenic shock; congestive heart failure

Abstract:

Objectives: To investigate the factors predicting the onset of major adverse cardiovascular events (MACEs) after primary percutaneous coronary intervention (pPCI) for ST-segment elevation myocardial infarction (STEMI) patients.

Background: apelin-12 has been regarded acting essential role in cardiovascular homeostasis. However, current knowledge of the optimal prognostic predictive value is limited.

Methods: 464 STEMI patients (63.0±11.9 years, 355 men) who underwent successful pPCI were enrolled. Patients were followed-up for 2.5 years. Multivariate cox regression analyses and receiver operating characteristic curve analysis were performed to determine the factors predicting MACEs.

Results: There were 118 patients (25.4%) who experienced MACEs in the follow-up period. Multivariate cox regression analysis demonstrated that low apelin-12 (HR=0.132, 95% CI=0.060-0.292, p < 0.001), low left ventricular ejection fraction (LVEF) (HR=0.965, 95% CI=0.941-0.991, p=0.007), low estimated glomerular filtration rate (eGFR) (HR=0.985, 95% CI=0.977-0.993, p < 0.001), Killip's classification > I (HR=0.610, 95% CI=0.408-0.912, p=0.016) and pathological Q-wave (HR=1.536, 95% CI=1.058-2.230, p=0.024) were independent predictors of 2.5 MACEs. Low apelin-12 could also predict worse in-hospital prognosis and showed advantage in predicting 2.5 year MACEs compared with Δapelin-12 (p=0.0115) and eGFR (p=0.0071) among patients with eGFR > 90 mL/min*1.73m^2. Further analysis prompt Δapelin-12 < 20% was usually associated with MACEs in patients whose apelin-12 admission below 0.76 ng/ml (p=0.0075).

Conclusions: STEMI patients receiving pPCI with lower apelin-12 are more likely to suffer MACEs in hospitalization and 2.5-year follow-up, especially for those with normal level of eGFR.

Key words: STEMI; Major adverse cardiovascular events; apelin-12; eGFR.

Strengths and limitations

1. Our study enrolled the purely STEMI patients receiving successful primary PCI and defined Δapelin-12 with apelin-12 elevation percent 72h after pPCI compared with apelin-12 level immediately prior to pPCI.

2. We analyzed the prognosis value of apelin-12 in predicting short-term (during

hospitalization) and long-term (2.5 years) MACEs **respectively** among patients with eGFR exceeding and below 90 mL/min*1.73m^2

3. Limitations: The relatively small cohort size included in our study may affect the statistical result, in hence a larger-scale study is warranted to further assess the risk of long-term MACEs after pPCI in STEMI patients with separate level of apelin-12.

4. The basic level of apelin ahead of STEMI onset is difficult to acquire, so the decline degree of apelin is unknown.

5. We focus on apelin-12, analysis on other forms of apelin would be desirable in future investigations.

INTRODUCTION

Although primary percutaneous coronary intervention (pPCI) could rescue ischemic myocardium by revascularization, ST elevation myocardial infarction (STEMI) is still responsible for the major cardiovascular mortality and morbidity worldwide, mainly due to mechanical complications, acute heart failure, and even cardiac shock after successful

procedure(1). The structural and functional alterations lead to progressive worsening of cardiac performance. Prognosis of STEMI with pPCI is influenced by several clinical, biochemical, and echocardiographic factors. To precisely identify survived patients with high risk for adverse clinical end-points in the follow-up period and formulate individualized prevention programs, more reliable novel biomarkers are urgently continuously needed(2, 3).

As the only known endogenous ligand for the human orphan G protein-coupled (APJ) receptor, apelin is synthesised as a peptide consisting of 77 amino acids adipocytokine, secreted by white adipose tissue and expressed in various cardiovascular tissues, including endothelial cells, coronary vessels, vascular smooth muscle cells, and cardiomyocytes(4). The apelin–APJ system has been regarded acting essential role in cardiovascular homeostasis(5). Apelin-12 may function its cardioprotective profile via the complex mechanism of improving hypertension, insulin resistance, obesity and reducing cardiovascular risk factors(6-8). However, whether serum apelin-12 level on admission provide additional prognostic information among STEMI patients receiving pPCI remains unknown. There is only limited evidence on apelin involvement in STEMI up to now, additionally, some articles suggest no prognostic value(9-11), individual research(12) even point out the inverse correlation between apelin-12 level and prognosis, the controversial result in real world worth pondering.

In this study, we aimed to determine the compliance of plasma apelin-12 levels to predict short-term and long-term major adverse coronary events (MACEs) in patients with STEMI following successful pPCI. Additionally, the combined utilization of estimated

glomerular filtration rate (eGFR) is further studied.

METHODS

Inclusion and Exclusion Criteria and diagnosis of MACEs

Patients were eligible and prospectively enrolled into our study if they presented with the onset of symptoms of STEMI in the First People's Hospital of Taizhou, Zhejiang, China between January 2010 and October 2014: persistent chest pain (>30 minutes), prolonged electrocardiogram (ECG) changes (including ischemic ST-segment elevation in 2 or more contiguous leads and/or depression) and significantly increased serum myocardial enzyme and troponin concentrations. Written informed consent was obtained, and the study was approved by the Research Ethics Committee of The First People's Hospital of Taizhou.

Exclusion criteria contains non-STEMI; severe vascular heart disease; balloon angioplasty alone, rescue PCI, conservative treatment without PCI, previous onset of ventricular fibrillation, cardiogenic shock, untreated 3rd or advanced degree of atrioventricular block, estimated life expectancy <12 months; secondary hypertension or endocrine diseases such as thyroid dysfunction or adrenal cortical dysfunction; history of cerebrovascular attack (within 1 year) or cerebrovascular attack with a significant residual neurologic deficit; a history of chronic hepatitis or cirrhosis; severe renal insufficiency needing dialysis; known contraindication to statins, heparin, aspirin, clopidogrel, contrast or glycoprotein IIb/IIIa inhibitor; recent serious infections, connective tissue disease, malignancy; active severe bleeding; significant gastrointestinal or genitourinary bleeding,

major surgery or trauma in recent 6 weeks; and incomplete clinical data.

Important definitions

MACEs was defined as the composite of cardiac death, recurrent target vessel myocardial infarction (RMI), and clinically driven target lesion revascularization (TLR), cardiogenic shock or demonstrated congestive heart failure (DCHF). We estimated eGFR with simplified Modification of Diet in Renal Disease (sMDRD) formula(13). We gave Δ apelin-12 the meaning of apelin-12 elevation percent 72h after pPCI compared with apelin-12 level immediately prior to pPCI.

Therapy Process

To begin with filtering the data using the eligibility and exclusion criteria, a total of 464 patients underwent successful pPCI. All patients were diagnosed definitely with STEMI and admitted to emergency room within 12 hours from onset. All patients received 300 mg oral aspirin and clopidogrel as well as standard heparin (initial 10,000 IU and boost as the operation time prolonged), postprocedure glycoprotein IIb/IIIa antagonists. Ultrasound scans were taken within 5 days after PCI (median 4.2 days). The parameters of 2D echocardiography and Doppler were measured by standard methods described as follows: using biplane Simpson's method for measuring left ventricular volumes and ejection fraction. Volumes were expressed as indices by normalizing with body surface area.

All the patients received 30 month follow-up after pPCI until MACEs; while few cases suffered hospitalization MACEs. Based on these, the authors stratified the patients into MACEs group and non-MACEs group and tried to identify the independent predictors of poor prognosis. Biochemical indicators were measured: blood routine (including

hemoglobin, neutrophil percent, hemoglobin, and platelet), coagulation routine, D-dimer, renal and hepatic function, apelin-12 preprocedure and 72h after pPCI, peak myocardial enzyme, lipid level and fast blood glucose on the 2nd day. We further stratified the patients into 2 subgroups according to the median value of apelin-12 level on admission, and into 3 subgroups according to tertiles of eGFR.

Apelin-12 Elisa Detection

Serum was isolated by centrifugation within 1h at 2500g for 10min, and stored at -80℃. Serum concentrations of apelin-12 were assayed using commercially available enzyme immunoassay kits (Phoenix Pharmaceuticals, Belmont, CA). Protocol was as follows: add 50μl/well of standard, sample, or positive control, 25μl of primary antibody and 25μl of biotinylated peptide. Incubate at room temperature (20℃–23℃) for 2 hours. Wash immunoplate 4 times with 350μl/well of 1×assay buffer. Add 100μl/well of Streptavidin-HRP solution and incubate at room temperature for 1h. Wash immunoplate 4 times with 350μl/well of 1×assay buffer. Add 100μl/well of 3,3', 5,5'-tetramethylbenzidine substrate solution and incubate at room temperature for 1h. Terminate reaction with 100μl/well of 2N HCl. The detection limit was 0.1mg/L, with 1.26% intra- and 5.4% inter-assay coefficients of variation respectively. The measurements were performed in triplicates.

Statistical Strategy

Continuous data were presented as mean±standard deviation or medians with interquartile ranges, whereas categorical data as percentage, unless otherwise denoted. univariate cox analysis and log-rank test were used for qualitative and quantitative

variables to determine whether there is significant difference between MACEs and non-MACEs. The variables selected to be tested in the multivariate cox proportional hazard analysis were those with P<0.1 in the above analysis. The adjusted hazard ratio and 95% confidence interval were calculated. Kaplan–Meier curves of MACE-free survival among 2 groups divided by the median apelin-12 were compared using the log-rank test. Additionally, the authors performed the receiver operating characteristic (ROC) analysis to identify the appropriate cutoff value of some potential predictive indicators. The cutoff point we acquired lied at the maximum sum of sensitivity and specificity. The ROC curves were conducted with the Medcalc 12.3.0.0. All statistical tests were 2-tailed, performed using SPSS 17.0 (SPSS, Inc, Chicago, IL), and a P-value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of Patients in Individual Groups

Of the 464 enrolled patients, 118 (25.4%) had MACEs in the 2.5-year follow-up. 19 (4.1%) patients suffered cardiac death, while 27 (5.8%) RMI; 85 (18.3%) received TLR due to RMI or progressive stenosis. 35 (7.5%) performed cardiogenic shock or demonstrated congestive heart failure onset. Among the MACEs group, 31 (6.7%) patients reached end point during hospitalization. Basic clinical characteristics, laboratory examinations, electrocardiogram results, angiographic and procedural characteristics are depicted in Table 1.

Multivariate Logistic Regression Analysis of Predictor of MACEs

Significant differences could be found between 2 groups in peak cardiac Troponin I (cTnI) [21.5 (9.3-32.4) vs 12.6 (3.0-28.8), p=0.014] and left ventricular ejection fraction (LVEF) (47.3%±9.4% vs 51.9%±7.3%, P=0.010). Consistent with our expectation, patients who suffered from MACEs usually coincided with lower apelin-12 on admission [0.69 (0.53-0.87) vs 0.79 (0.63-1.03), p < 0.001]. Anterior wall MI, pathological Q-wave and higher Killip's classification were more often present in the MACEs group as compared with survivals (p=0.003, 0.003, and 0.002, respectively). There was an borderline significance of Δapelin-12 [13.9 (5.6-17.6) vs 14.7 (5.3-22.3), p=0.092], neutrophil percent (76.8±12.6 vs 74.9±12.4, p=0.064), eGFR (89.7±25.8 vs 100.6±25.9, p=0.067) and previous MI proportion (16.1% vs 10.4%, p=0.071) among 2 groups.

Through the multivariate cox regression analysis, low apelin-12 (HR=0.132, 95% CI=0.060-0.292, p < 0.001), low eGFR (HR=0.985, 95% CI=0.977-0.993, p < 0.001), low LVEF (HR=0.965, 95% CI=0.941-0.991, p=0.007), and pathological Q-wave (HR=1.536, 95% CI=1.058-2.230, p=0.024) were independent predictors of MACEs within 2.5 years after pPCI, along with Killip's classification > I (HR=0.610, 95% CI=0.408-0.912, p=0.016), while anterior wall MI show its forecasting accuracy to some extent (HR=1.421, 95% CI=0.970-2.082, p=0.071) (Table 2).

