hsCRP and ET-1 expressions in patients with no-reflow phenomenon after Percutaneous Coronary Intervention

Min Liu¹, Tian Liang², Peiying Zhang³, Qing Zhang⁴, Lei Lu⁵, Zhongliang Wang⁶

ABSTRACT
Objective: To explore hsCRP and ET-1 expressions in patients with no-reflow phenomenon after percutaneous coronary intervention (PCI).

Methods: A total of 136 patients with single coronary artery disease receiving PCI were divided into a reflow group and a no-reflow group to compare the level use of ET-1 alone with combined level of ET-1 and hs-CRP in PCI regarding sensitivity, specificity, positive and negative predictive values and accuracy for postoperative no-reflow. The study was conducted between 2014-2016 at our hospital.

Results: Postoperative levels of ET-1 and hs-CRP in no-reflow group were significantly higher than those of reflow group (P<0.05). ET-1 level of reflow group peaked three hours after PCI and then declined. Serum level of hs-CRP decreased most obviously within three hours after PCI in reflow group and three - three days after PCI in no-reflow group. Left ventricular end-diastolic diameters of both groups after PCI were apparently lower than those before PCI, without significant inter-group difference (P>0.05). Left ventricular end-systolic diameters and left ventricular ejection fractions of both groups evidently increased after PCI, without significant inter-group differences either (P>0.05). Corrected TIMI frame count (CTFC) and wall motion score index of reflow group after PCI were significantly lower than those of no-reflow group (P<0.05). ET-1 level was positively correlated with CTFC (P<0.05). Multivariate linear regression showed hs-CRP was negatively correlated with the serum level (P<0.05) (r=-0.34).

Conclusion: hsCRP and ET-1 levels significantly increased in patients with no-reflow phenomenon.

KEYWORDS: Coronary artery disease, ET-1, hs-CRP, Percutaneous coronary intervention.

INTRODUCTION

In general, acute myocardial infarction can be classified into acute ST-segment elevation myocardial infarction (STEMI) and acute non-ST-segment elevation myocardial infarction.¹ ² For patients with STEMI, early rapid emergency thrombolytic therapy and/or percutaneous coronary intervention (PCI) can significantly reduce mortality and improve prognosis by clearing infarcted coronary arteries.³ However, thromboembolism, microthrombosis and other factors after PCI may cause no-recanalization of part of microvessels of the myocardium, resulting in no-reflow phenomenon and seriously affecting the clinical prognosis and outcomes of patients.
No-reflow phenomenon means that part of infarct-related artery (IRA) has no forward blood flow after PCI in the absence of dissection, thrombosis, spasm or distal embolization.\(^4\)

The pathophysiological mechanism of no-reflow remains unclear hitherto, but it has been attributed to severe local vascular spasm, obstruction, ischemia of corresponding tissues and organs (generally for 40 to 60 minutes), and release of many inflammatory factors. On this basis, no-reflow phenomenon easily occurs when the ischemic region cannot immediately be perfused with sufficient blood after recanalization for blood flow recovery.\(^5\)

Endothelin (ET), which exists commonly in vascular endothelial cells and also widely in various mammalian tissues and cells, is an important factor regulating cardiovascular function. It plays a crucial role in maintaining basic vascular tension and cardiovascular system homeostasis.\(^6\)

Endothelial cells are stimulated to synthesize and to release ET-1, mainly at the gene transcription level. ET-1 synthesis can be stimulated by epinephrine, thromboxane, vasopressin, angiotensin, insulin, cytokines and physical factors such as changes of vascular wall shear force and pressure and hypoxia. The process of stimulating ET-1 synthesis involves Ca\(^{2+}\)-dependent protein kinases.\(^7\) The factors that inhibit ET-1 synthesis include NO, PGI2, atrial natriuretic peptide, heparin, etc. With a very short half-life in plasma (<5 min), ET-1 can quickly bind tissue receptors. It can be rapidly decomposed by ET-degrading enzymes, mainly in lungs and kidneys.\(^8\)

High-sensitivity C-reactive protein (hs-CRP) has an extremely low content in the peripheral blood under normal conditions, but upon inflammation, such level can, as a stress reaction, markedly increase within a short time. It is well-documented that hs-CRP strongly indicate myocardial infarction and other inflammatory diseases.\(^9\) CRP is often synthesized by liver cells due to stimulation of interleukin-6 and other inflammatory molecules.\(^10\) In this study, we combined hs-CRP with ET-1 to detect their dynamic changes before and after PCI and the correlation, aiming to provide a scientific basis for the no-reflow phenomenon after PCI.