Survival analysis

Kaplan-Meier curves in patients with higher (> 0.76 ng/ml, n=229) and lower (< 0.76 ng/ml, n=235) apelin-12 during 2.5-year follow-up was showed in Figure 1. Significant differences in event-free survival were noted between patients with different apelin-12 on admission (p=0.018).

ROC Analysis of Predictor of MACEs

We conducted the ROC analysis but failed to find the area under curve (AUC) of any indicator for 2.5-year MACEs exceeding 0.7, although the p value for apelin-12, eGFR and LVEF alone was 0.0001, 0.0369 and 0.0015 respectively, while the AUC of Δapelin-12 for 2.5-year MACEs was 0.547 (95% CI= 0.500-0.593, p=0.0906, Figure 2). When we focused on in-hospital MACEs, no evidence predictive value was found except for LVEF < 52% (AUC=0.674, 95% CI=0.629-0.716, p=0.0005) and apelin-12 ≤ 0.64 ng/ml (AUC=0.623, 95%CI=0.577-0.667, p=0.0169, Figure 3 & Table 3). ROC analysis prompt Δapelin-12 provide relatively higher AUC with the cut-off point of 20% only in whose apelin-12 on admission≤0.76 ng/ml (p=0.0075, Table 3).

When we subgroup the patients according eGFR, we found eGFR exceeding 90 mL/min*1.73m^2 exist in 244 patients, among whom apelin-12 showed its advantage in predicting MACEs compared with Δ apelin-12 (p=0.0115) and eGFR (p=0.0071), moreover, LVEF only perform high predictive value in patients with eGFR > 90 mL/min*1.73m2 (AUC=0.628, 95%CI=0.564-0.689, p=0.0039, Table 4).

Discussion

In this study, we found the clinical outcome in STEMI patients receiving pPCI is closely associated with the pre-PCI apelin-12 concentration, especially among those with relatively normal renal function. In further subgroup analysis, we discovered the change of apelin during hospitalization could predict long-term prognosis only among patients with relatively lower apelin level on admission (apelin-12≤0.76 ng/ml).

Apelin present its potential role in assessing risk stratification among STEMI patients

mainly due to its pivotal action in the pathophysiology of both heart failure and ischemia/reperfusion injury(14). Apelin was found to increase contractility and reduce peripheral resistance via an endothelial nitric oxide (NO)-dependent signaling(15) in failing myocardial cell to postpone the pathological progress of heart failure(16). Apelin expression is demonstrated to decline in decompensated states, while maintain or mildly augment in stable chronic heart failure(17).

In patients with stable angina, plasma apelin was negatively associated with coronary artery stenosis severity marked by Gensini score and incidence of ACS independent of other cardiovascular risk factors(18). Weir et al.(19) showed that plasma apelin level reduces early after acute myocardial infarction and increases remarkably after revascularization, but remains depressed at 24 weeks, which could not be fully validated by our study due to the lack of long-term data, however, the regulation action against severe disease of apelin is further certified. We hypothesis the potential explanation of lower level of apelin following STEMI: (1) the demand of apelin among STEMI patients increase, so more apelin is consumed immediately after MI episodes; (2) product plunging due to MI. Recent study demonstrated the flow-mediated adjustment of the apelin/APJ system in human endothelial cells, apelin-12 expression is induced by shear stress independently of its ligand, especially when reperfusion(20). Besides, hypoxia-inducible factor (HIF)-mediated pathway participate in the apelin upregulation progress in myocardium, pulmonary circulation and skeletal muscle following systemic hypoxic exposure or myocardial injury(21), in turn, the apelin-APJ system could alleviate the myocardial reperfusion mediated oxidative stress and apoptosis by increasing superoxide

dismutase degradation, decreasing the generation of reactive oxygen species, along with upregulating eNOS levels and activating ERK1/2 phosphorylation signaling(22). Above could be partially responsible for the short-term poor outcome and tendency of long-term worse prognosis observed in patients with low levels of apelin.

As we know, apelin provide myocardial protection against ischemic damage by decreasing permeability of microvascular endothelial cells via upregulating the expression of Tie-2 and VEGFR2(23), and improving neovascularization via recruiting circulating Aplnr+ cells during early-phase myocardial repair as well(24). The Apelin/APJ system promotes angiogenesis and offer nutrients and oxygen to the ischemic area in MI animal models(25). Apelin gene therapy by myocardial injection ameliorates cardiac repair, improves cardiac metabolism via activating Sirt3 and up-regulating VEGF/VEGFR2 expression in post-MI mice(26, 27). Vice versa, apelin down-regulation exacerbates ischemia-reperfusion injury and myocardial infarction adverse remodeling(25). Apelin protects against angiotensin II-induced cardiovascular fibrosis and decreases plasminogen activator inhibitor type-1 production(28). The utilization rate of ACEI and β blockers were similar among patients with different prognosis in our study, which eliminates the drug-derived influence on angiotensin activity. Myeloid cell-derived Leucine-rich α2-glycoprotein attenuates adverse cardiac remodeling after MI via upregulating the expression of apelin receptor(29). Direct anti-inflammatory(30) and anti-atherogenic properties(31) are also reported as mechanisms of atherosclerotic lesions and aortic aneurysms prevention by apelin. Stress-induced apoptosis in serious cardiovascular diseases is inhibited by cardiac apelin expression elevation(32). TIMP3

maintains metabolic flexibility via apelin during cardiac stress seizure(33). Taken together, the above cardiovascular profile implies the beneficial effect of apelin on atherosclerosis, and makes apelin-APJ system a promising therapeutic target in acute coronary syndrome(34).

Liu et al(35) have certified the effect of serum apelin-12 in predicting 1-year outcomes following pPCI in patients with STEMI. Topuz et al(36) found the lower serum apelin-12 levels predict higher incidence of in-hospital MACEs after multivariate regression analysis and exist more in no-reflow group than in normal flow group, which was on account of NO dependent vasodilatation caused by apelin-12 in clinical studies(37) as well as in animal models(38). Abnormal level of apelin and a serious of adipokines observed in acute MI patients heralds high incidence of MACEs during 3-year follow-up(39). Low apelin seem to correlate with carotid plaque vulnerability in patients with carotid stenosis. The atorvastatin-induced apelin modification may beneficially affect carotid plaque stability(40).

Several limitations should still be addressed in this study. First, the relatively small cohort size included in our study may affect the statistical result, in hence a larger-scale study is warranted to further assess the risk of long-term MACEs after pPCI in STEMI patients with separate level of apelin-12. Second, the basic level of apelin ahead of STEMI onset is difficult to acquire, so the decline degree of apelin is unknown. Third, we focus on apelin-12, analysis on other forms of apelin would be desirable in future investigations.

CONCLUSION

In conclusion, we report that STEMI patients receiving pPCI with lower levels of apelin-12 are more likely to perform worse short-term and long-term prognosis after adjusting for other confounding clinical parameters. Additionally, apelin-12 shows its predominant value in predicting MACEs among patients with eGFR > 90 mL/min*1.73m^2.

a. contributorship statement

Xiaoyu Wu conceived and designed the study. Xiongneng Mou, Hui Lin, Yide Chen collect the statistics. Lingchang Yang, Ting Zheng, Haopeng Wu performed the statistical analysis. Lingchang Yang wrote the paper. Wenwei Xin and Xiaoyu Wu reviewed and edited the manuscript. All authors read and approved the manuscript.

b. competing interests

There is no competing interests in our study.

c. funding

The authors received no financial support for the research, authorship, and/or publication of this article.

d. data sharing statement

No additional unpublished data are available

Figure legends

Figure 1. Kaplan-Meier curves in STEMI patients with individual levels of apelin-12 during 2.5 year follow-up.

Figure 2. Receiver operating characteristic curve of apelin-12, eGFR, LVEF, and Δapelin-12 for predicting 2.5-year MACEs after pPCI among STEMI patients. LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; eGFR, estimated glomerular filtration rate.

Figure 3. Receiver operating characteristic curve of apelin-12, eGFR, LVEF, and Δapelin-12 for predicting in-hospital MACEs after pPCI among STEMI patients. LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; eGFR, estimated glomerular filtration rate.

References:

1. Kilic S, Ottervanger JP, Dambrink JH, Hoorntje JC, Koopmans PC, Gosselink AT, et al. The effect of thrombus aspiration during primary percutaneous coronary intervention on clinical outcome in daily clinical practice. Thromb Haemost. [Journal Article; Observational Study]. 2014 2014-01-01;111(1):165-71.
2. Steg PG, James SK, Atar D, Badano LP, Blomstrom-Lundqvist C, Borger MA, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. EUR HEART J. [Journal Article; Practice Guideline]. 2012 2012-10-01;33(20):2569-619.
3. Pedersen F, Butrymovich V, Kelbaek H, Wachtell K, Helqvist S, Kastrup J, et al. Short- and long-term cause of death in patients treated with primary PCI for STEMI. J AM COLL CARDIOL. [Journal Article; Research Support, Non-U.S. Gov't]. 2014 2014-11-18;64(20):2101-8.
4. Chandrasekaran B, Dar O, McDonagh T. The role of apelin in cardiovascular function and heart failure. EUR J HEART FAIL. [Journal Article; Review]. 2008 2008-08-01;10(8):725-32.
5. Yi L, Hou X, Zhou J, Xu L, Ouyang Q, Liang H, et al. HIF-1alpha genetic variants and protein expression confer the susceptibility and prognosis of gliomas. Neuromolecular Med. [Journal Article; Research Support, Non-U.S. Gov't]. 2014 2014-09-01;16(3):578-86.
6. Sonmez A, Celebi G, Erdem G, Tapan S, Genc H, Tasci I, et al. Plasma apelin and ADMA Levels in patients with essential hypertension. CLIN EXP HYPERTENS. [Journal Article; Research Support, Non-U.S. Gov't]. 2010 2010-05-01;32(3):179-83.
7. Boal F, Roumegoux J, Alfarano C, Timotin A, Calise D, Anesia R, et al. Apelin regulates FoxO3 translocation to mediate cardioprotective responses to myocardial injury and obesity. Sci Rep. [Journal Article; Research Support, Non-U.S. Gov't]. 2015 2015-11-06;5:16104.
8. Leal VO, Lobo JC, Stockler-Pinto MB, Farage NE, Calixto A, Geloneze B, et al. Apelin: a peptide involved in cardiovascular risk in hemodialysis patients? Ren Fail. [Comparative Study; Journal Article; Research Support, Non-U.S. Gov't]. 2012 2012-01-20;34(5):577-81.
9. Lakomkin SV, Tereshchenko SN, Sychev AV, Masenko VP, Tkachev GA, Gerasimova ON, et al. [Biomarkers in Heart Failure: Apelin Level Is Not Associated With Presence and Severity of the

Disease]. Kardiologiia. [English Abstract; Journal Article]. 2015 2015-01-20;55(2):37-41.

10. Cosansu K, Cakmak HA, Ikitimur B, Yildirim E, Can G, Karadag B, et al. Apelin in ST segment elevation and non-ST segment elevation acute coronary syndromes: a novel finding. KARDIOL POL. [Comparative Study; Journal Article; Research Support, Non-U.S. Gov't]. 2014 2014-01-20;72(3):239-45.

11. Kuklinska AM, Sobkowicz B, Sawicki R, Musial WJ, Waszkiewicz E, Bolinska S, et al. Apelin: a novel marker for the patients with first ST-elevation myocardial infarction. HEART VESSELS. [Journal Article; Research Support, Non-U.S. Gov't]. 2010 2010-09-01;25(5):363-7.

12. Sans-Rosello J, Casals G, Rossello X, Gonzalez DLPB, Vila M, Duran-Cambra A, et al. Prognostic value of plasma apelin concentrations at admission in patients with ST-segment elevation acute myocardial infarction. CLIN BIOCHEM. [Journal Article]. 2016 2016-11-24.

13. Manjunath G, Sarnak MJ, Levey AS. Prediction equations to estimate glomerular filtration rate: an update. Curr Opin Nephrol Hypertens. [Journal Article; Research Support, U.S. Gov't, P.H.S.; Review]. 2001 2001-11-01;10(6):785-92.

14. Tycinska AM, Lisowska A, Musial WJ, Sobkowicz B. Apelin in acute myocardial infarction and heart failure induced by ischemia. CLIN CHIM ACTA. [Journal Article; Review]. 2012 2012-02-18;413(3-4):406-10.

15. Scimia MC, Hurtado C, Ray S, Metzler S, Wei K, Wang J, et al. APJ acts as a dual receptor in cardiac hypertrophy. NATURE. [Journal Article; Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't]. 2012 2012-08-16;488(7411):394-8.

16. Chong KS, Gardner RS, Morton JJ, Ashley EA, McDonagh TA. Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. EUR J HEART FAIL. [Journal Article; Research Support, Non-U.S. Gov't]. 2006 2006-06-01;8(4):355-60.

17. Japp AG, Newby DE. The apelin-APJ system in heart failure: pathophysiologic relevance and therapeutic potential. BIOCHEM PHARMACOL. [Journal Article; Research Support, Non-U.S. Gov't; Review]. 2008 2008-05-15;75(10):1882-92.

18. Kadoglou NPE, Lampropoulos S, Kapelouzou A, Gkontopoulos A, Theofilogiannakos EK, Fotiadis G, et al. Serum levels of apelin and ghrelin in patients with acute coronary syndromes and established coronary artery disease — KOZANI STUDY. TRANSL RES. 2010 2010-05-01;155(5):238-46.

19. Weir RA, Chong KS, Dalzell JR, Petrie CJ, Murphy CA, Steedman T, et al. Plasma apelin concentration is depressed following acute myocardial infarction in man. EUR J HEART FAIL. [Comparative Study; Journal Article; Randomized Controlled Trial; Research Support, Non-U.S. Gov't]. 2009 2009-06-01;11(6):551-8.

20. Busch R, Strohbach A, Pennewitz M, Lorenz F, Bahls M, Busch MC, et al. Regulation of the endothelial apelin/APJ system by hemodynamic fluid flow. CELL SIGNAL. [Journal Article; Research Support, Non-U.S. Gov't]. 2015 2015-07-01;27(7):1286-96.

21. Ronkainen VP, Ronkainen JJ, Hanninen SL, Leskinen H, Ruas JL, Pereira T, et al. Hypoxia inducible factor regulates the cardiac expression and secretion of apelin. FASEB J. [Journal Article; Research Support, Non-U.S. Gov't]. 2007 2007-06-01;21(8):1821-30.

22. Zeng XJ, Zhang LK, Wang HX, Lu LQ, Ma LQ, Tang CS. Apelin protects heart against ischemia/reperfusion injury in rat. PEPTIDES. [Journal Article; Research Support, Non-U.S. Gov't]. 2009 2009-06-01;30(6):1144-52.

23. Zhang BH, Guo CX, Wang HX, Lu LQ, Wang YJ, Zhang LK, et al. Cardioprotective effects of

adipokine apelin on myocardial infarction. HEART VESSELS. [Journal Article; Research Support, Non-U.S. Gov't]. 2014 2014-09-01;29(5):679-89.

24. Tempel D, de Boer M, van Deel ED, Haasdijk RA, Duncker DJ, Cheng C, et al. Apelin enhances cardiac neovascularization after myocardial infarction by recruiting aplnr+ circulating cells. CIRC RES. [Journal Article; Research Support, Non-U.S. Gov't]. 2012 2012-08-17;111(5):585-98.

25. Wang W, McKinnie SM, Patel VB, Haddad G, Wang Z, Zhabyeyev P, et al. Loss of Apelin exacerbates myocardial infarction adverse remodeling and ischemia-reperfusion injury: therapeutic potential of synthetic Apelin analogues. J AM HEART ASSOC. [Journal Article; Research Support, Non-U.S. Gov't]. 2013 2013-07-01;2(4):e249.

26. Hou X, Zeng H, He X, Chen JX. Sirt3 is essential for apelin-induced angiogenesis in post-myocardial infarction of diabetes. J CELL MOL MED. [Journal Article; Research Support, N.I.H., Extramural]. 2015 2015-01-01;19(1):53-61.

27. Li L, Zeng H, Hou X, He X, Chen JX. Myocardial injection of apelin-overexpressing bone marrow cells improves cardiac repair via upregulation of Sirt3 after myocardial infarction. PLOS ONE. [Journal Article; Research Support, N.I.H., Extramural]. 2013 2013-01-20;8(9):e71041.

28. Siddiquee K, Hampton J, Khan S, Zadory D, Gleaves L, Vaughan DE, et al. Apelin protects against angiotensin II-induced cardiovascular fibrosis and decreases plasminogen activator inhibitor type-1 production. J HYPERTENS. [Journal Article; Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't]. 2011 2011-04-01;29(4):724-31.

29. Kumagai S, Nakayama H, Fujimoto M, Honda H, Serada S, Ishibashi-Ueda H, et al. Myeloid cell-derived LRG attenuates adverse cardiac remodelling after myocardial infarction. CARDIOVASC RES. [Journal Article; Research Support, Non-U.S. Gov't]. 2016 2016-02-01;109(2):272-82.

30. El-Shehaby AM, El-Khatib MM, Battah AA, Roshdy AR. Apelin: a potential link between inflammation and cardiovascular disease in end stage renal disease patients. Scand J Clin Lab Invest. [Journal Article]. 2010 2010-10-01;70(6):421-7.

31. Yu XH, Tang ZB, Liu LJ, Qian H, Tang SL, Zhang DW, et al. Apelin and its receptor APJ in cardiovascular diseases. CLIN CHIM ACTA. [Journal Article; Research Support, Non-U.S. Gov't; Review]. 2014 2014-01-20;428:1-8.

32. Ustunel I, Acar N, Gemici B, Ozbey O, Edizer I, Soylu H, et al. The effects of water immersion and restraint stress on the expressions of apelin, apelin receptor (APJR) and apoptosis rate in the rat heart. ACTA HISTOCHEM. [Journal Article; Research Support, Non-U.S. Gov't]. 2014 2014-06-01;116(5):675-81.

33. Stohr R, Kappel BA, Carnevale D, Cavalera M, Mavilio M, Arisi I, et al. TIMP3 interplays with apelin to regulate cardiovascular metabolism in hypercholesterolemic mice. Mol Metab. [Journal Article]. 2015 2015-10-01;4(10):741-52.

34. Pisarenko OI, Serebryakova LI, Studneva IM, Pelogeykina YA, Tskitishvili OV, Bespalova ZD, et al. Effects of structural analogues of apelin-12 in acute myocardial infarction in rats. J Pharmacol Pharmacother. [Journal Article]. 2013 2013-07-01;4(3):198-203.

35. Liu HT, Chen M, Yu J, Li WJ, Tao L, Li Y, et al. Serum apelin level predicts the major adverse cardiac events in patients with ST elevation myocardial infarction receiving percutaneous coronary intervention. Medicine (Baltimore). [Journal Article]. 2015 2015-01-01;94(4):e449.

36. Topuz M, Oz F, Akkus O, Sen O, Topuz AN, Bulut A, et al. Plasma apelin-12 levels may predict in-hospital major adverse cardiac events in ST-elevation myocardial infarction and the relationship between apelin-12 and the neutrophil/lymphocyte ratio in patients undergoing primary coronary

intervention. Perfusion. [Journal Article]. 2017 2017-04-01;32(3):206-13.

37. Japp AG, Cruden NL, Barnes G, van Gemeren N, Mathews J, Adamson J, et al. Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure. CIRCULATION. [Journal Article; Randomized Controlled Trial; Research Support, Non-U.S. Gov't]. 2010 2010-04-27;121(16):1818-27.

38. Zhang M, Mahoney E, Zuo T, Manchanda PK, Davuluri RV, Kirschner LS. Protein kinase A activation enhances beta-catenin transcriptional activity through nuclear localization to PML bodies. PLOS ONE. [Journal Article; Research Support, N.I.H., Extramural]. 2014 2014-01-20;9(10):e109523.

39. Grzywocz P, Mizia-Stec K, Wybraniec M, Chudek J. Adipokines and endothelial dysfunction in acute myocardial infarction and the risk of recurrent cardiovascular events. J Cardiovasc Med (Hagerstown). [Comparative Study; Journal Article]. 2015 2015-01-01;16(1):37-44.

40. Kadoglou NP, Sailer N, Moumtzouoglou A, Kapelouzou A, Gerasimidis T, Kostakis A, et al. Adipokines: a novel link between adiposity and carotid plaque vulnerability. EUR J CLIN INVEST. [Journal Article; Research Support, Non-U.S. Gov't]. 2012 2012-12-01;42(12):1278-86.

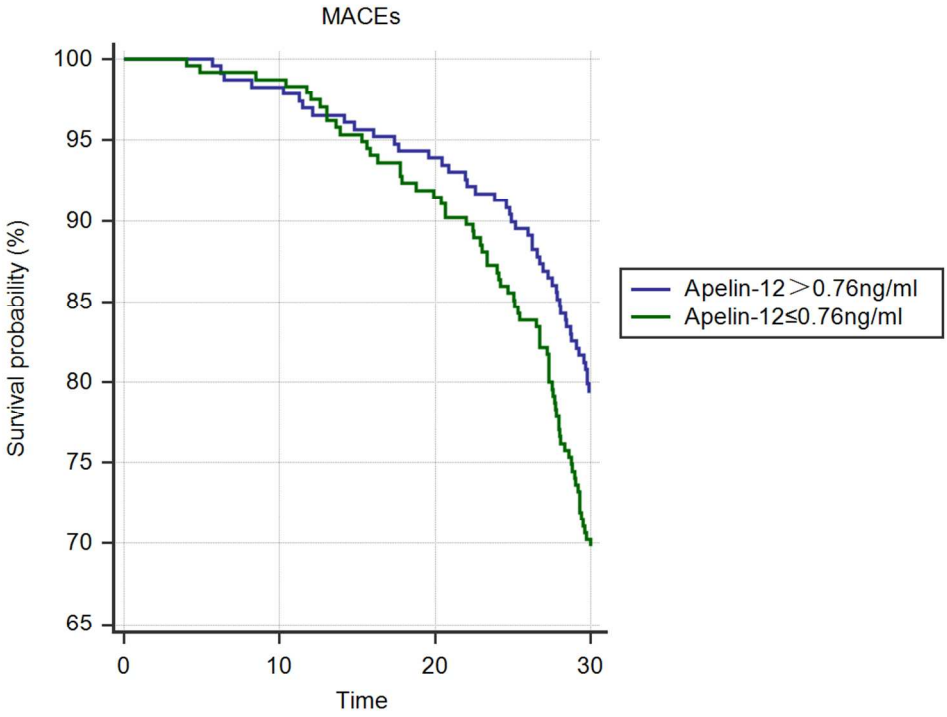

Figure 1. Kaplan-Meier curves in STEMI patients with individual levels of apelin-12 during 2.5 year follow-up.

101x76mm (300 x 300 DPI)

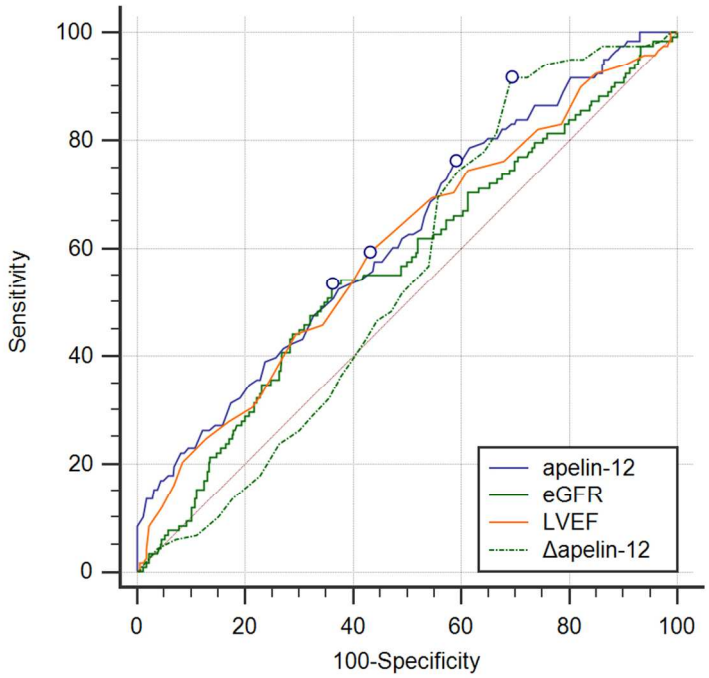

Figure 2. Receiver operating characteristic curve of apelin-12, eGFR, LVEF, and Δapelin-12 for predicting 2.5-year MACEs after pPCI among STEMI patients. LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; eGFR, estimated glomerular filtration rate.