**METHODS**

A total of 136 patients with single coronary artery disease admitted in our hospital from June 2014 to August 2016 were selected, including 78 males and 58 females aged 42-79 years old, \((59.4 \pm 18.7)\) on average. The patients after PCI were then divided into a reflow group (98 cases) and a no-reflow group (38 cases).

**Inclusion criteria:** All enrolled patients which met the STEMI diagnostic criteria developed by the American College of Cardiology/American Heart Association.\(^2\) They suffered from various degrees of typical precordial pain or discomfort, finally diagnosed as single coronary artery disease by coronary angiography. This study was been approved by the ethics committee of our hospital. The patients and their families had signed informed consent.

**Exclusion criteria:**
1. Complicated with other cardiovascular diseases such as endocarditis, valvular lesions and congestive heart failure;
2. Use of immunosuppressive agents;
3. Acute and chronic bacterial and/or viral infections;
4. Autoimmune diseases;
5. Connective tissue diseases;
6. Malignant tumors;
7. Liver and kidney dysfunctions;
8. Chronic muscular diseases;
9. Atorvastatin allergy;
10. Peripheral vascular diseases, chronic heart failure, thyroid diseases and surgeries within the last six months owing to major injuries;
11. Myocardial infarction, percutaneous transluminal coronary angioplasty or coronary artery bypass grafting within the last six months, recent use of adrenal cortex hormones or other immunomodulators, incompliance of patients or their families, and history of mental illness.

**PCI method:** The IRA lesion was sucked 3-5 times with a thrombus suction catheter, which was increased when necessary to lower the thrombus load effectively and to open the forward blood flow. Afterwards, most of intracoronary thrombosis was eliminated to wipe out continuous retention of contrast agent, floating thrombus or residual of IRA distal thrombus fragments. After 50-100 μg nitroglycerin was intracoronarily injected, delayed angiography was performed. According to the vascular diameter, stent implantation was started when the stent successfully passed or the residual stenosis was less than 70%. Alternatively, pre-dilation was conducted before implantation at an appropriate pressure by using a balloon (5-14 atm). Prior to stent implantation or balloon pre-dilation, the contrast agent amount and the angiography number were minimized, and the interval between two angiographies was extended.
Method for determining immediate postoperative perfusion of epicardial blood vessels and myocardium: Two experienced interventional surgeons analyzed the TIMI blood flow grade, corrected TIMI frame count (CTFC) and wall motion score index (WMSI) of IRA.

Criteria for successful treatment: In at least two orthogonal projections, angiography showed that the stent well adhered to the vascular wall and the residual stenosis was <20%, with TIMI grade 3 and without severe clinical complications such as sudden cardiac death, myocardial infarction and acute coronary artery bypass.

Angiographic criteria for no-reflow phenomenon: Angiography disclosed no or obviously decelerated forward blood flow after PCI, with TIMI grade 2.

Serological detection: Fasting cubital venous blood (8-12 ml) was drawn, of which 2 ml was used to detect hs-CRP levels before as well as half an hour, three hours and three days after PCI by rapid fluorescence immunoassay (Triage meter), and the remaining 4-6 ml was centrifuged at 1,200 r/minutes for 10 minutes. The resulting serum was stored in a refrigerator at -80°C, and the level of ET-1 was detected before as well as half an hour, three hours and three days after PCI by the enzyme-linked immunosorbent assay.

Echocardiographic examination: Echocardiography was performed by two experienced ultrasound physicians strictly according to the guideline of American Society of Echocardiography. All patients received echocardiography by color Doppler diagnosis using a Philips iE33 ultrasound machine (probe frequency: 2.5 MHz) before PCI, on the first day after PCI and during follow-up respectively. The left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD) and left ventricular ejection fraction (LVEF) were recorded for statistical analysis.

Statistical analysis: All data were analyzed by SPSS19.0. The qualitative data were compared by the χ^2 test, and the ineligible fourfold table data were subjected to the Fisher’s exact test. The quantitative data were compared by the analysis of variance. All detected factors were subjected to the Pearson’s correlation analysis. P<0.05 was considered statistically significant.

RESULTS

Baseline clinical data: The reflow and no-reflow groups had similar gender ratio, age, fasting blood glucose level, LDL, HDL, BMI and MAP (P>0.05) (Table-I).

Postoperative biochemical indices: The postoperative levels of ET-1 and hs-CRP in the peripheral blood of no-reflow group were both significantly higher than those of the reflow group (P<0.05) (Table-II). The ET-1 level of the reflow group peaked three hours after PCI and then declined. The serum level of hs-CRP decreased most obviously within three hours after PCI in the reflow group and three hours-three days after PCI in the no-reflow group.

| Index          | Group               | Case No. | 0.5 h before | 0.5 h after | three hours after | three days after | F     | P       |
|----------------|---------------------|----------|--------------|-------------|------------------|-----------------|-------|---------|
| ET-1 (mg/L)    | Reflow group        | 98       | 128.8±22.8   | 71.3±18.4   | 57.2±16.8        | 52.3±12.7       | 4.18  | 0.01    |
|                | No-reflow group     | 38       | 159.4±69.5   | 99.4±15.9   | 91.7±31.5        | 91.3±32.4       | 0.19  | 0.31    |
| T value        | -                   | 0.76     | 20.37        | 27.3        |                  | 31.4            | -     | -       |
| P value        | -                   | 0.45     | 0.01         | 0.02        |                  | 0.01            | -     | -       |
| hs-CRP (mg/L)  | Reflow group        | 98       | 39.7±14.6    | 42.8±11.9   | 22.3±2.15        | 11.4±0.8        | 6.88  | 0.01    |
|                | No-reflow group     | 38       | 41.2±10.65   | 44.2±10.8   | 43.2±16.5        | 23.8±1.24       | 0.48  | 0.41    |
| T value        | -                   | 0.82     | 0.39         | 26.4        |                  | 19.8            | -     | -       |
| P value        | -                   | 0.43     | 0.62         | 0.02        |                  | 0.03            | -     | -       |
Postoperative echocardiographic indices: LVEDDs of the two groups after PCI were apparently lower than those before PCI, but there was no significant inter-group difference (P>0.05). LVESDs and LVEFs of both groups evidently increased after PCI compared with those before PCI, without significant inter-group difference either (P>0.05) (Table-III).

Immediate postoperative perfusion of epicardial blood vessels and myocardium: CTFC and WMSI of the reflow group after PCI were significantly lower than those of the no-reflow group (P<0.05) (Table-IV).

Correlations between serum ET-1 level and other factors: The Pearson’s correlation analysis showed that ET-1 level was positively correlated with CTFC in the patients (P<0.05) (Table-V). Multivariate linear regression analysis was performed with ET-1 level as the dependent variable and gender, age, mean arterial pressure, CTFC and WMSI as independent variables. Serum hs-CRP level was negatively correlated with ET-1 level (P<0.05) (Table-VI), with a correlation coefficient r of -0.34.

DISCUSSION

No-reflow phenomenon was first found in the canine experiment. Animal experiments have shown that in the local myocardial ischemia model caused by ligation of canine coronary artery, the ligated artery is reopened for blood flow, when the ischemic area cannot be fully perfused. At present, no-reflow is a phenomenon that in case of severe spasm and obstruction in local blood vessels, the corresponding tissues and organs appear ischemic (40-60 min generally), when if the blood vessels are recanalized to restore blood flow, but the ischemic area cannot get sufficient blood perfusion. No-reflow phenomenon is commonly seen in the myocardium, but can also be found in...
the brain, kidneys, skeletal muscles, etc. No-reflow
is actually caused by the duration of ischemic
time of tissue damage and the superimposition of
extent.\(^\text{13}\) The main reason for no-reflow is swelling
of microvascular endothelial cells, increased
interstitial pressure due to exudate in extravascular
microvascular extracellular matrix, and clogging
of microvessels caused by platelet aggregation
and/or leukocyte impaction.\(^\text{14}\) In the course of no-
reflow development, biological indicators such as
endothelin and C-reactive protein play a crucial
regulatory role.\(^\text{15}\)

ET is an important factor in regulating
cardiovascular function, which plays a key role in
maintaining basal vascular tension and the stability
of cardiovascular system.\(^\text{16}\) ET is a polypeptide
composed of 21 amino acids, with a molecular
weight of 2400 D. Its N-terminus is two disulfide
bonds linking the cysteine in sites 1-15 and 3-11,
and its C-terminus is some hydrophobic amino
acid residues. The N-terminal structure determines
its affinity with the receptor, and the C-terminal
structure the binding site with the receptor.\(^\text{17}\) ET-1
also has two isomeric families, i.e. ET-2 and ET-3,
which differ in the residues of individual amino
carboxylic acids. ET-1 plays a major role in the cardiovascular
system.\(^\text{18}\) In coronary atherosclerotic heart diseases,
coronary endothelial cells are stimulated to
synthesize and release ET-1, whose regulation is
at the level of genetic transcription. Factors that
stimulate ET-1 synthesis include epinephrine,
thromboxane, vasopressin, angiotensin, insulin,
and cytokines, as well as physical factors such as
changes of vascular wall shear stress and
pressure, and anoxia. And the stimulating process
of ET-1 synthesis needs the involvement of Ca\(^{2+}\)
(dependent protein kinase C).\(^\text{19}\) Factors that inhibit
ET-1 synthesis include NO, PGI\(_2\), atrial natriuretic
peptide, heparin, etc. ET-1 half-life in plasma is very
short (<5 minutes), which can quickly combine with
tissue receptors and decomposed by ET-degrading
enzymes, with its sites of clearance mainly in the
lungs and kidneys.\(^\text{20}\) Therefore, ET-1 can be used
as one of the serological markers of CHD.\(^\text{21}\) In
addition, the Centers for Disease Control and
Prevention and the American Heart Association
(suggest that cardiovascular risks of patients can be
categorized according to hs-CRP levels: <1 mg/L
for relatively low risk, 1.0 to 3.0 mg/L for moderate
risk, >3.0 mg/L for high risk.\(^\text{22}\) The two indicators
are clinically important for evaluating no-reflow in
myocardial infarction, but their relevance remains
unclear.\(^\text{23}\)