101x76mm (300 x 300 DPI)

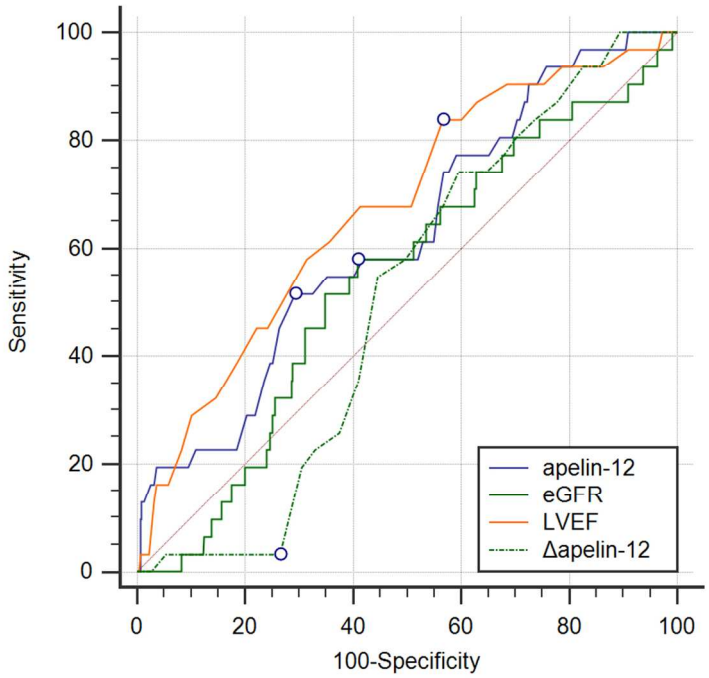

Figure 3. Receiver operating characteristic curve of apelin-12, eGFR, LVEF, and Δapelin-12 for predicting in-hospital MACEs after pPCI among STEMI patients. LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; eGFR, estimated glomerular filtration rate

101x76mm (300 x 300 DPI)

TABLE 1. Basic clinical characteristics, laboratory examinations, electrocardiogram results, angiographic and procedural characteristic

	MACEs (N=118)	Non-MACEs (N=346)	P value
Clinical characteristics			
Age, yr	67.0±12.2	61.7±11.6	0.252
Female, n (%)	33 (28.0)	76 (22.0)	0.115
Heart rate, beats per min	79.5±19.5	76.1±16.6	0.118
SBP, mm Hg	132.2±26.6	131.6±25.0	0.277
Anterior wall MI, n (%)	72 (61.0)	159 (46.0)	0.003 [a]
Killip's classification > I, n (%)	41 (34.7)	71 (20.5)	0.002[a]
Diabetes mellitus, n (%)	41 (34.7)	109 (31.5)	0.294
Hypertension, n (%)	72 (61.0)	193 (55.8)	0.188
Previous MI, n (%)	19 (16.1)	36 (10.4)	0.071
Lab examination			
Apelin-12, ng/ml	0.69 (0.53-0.87)	0.79 (0.63-1.03)	< 0.001 [a]
Δapelin-12 (%)	13.9 (5.6-17.6)	14.7 (5.3-22.3)	0.092
WBC $\times 10^9$/L	10.6±3.86	9.86±3.58	0.161
Neutrophil (%)	76.8±12.6	74.9±12.4	0.064
Hemoglobin, g/L	139.4±16.7	145.3±17.1	0.546
Platelet $\times 10^9$/L	240.2±60.1	227.8±57.2	0.264
Albumin, g/L	37.9±3.9	38.0±3.8	0.424
TC, mmol/L	5.87±0.99	5.57±1.17	0.469

TG, mmol/L	1.06±0.65	1.12±0.90	0.261
HDL-C, mmol/L	1.25±0.28	1.18±0.27	0.982
LDL-C, mmol/L	3.07±0.72	3.03±0.73	0.744
FBG, mmol/L	7.67±2.68	7.66±2.48	0.207
BUN, mmol/L	6.78±1.88	6.72±2.14	0.387
Creatinine, mmol/L	76.3±15.6	74.1±21.7	0.392
Uric acid, mmol/L	333.3±80.7	338.5±72.9	0.153
eGFR mL/min*1.73m^2	89.7±25.8	100.6±25.9	0.067
D-Dimer, mg/L	0.7 (0.2-1.6)	1.0 (0.2-1.7)	0.247
Peak CK-MB, U/L	131.5 (51.6-208.5)	103.0 (39.3-193.4)	0.252
Peak cTnI, ng/ml	21.5 (9.3-32.4)	12.6 (3.0-28.8)	0.014 [a]
Treatment			
ACEIs/ARBs, n (%)	94 (79.7)	294 (85.0)	0.116
b-blocker, n (%)	65 (55.1)	211 (61.0)	0.154
CCBs, n (%)	29 (24.6)	101 (29.2)	0.200
Statins, n (%)	97 (82.2)	288 (83.2)	0.448
Diuretics, n (%)	19 (16.1)	55 (15.9)	0.530
Echocardiogram and electrocardiogram			
LAD, mm	38.5±5.3	37.0±5.7	0.311
LVEDD, mm	52.0±6.4	49.9±6.1	0.273
LVEF, %	47.3±9.4	51.9±7.3	0.010 [a]

Pathological Q-wave, n (%)	70 (59.3)	153 (44.2)	0.003 [a]
GENSINI	85.0 (48.8-100.1)	66.9 (37.2-101.7)	0.129
Culprit vessels, n (%)			
LAD	64 (54.2)	169 (48.8)	
LCX	18 (15.3)	54 (15.6)	0.557
RCA	36 (30.5)	123 (35.5)	
Stent number	1.33±0.55	1.39±0.57	0.524

Data are n/N (%) or mean±standard deviation or median (25th to 75th percentile).

[a] $P < 0.05$.

ACEIs, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BUN, blood urea nitrogen; CCBs, calcium channel blockers; CK-MB, creatinine kinase MB; cTnI, cardiac Troponin I; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HDL-C, high density lipoprotein; LAD, left anterior descending; LAD, left atrial diameter; LCX, left circumflex coronary artery; LDL-C, low-density lipoprotein; LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; MI, myocardial infarction; RCA, right coronary artery; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyeride; WBC, white blood cells.

TABLE 2. Multivariate cox regression analysis for predictor of MACEs

Variables	P	OR	95% CI
Anterior wall MI	0.071	1.421	0.970-2.082
Previous MI	0.708	1.107	0.650-1.884
Apelin-12	< 0.001 [a]	0.132	0.060-0.292
Δapelin-12 (%)	0.411	0.991	0.970-1.012
Neutrophil (%)	0.186	1.011	0.995-1.027
eGFR	< 0.001 [a]	0.985	0.977-0.993
cTnI	0.203	1.017	0.991-1.044
LVEF	0.007 [a]	0.965	0.941-0.991
Pathological Q-wave	0.024 [a]	1.536	1.058-2.230
Killip's classification > I	0.016	0.610	0.408-0.912

[a] Statistically significant value (P < 0.05).

CI, confidence interval; cTnI, cardiac Troponin I; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; MI, myocardial infarction; OR, odds ratio.

TABLE 3. ROC analysis for in-hospital and 2.5-year MACEs

Parameters	AUC	95% CI	P	Threshold	Sensitivity，%	Specificity，%
2.5-year MACEs						
Δapelin-12	0.547	0.500-0.593	0.0906	20%	91.53	30.64
apelin-12	0.619	0.573-0.663	0.0001[a]	0.87	75.36	42.03
eGFR	0.565	0.518-0.611	0.0369[a]	86.13	53.39	63.87
LVEF	0.597	0.551-0.642	0.0015[a]	50%	59.32	56.94
Apelin-12 > 0.76 ng/ml						
Δapelin-12	0.530	0.454-0.605	0.4767	17%	92.31	39.81
Apelin-12≤0.76 ng/ml						
Δapelin-12	0.613	0.547-0.675	0.0075 [a]	20%	100.00	30.77
In-hospital MACEs						

Δapelin-12	0.507	0.460-0.553	0.8711	20%	3.23	73.44
apelin-12	0.623	0.577-0.667	0.0169 [a]	0.64	51.61	70.67
eGFR	0.543	0.497-0.589	0.4021	86.98	58.06	59.12
LVEF	0.674	0.629-0.716	0.0005[a]	52%	83.87	43.42

[a] Statistically significant value ($P < 0.05$).

TABLE 4. ROC analysis for 2.5-year MACEs among patients with separate level of renal function

Parameters	AUC	95% CI	P	Threshold	Sensitivity，%	Specificity，%
eGFR > 90 mL/min*1.73m^2（n=244）						
Δapelin-12	0.524	0.459-0.588	0.5556	20%	90.57	29.84
apelin-12	0.664	0.601-0.723	0.0001[a]	0.65	66.04	58.12
eGFR	0.508	0.443-0.572	0.8634	91.67	100	7.33
LVEF	0.628	0.564-0.689	0.0039[a]	50%	62.26	61.78
eGFR < 90 mL/min*1.73m^2（n=220）						
Δapelin-12	0.566	0.498-0.633	0.0885	20%	92.31	31.61
apelin-12	0.654	0.587-0.716	0.0001[a]	0.89	67.69	60.65
eGFR	0.562	0.494-0.629	0.1186	86.13	96.92	19.35
LVEF	0.561	0.492-0.627	0.1651	51%	63.08	48.39

[a] Statistically significant value compared with apelin-12 ($P < 0.05$).

BMJ Open

Predictive value of apelin-12 in ST-elevation myocardial infarction patients with different renal function: a prospective observational study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-018595.R1
Article Type:	Research
Date Submitted by the Author:	17-Sep-2017
Complete List of Authors:	Yang, Lingchang ; Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China Zheng, Ting ; Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China Wu, Haopeng ; Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China Xin, Wenwei ; Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China Mou, Xiongneng ; Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China Lin, Hui ; Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China Chen, Yide ; Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China Wu, Xiaoyu; Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Diagnostics, Emergency medicine
Keywords:	Adult cardiology < CARDIOLOGY, Ischaemic heart disease < CARDIOLOGY, Clinical trials < THERAPEUTICS

Predictive value of apelin-12 in ST-elevation myocardial infarction patients with different renal function: a prospective observational study

Running head: apelin-12 predict MACEs

Lingchang Yang MD, Ting Zheng MD, Haopeng Wu MD, Wenwei Xin MD, Xiongneng Mou MD, Hui Lin MD, Yide Chen MD, Xiaoyu Wu MD

Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China

Correspondence: Xiaoyu Wu Email: acmilancaca@126.com

Department of Emergency Medicine, The First People's Hospital of Taizhou, Zhejiang, 318020, China

Address: Hengjie Road 218, Huangyan District

Telephone: +86-18920815067

fax numbers: 0576-3979579

Word count: 2470

Abstract:

Objectives: To investigate factors predicting the onset of major adverse cardiovascular events (MACEs) after primary percutaneous coronary intervention (pPCI) for ST-segment elevation myocardial infarction (STEMI) patients.

Background: Apelin-12 plays an essential role in cardiovascular homeostasis. However, current knowledge of its predictive prognostic value is limited.

Methods: 464 STEMI patients (63.0±11.9 years, 355 men) who underwent successful pPCI were enrolled and followed for 2.5 years. Multivariate cox regression analysis and receiver operating characteristic (ROC) curve analysis were performed to determine the factors predicting MACEs.

Results: One hundred and eighteen patients (25.4%) experienced MACEs in the follow-up period. Multivariate cox regression analysis found low apelin-12 (HR=0.132, 95% CI=0.060-0.292, $p < 0.001$), low left ventricular ejection fraction (LVEF) (HR=0.965, 95%

CI=0.941-0.991,p=0.007), low estimated glomerular filtration rate (eGFR) (HR=0.985, 95% CI=0.977-0.993, p < 0.001), Killip's classification > I (HR=0.610, 95% CI=0.408-0.912,p=0.016), and pathological Q-wave (HR=1.536, 95% CI=1.058-2.230,p=0.024) were independent predictors of MACEs in the 2.5 year follow-up period. Low apelin-12 also predicted poorer in-hospital prognosis and MACEs in the 2.5 years follow-up period compared with Δapelin-12 (p=0.0115) and eGFR (p=0.0071) among patients with eGFR > 90 mL/min*1.73m^2. Further analysis showed Δapelin-12 < 20% was associated with MACEs in patients whose apelin-12 was below 0.76 ng/ml (p=0.0075) upon admission.

Conclusions: STEMI patients receiving pPCI with lower apelin-12 are more likely to suffer MACEs in hospital and 2.5-years post procedure, particularly in those with normal eGFR levels.

Key words: STEMI; Major adverse cardiovascular events; apelin-12; eGFR.

Strengths and limitations

1. Only STEMI patients receiving successful primary PCI and defined Δapelin-12 with apelin-12 elevation percent 72h after pPCI compared with apelin-12 level immediately prior to pPCI were enrolled.
2. The prognosis value of apelin-12 in predicting short-term (during hospitalization) and long-term (2.5 years) MACEs respectively was analyzed among patients with eGFR exceeding and below 90 mL/min*1.73m^2.
3. Limitations: The relatively small cohort size may affect the statistical results, therefore a larger-scale study is warranted.
4. The basic level of apelin prior to STEMI onset is difficult to measure; therefore the degree of reduction in apelin is unknown.
5. The present study focuses only on apelin-12, and the analysis of other forms of apelin is suggested.

INTRODUCTION

ST elevation myocardial infarction (STEMI) following successful primary percutaneous coronary intervention (pPCI) is the leading cause of mortality and morbidity worldwide in major adverse coronary events (MACEs) due to mechanical complications, acute heart failure, and cardiac shock after successful procedure[1]. Structural and functional alterations lead to progressive worsening of cardiac performance. The prognosis of STEMI following pPCI is influenced by several clinical, biochemical, and echocardiographic factors. Novel, more reliable biomarkers are urgently needed to precisely identify patients at high risk for adverse clinical outcomes in the follow-up period after pPCI and to aid in the development of individualized prevention programs [2, 3].

Apelin, a 77-amino acid peptide is the endogenous ligand for the human orphan G protein-coupled receptor (APJ) and is secreted by white adipose tissue. It is expressed in various cardiovascular tissues, including endothelial cells, coronary vessels, vascular smooth muscle cells, and cardiomyocytes[4]. The apelin–APJ system plays a role in cardiovascular homeostasis[5]. Apelin-12 may employ its cardio protective profile via the complex mechanism of improving

hypertension, insulin resistance, obesity, and cardiovascular risk factors[6-8]. However, the utility of serum apelin-12 level on admission in providing additional prognostic information among STEMI patients receiving pPCI remains unknown. There is limited evidence examining the involvement of apelin in STEMI and some research suggests it has no prognostic value[9-11], and one study found an inverse correlation between the level of apelin-12 and prognosis [12]. These results highlight the need for additional research.

The present study aims to determine the ability of plasma apelin-12 levels to predict short-term and long-term MACEs in patients with STEMI following successful pPCI. Further, the combined utilization of estimated glomerular filtration rate (eGFR) is examined.

METHODS

Inclusion and Exclusion Criteria and definition of MACEs

Patients were enrolled in the study if they presented with the onset of symptoms of STEMI at the First People's Hospital of Taizhou, Zhejiang, China between January 2010 and October 2014. STEMI symptoms included: persistent chest pain (>30 minutes), prolonged electrocardiogram (ECG) changes (including ischemic ST-segment elevation in two or more contiguous leads and/or depression), and significantly increased serum myocardial enzyme and troponin concentrations. Written informed consent was obtained, and the Research Ethics Committee of The First People's Hospital of Taizhou approved the study.

Exclusion criteria included non-STEMI; severe vascular heart disease; balloon angioplasty alone; rescue PCI; conservative treatment without PCI; previous onset of ventricular fibrillation; cardiogenic shock; untreated 3rd or advanced degree of atrioventricular block; estimated life expectancy <12 months; secondary hypertension; endocrine diseases such as thyroid dysfunction or adrenal cortical dysfunction; history of cerebrovascular attack (within one year) or cerebrovascular attack with a significant residual neurologic deficit; a history of chronic hepatitis or cirrhosis; severe renal insufficiency needing dialysis; known contraindication to statins, heparin, aspirin, clopidogrel, contrast or glycoprotein IIb/IIIa inhibitor (GPI); recent serious infection, connective tissue disease; malignancy; active severe bleeding; significant gastrointestinal or genitourinary bleeding; major surgery or trauma within six weeks; and incomplete clinical data.

Important definitions

A MACE is defined as the composite of cardiac death, recurrent target vessel myocardial infarction (RMI); clinically driven target lesion revascularization (TLR); cardiogenic shock; or demonstrated congestive heart failure (DCHF). The eGFR was estimated using the simplified Modification of Diet in Renal Disease (sMDRD) formula[13]. Δapelin-12 was defined as the level of apelin-12 elevation 72h after pPCI compared with apelin-12 level immediately before pPCI.

Therapy Process

A total of 464 patients underwent successful pPCI all of whom were diagnosed with STEMI and admitted to emergency room within 12 hours from onset. All patients received 300 mg oral aspirin and clopidogrel as well as standard heparin (initial 10,000 IU and boost during surgery), patients with high thrombotic burden utilize the GPI (uniformly tirofiban in our center), which was determined by our interventional physician. Ultrasound scans were taken within five days after PCI (median 4.2 days). The parameters of 2D echocardiography and Doppler were measured using standard methods (i.e. using biplane Simpson's method for measuring left ventricular

volumes and ejection fraction). Volumes were expressed as indices by normalizing with body surface area.

All the patients received 30-month follow-up after pPCI until MACEs. A small number of patients suffered MACEs requiring hospitalization. Patients were organized into MACEs group and non-MACEs group and independent predictors of poor prognosis were identified. The following biochemical indicators were measured: blood (including hemoglobin, neutrophil percent, hemoglobin, and platelet), coagulation, D-dimer, renal and hepatic function, Δapelin-12, peak myocardial enzyme, lipid level and fast blood glucose on the second day. Patients were further stratified into two subgroups according to the median value of apelin-12 level on admission, and into three further subgroups according to tertiles of eGFR.

Apelin-12 Elisa Detection

Serum was isolated by centrifugation within 1h at 2500g for 10 min, and stored at -80℃. Serum concentrations of apelin-12 were assayed using commercially available enzyme immunoassay kits (Phoenix Pharmaceuticals, Belmont, CA). The protocol was as follows: add 50μl/well of standard, sample, or positive control, 25μl of primary antibody and 25μl of biotinylated peptide; incubate at room temperature (20℃–23℃) for two hours; wash immunoplate four times with 350μl/well of 1×assay buffer; add 100μl/well of Streptavidin-HRP solution and incubate at room temperature for one hour. Wash immunoplate four times with 350μl/well of 1×assay buffer. Add 100μl/well of 3,3', 5,5'-tetramethylbenzidinesubstrate solution and incubate at room temperature for one hour. Terminate reaction with 100μl/well of 2N HCl. The detection limit was 0.1mg/L, with 1.26% and 5.4% intra- and inter-assay coefficients of variation respectively. The measurements were performed in triplicates.

Statistical Strategy

Continuous data were presented as mean ±standard deviation or medians with interquartile ranges, whereas categorical data were presented as a percentage, unless otherwise denoted. Univariate cox analysis and log-rank test were used for qualitative and quantitative variables to determine whether there is significant difference between the MACE and non-MACE groups. Variables with $P<0.1$ in the above analysis were selected for the multivariate cox proportional hazard analysis. The adjusted hazard ratio and 95% confidence interval were calculated. The Log-rank test was used to compare Kaplan–Meier curves of MACE-free survival in the two groups, divided by the median apelin-12. Finally, the receiver operating characteristic (ROC) analysis was used to identify the appropriate cutoff value of potential predictive indicators. The cutoff point was the maximum sum of sensitivity and specificity. The ROC curves were conducted with the Medcalc 12.3.0.0. All statistical tests were 2-tailed, performed using SPSS 17.0 (SPSS, Inc, Chicago,IL), and a P-value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of Patients in Individual Groups

Of the 464 enrolled patients, 118 (25.4%) had MACEs in the 2.5-year follow-up. Nineteen (4.1%) patients suffered cardiac death, while 27 (5.8%) RMI; 85 (18.3%) received TLR due to RMI or progressive stenosis. Thirty-five (7.5%) performed cardiogenic shock or demonstrated congestive heart failure onset. Among the MACEs group, 31 (6.7%) patients reached end point during hospitalization. 21 (4.5%) patients loss to follow up after discharge (Figure 1). Basic clinical characteristics, laboratory examinations, electrocardiogram results, angiographic, and

procedural characteristics are depicted in Table 1.

TABLE 1. Basic clinical characteristics, laboratory examinations, electrocardiogram results, angiographic and procedural characteristic

	MACEs (N=118)	Non-MACEs (N=346)	P value
Clinical characteristics			
Age, yr	67.0±12.2	61.7±11.6	0.252
Female, n (%)	33 (28.0)	76 (22.0)	0.184
Heart rate, beats per min	79.5±19.5	76.1±16.6	0.118
SBP, mm Hg	132.2±26.6	131.6±25.0	0.277
Anterior wall MI, n (%)	72 (61.0)	159 (46.0)	0.005 [a]
Killip's classification > I, n (%)	41 (34.7)	71 (20.5)	0.002[a]
Diabetes mellitus, n (%)	41 (34.7)	109 (31.5)	0.515
Hypertension, n (%)	72 (61.0)	193 (55.8)	0.321
Previous MI, n (%)	19 (16.1)	36 (10.4)	0.098
Lab examination			
Apelin-12, ng/ml	0.69 (0.53-0.87)	0.79 (0.63-1.03)	< 0.001 [a]
Δapelin-12 (%)	13.9 (5.6-17.6)	14.7 (5.3-22.3)	0.092
WBC ×10⁹/L	10.6±3.86	9.86±3.58	0.161
Neutrophil (%)	76.8±12.6	74.9±12.4	0.064
Hemoglobin, g/L	139.4±16.7	145.3±17.1	0.546
Platelet ×10⁹/L	240.2±60.1	227.8±57.2	0.264
Albumin, g/L	37.9±3.9	38.0±3.8	0.424

TC, mmol/L	5.87±0.99	5.57±1.17	0.469
TG, mmol/L	1.06±0.65	1.12±0.90	0.261
HDL-C, mmol/L	1.25±0.28	1.18±0.27	0.982
LDL-C, mmol/L	3.07±0.72	3.03±0.73	0.744
FBG, mmol/L	7.67±2.68	7.66±2.48	0.207
BUN, mmol/L	6.78±1.88	6.72±2.14	0.387
Creatinine, mmol/L	76.3±15.6	74.1±21.7	0.392
Uric acid, mmol/L	333.3±80.7	338.5±72.9	0.153
eGFR mL/min*1.73m^2	89.7±25.8	100.6±25.9	0.067
D-Dimer, mg/L	0.7 (0.2-1.6)	1.0 (0.2-1.7)	0.247
Peak CK-MB, U/L	131.5 (51.6-208.5)	103.0 (39.3-193.4)	0.252
Peak cTnI, ng/ml	21.5 (9.3-32.4)	12.6 (3.0-28.8)	0.014 [a]
Treatment			
ACEIs/ARBs, n (%)	94 (79.7)	294 (85.0)	0.178
b-blocker, n (%)	65 (55.1)	211 (61.0)	0.260
CCBs, n (%)	29 (24.6)	101 (29.2)	0.335
Statins, n (%)	97 (82.2)	288 (83.2)	0.796
Diuretics, n (%)	19 (16.1)	55 (15.9)	0.958
tirofiban, n (%)	15 (12.7)	52 (15.0)	0.536
Echocardiogram and electrocardiogram			
LAD, mm	38.5±5.3	37.0±5.7	0.311

LVEDD, mm	52.0±6.4	49.9±6.1	0.273
LVEF, %	47.3±9.4	51.9±7.3	0.010 [a]
Pathological Q-wave, n (%)	70 (59.3)	153 (44.2)	0.005 [a]
GENSINI	85.0 (48.8-100.1)	66.9 (37.2-101.7)	0.129
Culprit vessels, n (%)			
LAD	64 (54.2)	169 (48.8)	
LCX	18 (15.3)	54 (15.6)	0.557
RCA	36 (30.5)	123 (35.5)	
Stent number	1.33±0.55	1.39±0.57	0.524

Data are n/N (%) or mean±standard deviation or median (25th to 75th percentile).

[a] $P < 0.05$.

ACEIs, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BUN, blood urea nitrogen; CCBs, calcium channel blockers; CK-MB, creatinine kinase MB; cTnI, cardiac Troponin I; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HDL-C, high density lipoprotein; LAD, left anterior descending; LAD, left atrial diameter; LCX, left circumflex coronary artery; LDL-C, low-density lipoprotein; LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; MI, myocardial infarction; RCA, right coronary artery; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyeride; WBC, white blood cells.

Multivariate Logistic Regression Analysis of Predictor of MACEs

Significant differences were found between the two groups in peak cardiac Troponin I (cTnI) [21.5 (9.3-32.4) vs 12.6 (3.0-28.8), p=0.014] and left ventricular ejection fraction (LVEF) (47.3%±9.4%vs 51.9%±7.3%, P=0.010). Consistent with the hypotehsis, patients who suffered

from MACEs had lower apelin-12 on admission [0.69 (0.53-0.87)vs 0.79 (0.63-1.03), p < 0.001]. Anterior wall MI, pathological Q-wave, and higher Killip's classification were found more in the MACEs group compared to survivals (p=0.005, 0.005, and 0.002, respectively). Differences in Δapelin-12 [13.9(5.6-17.6) vs 14.7 (5.3-22.3), p=0.092], neutrophil percent (76.8±12.6 vs 74.9±12.4, p=0.064), eGFR (89.7±25.8 vs 100.6±25.9, p=0.067) and previous MI proportion (16.1% vs 10.4%, p=0.098) were approaching significance between the two groups.

Through the multivariate cox regression analysis, low apelin-12 (HR=0.132, 95% CI=0.060-0.292, p < 0.001), low eGFR (HR=0.985, 95% CI=0.977-0.993, p < 0.001), low LVEF (HR=0.965, 95% CI=0.941-0.991,p=0.007), and pathological Q-wave (HR=1.536, 95% CI=1.058-2.230,p=0.024) were independent predictors of MACEs within 2.5 years after pPCI, along with Killip's classification > I (HR=0.610, 95% CI=0.408-0.912,p=0.016). Anterior wall MI was moderately predictive of MACEs within 2.5 years (HR=1.421, 95% CI=0.970-2.082,p=0.071) (Table 2).

TABLE 2. Multivariate cox regression analysis for predictor of MACEs

Variables	P	OR	95% CI
Anterior wall MI	0.071	1.421	0.970-2.082
Previous MI	0.708	1.107	0.650-1.884
Apelin-12	< 0.001 [a]	0.132	0.060-0.292
Δapelin-12 (%)	0.411	0.991	0.970-1.012
Neutrophil (%)	0.186	1.011	0.995-1.027
eGFR	< 0.001 [a]	0.985	0.977-0.993
cTnI	0.203	1.017	0.991-1.044
LVEF	0.007 [a]	0.965	0.941-0.991
Pathological Q-wave	0.024 [a]	1.536	1.058-2.230
Killip's classification > I	0.016	0.610	0.408-0.912

[a] Statistically significant value (P < 0.05).

CI, confidence interval; cTnI, cardiac Troponin I; eGFR, estimated glomerular filtration rate;

LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; MI, myocardial infarction; OR, odds ratio.

Survival analysis

Kaplan-Meier curves in patients with higher (> 0.76 ng/ml, n=229) and lower (< 0.76 ng/ml, n=235) apelin-12 during 2.5-year follow-up (without MACEs during hospitalization) is shown in Figure 2. Significant differences in event-free survival were noted between patients with differing apelin-12 on admission (p=0.018).

ROC Analysis of Predictor of MACEs

ROC analysis failed to find the area under curve (AUC) of any indicator for 2.5-year MACEs exceeding 0.7, although the p value for apelin-12, eGFR and LVEF alone was 0.0001, 0.0369 and 0.0015 respectively, while the AUC of Δapelin-12 for 2.5-year MACEs was 0.547 (95% CI= 0.500-0.593, p=0.0906, Figure 3). When only in-hospital MACEs were observed, there was no evidence of predictive value except for LVEF < 52% (AUC=0.674, 95%CI=0.629-0.716, p=0.0005) and apelin-12≤0.64ng/ml (AUC=0.623, 95%CI=0.577-0.667, p=0.0169, Figure 4 & Table 3). ROC analysis ofΔapelin-12 found a higher AUC with a cut-off point of 20% only for those with apelin-12 on admission ≤0.76 ng/ml (p=0.0075, Table 3).

TABLE 3. ROC analysis for in-hospital and 2.5-year MACEs

Parameters	AUC	95% CI	P	Threshold	Sensitivity , %	Specificity , %
2.5-year MACEs						
Δapelin-12	0.547	0.500-0.593	0.0906	20%	91.53	30.64
apelin-12	0.619	0.573-0.663	0.0001[a]	0.87	75.36	42.03
eGFR	0.565	0.518-0.611	0.0369[a]	86.13	53.39	63.87

LVEF	0.597	0.551-0.642	0.0015[a]	50%	59.32	56.94
Apelin-12 > 0.76 ng/ml						
Δapelin-12	0.530	0.454-0.605	0.4767	17%	92.31	39.81
Apelin-12≤ 0.76 ng/ml						
Δapelin-12	0.613	0.547-0.675	0.0075[a]	20%	100.00	30.77
In-hospital MACEs						
Δapelin-12	0.507	0.460-0.553	0.8711	20%	3.23	73.44
apelin-12	0.623	0.577-0.667	0.0169[a]	0.64	51.61	70.67
eGFR	0.543	0.497-0.589	0.4021	86.98	58.06	59.12
LVEF	0.674	0.629-0.716	0.0005[a]	52%	83.87	43.42

[a] Statistically significant value (P < 0.05).

eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; AUC, area under curves

When patients were further subdivided according to eGFR, it was found that 224 patient with eGFR over 90 mL/min*1.73m^2. Among these patients, apelin-12 had a predictive advantage for MACEs compared with Δapelin-12 (p=0.0115) and eGFR (p=0.0071). Moreover, LVEF was only predictive in patients with eGFR > 90 mL/min*1.73m2 (AUC=0.628, 95%CI=0.564-0.689, p=0.0039, Table 4), in other words, among patients with eGFR over 90 mL/min*1.73m2 , apelin-12 perform the most ideal prognostic factor.

TABLE 4. ROC analysis for 2.5-year MACEs among patients with separate level of renal function

Parameters	AUC	95% CI	P	Threshold	Sensitivity ,%	Specificity , %
eGFR > 90 mL/min*1.73m^2 (n=244)						
Δapelin-12	0.524	0.459-0.588	0.5556	20%	90.57	29.84
apelin-12	0.664	0.601-0.723	0.0001[a]	0.65	66.04	58.12
eGFR	0.508	0.443-0.572	0.8634	91.67	100	7.33
LVEF	0.628	0.564-0.689	0.0039[a]	50%	62.26	61.78
eGFR < 90 mL/min*1.73m^2 (n=220)						

Δapelin-12	0.566	0.498-0.633	0.0885	20%	92.31	31.61
apelin-12	0.654	0.587-0.716	0.0001[a]	0.89	67.69	60.65
eGFR	0.562	0.494-0.629	0.1186	86.13	96.92	19.35
LVEF	0.561	0.492-0.627	0.1651	51%	63.08	48.39

[a] Statistically significant value compared with apelin-12 (P < 0.05).

eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; AUC, area under curves

Discussion

The clinical outcomes in STEMI patients receiving pPCI are closely associated with the pre-PCI apelin-12 concentration, particularly among those with normal renal function. In further subgroup analysis, the change of apelin during hospitalization was found to predict long-term prognosis among patients with low apelin level upon admission (apelin-12≤0.76 ng/ml).

Apelin may then play a role in assessing risk stratification among STEMI patients given it's role in the pathophysiology of both heart failure and ischemia/reperfusion injury[14]. Apelin has been found to increase contractility and reduce peripheral resistance via endothelial nitric oxide (NO)-dependent signaling[15] in failing myocardial cells to slow the pathological progress of heart failure[16]. Apelin expression has been found to decline in decompensated states, whereas it is maintained or augmented in stable chronic heart failure[17].

In patients with stable angina, plasma apelin was negatively associated with coronary artery stenosis severity independent of other cardiovascular risk factors[18]. Weir and colleagues demonstrated plasma apelin level is reduced immediately after acute myocardial infarction but increases markedly after revascularization. Despite this, it remains depressed at 24 weeks[19]. A potential explanation of lower levels of apelin following STEMI include:(1) the demand of apelin among STEMI patients increases, therefore more apelin is consumed immediately after MI episodes; (2) product plunging due to MI. A recent study demonstrated the flow-mediated adjustment of the apelin/APJ system in human endothelial cells and found apelin-12 expression is induced by shear stress independently of its ligand, particularly during reperfusion[20]. Further,

hypoxia-inducible factor (HIF)-mediated pathways participate in the apelin up-regulation in myocardium, pulmonary circulation, and skeletal muscles following systemic hypoxic exposure or myocardial injury[21]. Therefore, the apelin-APJ system may alleviate the myocardial reperfusion mediated oxidative stress and apoptosis by increasing superoxide dismutase degradation, thereby decreasing the generation of reactive oxygen species, along with up-regulating eNOS levels and activating ERK1/2 phosphorylation signaling[22]. The above results may be responsible for the poor short-term outcomes and long-term prognosis observed in patients with low levels of apelin.

Apelin can provide myocardial protection against ischemic damage by decreasing permeability of microvascular endothelial cells via up-regulating the expression of Tie-2 and VEGFR2[23], and improving neovascularization via recruiting circulating Aplnr+ cells during early-phase myocardial repair [24]. The Apelin/APJ system promotes angiogenesis and provides nutrients and oxygen to the ischemic area in MI animal models[25]. Apelin gene therapy by myocardial injection ameliorates cardiac repair, improves cardiac metabolism via activating Sirt3, and up-regulating VEGF/VEGFR2 expression in post-MI mice[26, 27]. Conversely, apelin down-regulation exacerbates ischemia-reperfusion injury and myocardial infarction adverse remodeling[25]. A recently published study proved apelin 12 is able to protect pro-thrombotic effects of other adipokine such as apelin-13[28]. Apelin protects against angiotensin II-induced cardiovascular fibrosis and decreases plasminogen activator inhibitor type-1 production[29]. The utilization rate of ACEI and β blockers was similar among patients with different prognosis in the present study, eliminating the drug-derived influence on angiotensin activity. Myeloid cell-derived Leucine-rich α2-glycoprotein attenuates adverse cardiac remodeling after MI via up-regulating the expression of apelin receptor[30]. Direct anti-inflammatory[31] and anti-atherogenic properties[32] are also reported as mechanisms of atherosclerotic lesions and aortic aneurysms prevention by apelin. Stress-induced apoptosis in serious cardiovascular diseases is inhibited by cardiac apelin expression elevation[33]. TIMP3 maintains metabolic flexibility via apelin during cardiac stress seizure[34]. Taken together, the above cardiovascular profile suggests a beneficial effect of apelin on atherosclerosis, and makes apelin-APJ system a promising therapeutic target in acute coronary syndrome[35].

Liu and colleagues[36] have demonstrated the effect of serum apelin-12 in predicting one-year outcomes following pPCI in patients with STEMI. Topuz and colleagues[37] found lower levels of serum apelin-12 predicts higher incidence of in-hospital MACEs after multivariate regression analysis and is more likely in no-reflow vs normal flow group. This is a result of NO-dependent vasodilatation caused by apelin-12 in clinical studies[38] as well as in animal models[39]. Abnormal level of apelin and a series of adipokines observed in acute MI patients resulted in a high incidence of MACEs during three-year follow-up[40]. Low apelin appears to correlate with carotid plaque vulnerability in patients with carotid stenosis. Atorvastatin-induced apelin modification may beneficially affect carotid plaque stability[41].

We hypothesis the potential explanation of the subgroup analysis according to different renal function is that patients with relatively normal level of eGFR fail to perform enough discrepancy to distinguish high-risk patients, to these patients, our novel index apelin-12 show its superiority in predicting MACEs.

The present study is not without limitations. The study cohort was relatively small. which may affect the statistical results. A larger-scale study is warranted to further assess the risk of

long-term MACEs after pPCI in STEMI patients. The base level of apelin prior to STEMI onset is difficult to obtain, therefore the degree of apelin reduction is unknown. Finally this study only focused on apelin-12, analysis on other forms of apelin is recommended.

CONCLUSION

In conclusion, STEMI patients receiving pPCI with lower levels of apelin-12 are more likely to have poor short-term and long-term prognosis after adjusting for other clinical parameters. Further, apelin-12 may be beneficial in predicting MACEs among patients with eGFR > 90 mL/min*1.73m^2.

a. Contributor statement

Xiaoyu Wu conceived and designed the study. XiongnengMou, Hui Lin, Yide Chen collect the statistics. Lingchang Yang, Ting Zheng, Haopeng Wu performed the statistical analysis. Lingchang Yang wrote the paper. Wenwei Xin and Xiaoyu Wu reviewed and edited the manuscript. All authors read and approved the manuscript.

b. competing interests

There is no competing interests in our study.

c. funding

The authors received no financial support for the research,authorship, and/or publication of this article.

d. data sharing statement

No additional unpublished data are available

Figure legends

Figure 1. patients selecting process and results reported.

Figure 2. Kaplan-Meier curves in STEMI patients with individual levels of apelin-12during 2.5 years follow-up (without MACEs during hospitalization).

Figure 3. Receiver operating characteristic curve of apelin-12, eGFR, LVEF, and Δapelin-12 for predicting 2.5-year MACEs after pPCI among STEMI patients. LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; eGFR, estimated glomerular filtration rate.

Figure 4. Receiver operating characteristic curve of apelin-12, eGFR, LVEF, and Δapelin-12 for predicting in-hospital MACEs after pPCI among STEMI patients. LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; eGFR, estimated glomerular filtration rate.

References:

[1] Kilic S, Ottervanger JP, Dambrink JH, Hoorntje JC, et al. The effect of thrombus aspiration during primary percutaneous coronary intervention on clinical outcome in daily clinical practice. Thromb Haemost. 2014;**111**(1):165-171 'doi': 10.1160/TH13-05-0433['2014-01-01].

[2] Steg PG, James SK, Atar D, Badano LP, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. Eur Heart J. 2012;**33**(20):2569-2619 'doi': 10.1093/eurheartj/ehs215['2012-10-01].

[3] Pedersen F, Butrymovich V, Kelbaek H, Wachtell K, et al. Short- and long-term cause of death in

patients treated with primary PCI for STEMI. J Am Coll Cardiol. 2014;**64**(20):2101-2108 'doi': 10.1016/j.jacc.2014.08.037['Copyright (c) 2014 American College of Cardiology Foundation. Published by

Elsevier Inc. All rights reserved.:2014-11-18].

[4] Chandrasekaran B, Dar O, McDonagh T. The role of apelin in cardiovascular function and heart failure. Eur J Heart Fail. 2008;**10**(8):725-732 'doi': 10.1016/j.ejheart.2008.06.002['2008-08-01].

[5] Yi L, Hou X, Zhou J, Xu L, et al. HIF-1alpha genetic variants and protein expression confer the susceptibility and prognosis of gliomas. Neuromolecular Med. 2014;**16**(3):578-586 'doi': 10.1007/s12017-014-8310-1['2014-09-01].

[6] Sonmez A, Celebi G, Erdem G, Tapan S, et al. Plasma apelin and ADMA Levels in patients with essential hypertension. Clin Exp Hypertens. 2010;**32**(3):179-183 'doi': 10.3109/10641960903254505['2010-05-01].

[7] Boal F, Roumegoux J, Alfarano C, Timotin A, et al. Apelin regulates FoxO3 translocation to mediate cardioprotective responses to myocardial injury and obesity. Sci Rep. 2015;**5**:16104 'doi': 10.1038/srep16104['2015-11-06].

[8] Leal VO, Lobo JC, Stockler-Pinto MB, Farage NE, et al. Apelin: a peptide involved in cardiovascular risk in hemodialysis patients? Ren Fail. 2012;**34**(5):577-581 'doi': 10.3109/0886022X.2012.668490['2012-01-20].

[9] Lakomkin SV, Tereshchenko SN, Sychev AV, Masenko VP, et al. [Biomarkers in Heart Failure: Apelin Level Is Not Associated With Presence and Severity of the Disease]. Kardiologiia. 2015;**55**(2):37-412015-01-20].

[10] Cosansu K, Cakmak HA, Ikitimur B, Yildirim E, et al. Apelin in ST segment elevation and non-ST segment elevation acute coronary syndromes: a novel finding. Kardiol Pol. 2014;**72**(3):239-245 'doi': 10.5603/KP.a2013.0251['2014-01-20].

[11] Kuklinska AM, Sobkowicz B, Sawicki R, Musial WJ, et al. Apelin: a novel marker for the patients with first ST-elevation myocardial infarction. Heart Vessels. 2010;**25**(5):363-367 'doi': 10.1007/s00380-009-1217-3['2010-09-01].

[12] Sans-Rosello J, Casals G, Rossello X, Gonzalez DLPB, et al. Prognostic value of plasma apelin concentrations at admission in patients with ST-segment elevation acute myocardial infarction. Clin Biochem. 2016 'doi': 10.1016/j.clinbiochem.2016.11.018['Copyright A(c) 2016 The Canadian Society of Clinical Chemists. Published by

Elsevier Inc. All rights reserved.:2016-11-24].

[13] Manjunath G, Sarnak MJ, Levey AS. Prediction equations to estimate glomerular filtration rate: an update. Curr Opin Nephrol Hypertens. 2001;**10**(6):785-7922001-11-01].

[14] Tycinska AM, Lisowska A, Musial WJ, Sobkowicz B. Apelin in acute myocardial infarction and heart failure induced by ischemia. Clin Chim Acta. 2012;**413**(3-4):406-410 'doi': 10.1016/j.cca.2011.11.021['Copyright (c) 2011 Elsevier B.V. All rights reserved.:2012-02-18].

[15] Scimia MC, Hurtado C, Ray S, Metzler S, et al. APJ acts as a dual receptor in cardiac hypertrophy. Nature. 2012;**488**(7411):394-398 'doi': 10.1038/nature11263['2012-08-16].

[16] Chong KS, Gardner RS, Morton JJ, Ashley EA, McDonagh TA. Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. Eur J Heart Fail. 2006;**8**(4):355-360 'doi': 10.1016/j.ejheart.2005.10.007['2006-06-01].

[17] Japp AG, Newby DE. The apelin-APJ system in heart failure: pathophysiologic relevance and therapeutic potential. Biochem Pharmacol. 2008;**75**(10):1882-1892 'doi':

10.1016/j.bcp.2007.12.015['2008-05-15].

[18] Kadoglou NPE, Lampropoulos S, Kapelouzou A, Gkontopoulos A, et al. Serum levels of apelin and ghrelin in patients with acute coronary syndromes and established coronary artery disease—KOZANI STUDY. Translational Research. 2010;**155**(5):238-246 'doi': 10.1016/j.trsl.2010.01.004['2010-05-01].

[19] Weir RA, Chong KS, Dalzell JR, Petrie CJ, et al. Plasma apelin concentration is depressed following acute myocardial infarction in man. Eur J Heart Fail. 2009;**11**(6):551-558 'doi': 10.1093/eurjhf/hfp043['2009-06-01].

[20] Busch R, Strohbach A, Pennewitz M, Lorenz F, et al. Regulation of the endothelial apelin/APJ system by hemodynamic fluid flow. Cell Signal. 2015;**27**(7):1286-1296 'doi': 10.1016/j.cellsig.2015.03.011['Copyright (c) 2015 Elsevier Inc. All rights reserved.:2015-07-01].

[21] Ronkainen VP, Ronkainen JJ, Hanninen SL, Leskinen H, et al. Hypoxia inducible factor regulates the cardiac expression and secretion of apelin. FASEB J. 2007;**21**(8):1821-1830 'doi': 10.1096/fj.06-7294com['2007-06-01].

[22] Zeng XJ, Zhang LK, Wang HX, Lu LQ, Ma LQ, Tang CS. Apelin protects heart against ischemia/reperfusion injury in rat. Peptides. 2009;**30**(6):1144-1152 'doi': 10.1016/j.peptides.2009.02.010['2009-06-01].

[23] Zhang BH, Guo CX, Wang HX, Lu LQ, et al. Cardioprotective effects of adipokine apelin on myocardial infarction. Heart Vessels. 2014;**29**(5):679-689 'doi': 10.1007/s00380-013-0425-z['2014-09-01].

[24] Tempel D, de Boer M, van Deel ED, Haasdijk RA, et al. Apelin enhances cardiac neovascularization after myocardial infarction by recruiting aplnr+ circulating cells. Circ Res. 2012;**111**(5):585-598 'doi': 10.1161/CIRCRESAHA.111.262097['2012-08-17].

[25] Wang W, McKinnie SM, Patel VB, Haddad G, et al. Loss of Apelin exacerbates myocardial infarction adverse remodeling and ischemia-reperfusion injury: therapeutic potential of synthetic Apelin analogues. J Am Heart Assoc. 2013;**2**(4):e249 'doi': 10.1161/JAHA.113.000249['2013-07-01].

[26] Hou X, Zeng H, He X, Chen JX. Sirt3 is essential for apelin-induced angiogenesis in post-myocardial infarction of diabetes. J Cell Mol Med. 2015;**19**(1):53-61 'doi': 10.1111/jcmm.12453['(c) 2014 The Authors. Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.:2015-01-01].

[27] Li L, Zeng H, Hou X, He X, Chen JX. Myocardial injection of apelin-overexpressing bone marrow cells improves cardiac repair via upregulation of Sirt3 after myocardial infarction. PLoS One. 2013;**8**(9):e71041 'doi': 10.1371/journal.pone.0071041['2013-01-20].

[28] Cirillo P, Ziviello F, Pellegrino G, Conte S, et al. The adipokine apelin-13 induces expression of prothrombotic tissue factor. Thromb Haemost. 2015;**113**(2):363-372 'doi': 10.1160/TH14-05-0451['2015-02-01].

[29] Siddiquee K, Hampton J, Khan S, Zadory D, et al. Apelin protects against angiotensin II-induced cardiovascular fibrosis and decreases plasminogen activator inhibitor type-1 production. J Hypertens. 2011;**29**(4):724-731 'doi': 10.1097/HJH.0b013e32834347de['2011-04-01].

[30] Kumagai S, Nakayama H, Fujimoto M, Honda H, et al. Myeloid cell-derived LRG attenuates adverse cardiac remodelling after myocardial infarction. Cardiovasc Res. 2016;**109**(2):272-282 'doi': 10.1093/cvr/cvv273['Published on behalf of the European Society of Cardiology. All rights reserved. (c) The Author 2015. For permissions please email: journals.permissions@oup.com.:2016-02-01].

[31] El-Shehaby AM, El-Khatib MM, Battah AA, Roshdy AR. Apelin: a potential link between inflammation and cardiovascular disease in end stage renal disease patients. Scand J Clin Lab Invest. 2010;**70**(6):421-427 'doi': 10.3109/00365513.2010.504281['2010-10-01].

[32] Yu XH, Tang ZB, Liu LJ, Qian H, et al. Apelin and its receptor APJ in cardiovascular diseases. Clin Chim Acta. 2014;**428**:1-8 'doi': 10.1016/j.cca.2013.09.001['(c) 2013. Elsevier B.V. All rights reserved.:2014-01-20].

[33] Ustunel I, Acar N, Gemici B, Ozbey O, et al. The effects of water immersion and restraint stress on the expressions of apelin, apelin receptor (APJR) and apoptosis rate in the rat heart. Acta Histochem. 2014;**116**(5):675-681 'doi': 10.1016/j.acthis.2013.12.004['Copyright (c) 2013 Elsevier GmbH. All rights reserved.:2014-06-01].

[34] Stohr R, Kappel BA, Carnevale D, Cavalera M, et al. TIMP3 interplays with apelin to regulate cardiovascular metabolism in hypercholesterolemic mice. Mol Metab. 2015;**4**(10):741-752 'doi': 10.1016/j.molmet.2015.07.007['2015-10-01].

[35] Pisarenko OI, Serebryakova LI, Studneva IM, Pelogeykina YA, et al. Effects of structural analogues of apelin-12 in acute myocardial infarction in rats. J Pharmacol Pharmacother. 2013;**4**(3):198-203 'doi': 10.4103/0976-500X.114600['2013-07-01].

[36] Liu HT, Chen M, Yu J, Li WJ, et al. Serum apelin level predicts the major adverse cardiac events in patients with ST elevation myocardial infarction receiving percutaneous coronary intervention. Medicine (Baltimore). 2015;**94**(4):e449 'doi': 10.1097/MD.0000000000000449['2015-01-01].

[37] Topuz M, Oz F, Akkus O, Sen O, et al. Plasma apelin-12 levels may predict in-hospital major adverse cardiac events in ST-elevation myocardial infarction and the relationship between apelin-12 and the neutrophil/lymphocyte ratio in patients undergoing primary coronary intervention. Perfusion. 2017;**32**(3):206-213 'doi': 10.1177/0267659116676335['2017-04-01].

[38] Japp AG, Cruden NL, Barnes G, van Gemeren N, et al. Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure. Circulation. 2010;**121**(16):1818-1827 'doi': 10.1161/CIRCULATIONAHA.109.911339['2010-04-27].

[39] Zhang M, Mahoney E, Zuo T, Manchanda PK, Davuluri RV, Kirschner LS. Protein kinase A activation enhances beta-catenin transcriptional activity through nuclear localization to PML bodies. PLoS One. 2014;**9**(10):e109523 'doi': 10.1371/journal.pone.0109523['2014-01-20].

[40] Grzywocz P, Mizia-Stec K, Wybraniec M, Chudek J. Adipokines and endothelial dysfunction in acute myocardial infarction and the risk of recurrent cardiovascular events. J Cardiovasc Med (Hagerstown). 2015;**16**(1):37-44 'doi': 10.2459/JCM.0000000000000042['2015-01-01].

[41] Kadoglou NP, Sailer N, Moumtzouoglou A, Kapelouzou A, et al. Adipokines: a novel link between adiposity and carotid plaque vulnerability. Eur J Clin Invest. 2012;**42**(12):1278-1286 'doi': 10.1111/j.1365-2362.2012.02728.x['(c) 2012 The Authors. European Journal of Clinical Investigation (c) 2012

Stichting European Society for Clinical Investigation Journal Foundation.:2012-12-01].

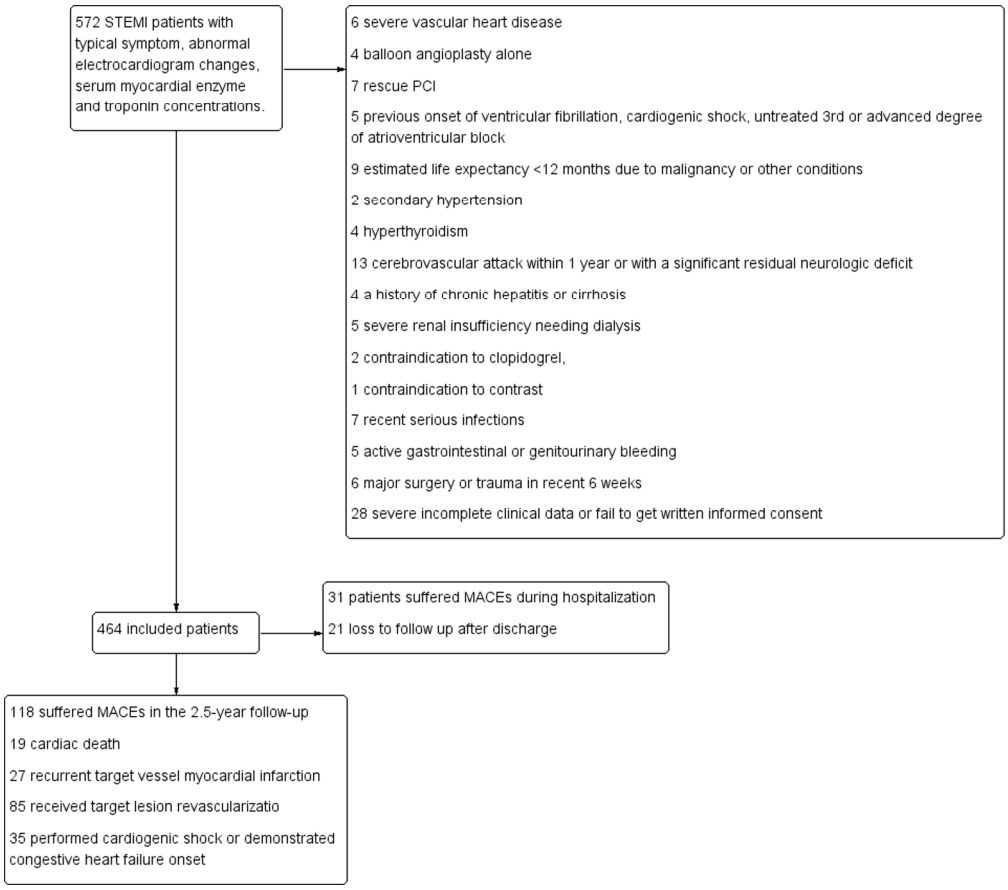

Figure 1. patients selecting process and results reported.

252x222mm (300 x 300 DPI)

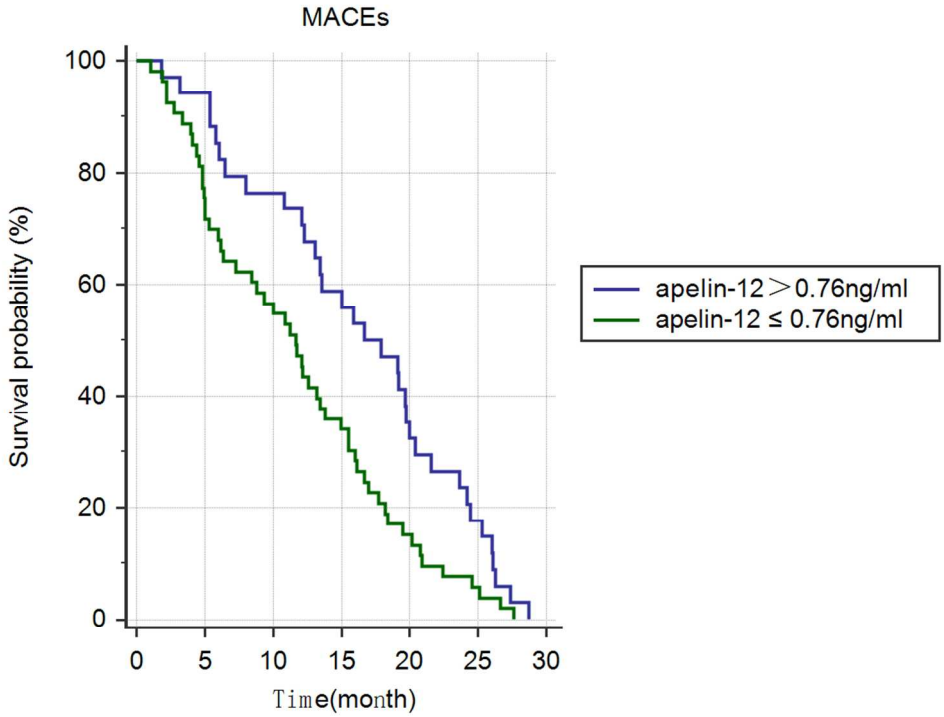

Figure 2. Kaplan-Meier curves in STEMI patients with individual levels of apelin-12during 2.5 years follow-up (without MACEs during hospitalization).

101x76mm (300 x 300 DPI)

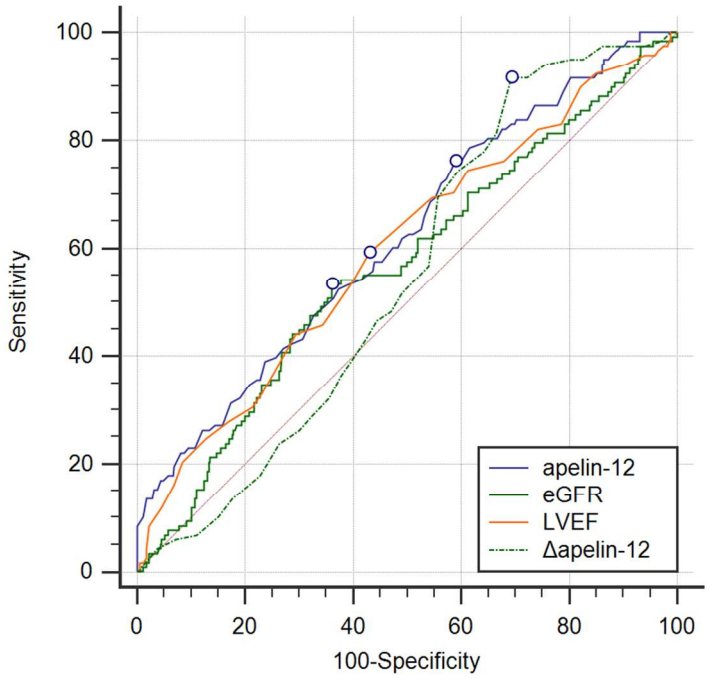

Figure 3. Receiver operating characteristic curve of apelin-12, eGFR, LVEF, and Δapelin-12 for predicting 2.5-year MACEs after pPCI among STEMI patients. LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; eGFR, estimated glomerular filtration rate.

101x76mm (300 x 300 DPI)

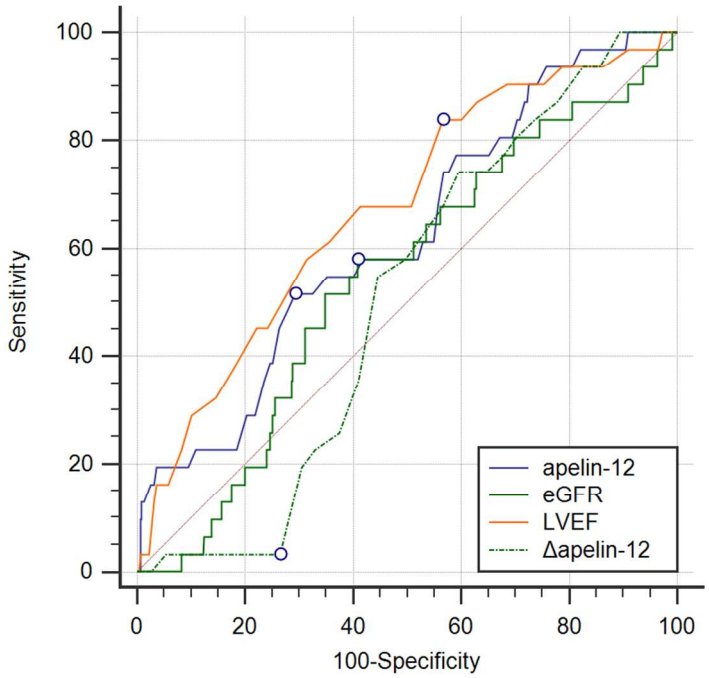

Figure 4. Receiver operating characteristic curve of apelin-12, eGFR, LVEF, and Δapelin-12 for predicting in-hospital MACEs after pPCI among STEMI patients. LVEF, left ventricular ejection fraction; MACEs, major adverse cardiovascular events; eGFR, estimated glomerular filtration rate

101x76mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	2-3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	2-3
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	2-3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*2-3*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	2-3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	2-4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	3-4
		(*b*) Describe any methods used to examine subgroups and interactions	3-4
		(*c*) Explain how missing data were addressed	3-4
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4-5
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4-5
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	4
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	4-5
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	4-5
Discussion			
Key results	18	Summarise key results with reference to study objectives	5-6
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.