In our study, the levels of ET-1 and hs-CRP in
peripheral blood after PCI in the no-reflow group
were significantly higher than those in the reflow
group, with the difference statistically significant
(P<0.05). The ET-1 level in the reflow group peaked
at three hours after PCI and then declined. The level
of ET-1 did not show any significant change in both
the reflow group and the no-reflow group (P>0.05).
The level of serum hs-CRP had the largest amplitude
of decrease within three hours after PCI in the reflow
group and at three hours-three days after PCI in
the no-reflow group respectively. The results were
consistent with those of Ding et al.\(^\text{24}\) LVEDDs of the
two groups after PCI were significantly lower than
those before PCI, between which, however, there
was no significant difference (P>0.05). LVESDs and
LVEFs of the two groups after PCI were significantly
increased, with the differences not statistically
significant (P>0.05), suggesting that whether there
is reflow or not has no significant short-term impact
on cardiac remodeling. We believe that this may be
due to the reason that in addition to increased left
ventricular end-diastolic diameter and increases
ejection fraction caused by impaired ischemic
myocardial contractility, cardiac remodeling can also
be regulated by multiple neuroendocrine factors,
such as vasopressin and atrial natriuretic peptide.
CTFC and WMSI of the reflow group after PCI were
significantly lower than those in the no-reflow group,
with the differences being statistically significant
(P<0.05). The Pearson’s correlation analysis showed
that the ET-1 level was positively correlated with
CTFC in STEMI patients (P<0.05). The multiple
linear regression analysis was made with ET-1 level
as the dependent variable, gender, age, MAP, CTF
and WMSI as independent variables, and the results
showed that hs-CRP might be a factor influencing
the serum level of STEMI patients (P<0.05), between
which there was a negative correlation, with a

| Variable | β    | SE   | β'   | t    | P   | (95%CI) |
|----------|------|------|------|------|-----|--------|
|          |      |      |      |      |     |        |
| Upper limit | Lower limit |
| hs-CRP   | 0.531| 0.143| 0.784| 0.432| 0.02| 0.16   |
| WMSI     | 0.381| 0.109| 0.692| 0.692| 0.28| 0.48   | 0.79   |
correlation coefficient of r=−0.34. These results suggested that the combined monitoring of ET-1 and hs-CRP levels after PCI may have important clinical value for indicating postoperative reflow.

**CONCLUSION**

In conclusion, hsCRP and ET-1 levels significantly increased in patients with no-reflow phenomenon.

**Source of funding:** None.

**Declaration of interest:** All authors have no conflict of interest regarding this paper.

**REFERENCES**

1. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. Circulation. 2012;126(16):2020-2035. doi: 10.1161/CIR.0b013e31826e1058.
2. Wright RS, Anderson JL, Adams CD, Bridges CR, Casey DE Jr, Ettinger SM, et al. 2011 ACCF/AHA focused update incorporated into the ACC/AHA 2007 Guidelines for the Management of Patients with Unstable Angina/Non-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. 2011;57:e215-367. doi: 10.1016/j.jacc.2011.02.011.
3. Abdel MI, Dantani N, Ihara C, Aimone-Gastin I, Angioï P, Pak J, et al. Brief ischemia-reperfusion induces stunning of the canine coronary artery. Circulation. 2009;120(18):1838-1842. doi: 10.1161/CIRCULATIONAHA.109.842519.
4. Ensinck EP, Pak J, Hildner DJM, Dantani N, Ihara C, Aimone-Gastin I, et al. Effect of no-reflow during primary percutaneous coronary intervention on coronary microcirculation instable angina patients. J Med Sci. 2016;52:38-44. doi: 10.4068/cmj.2016.52.1.38.
5. Cenko E, Ricci B, Kedev S, Kalpak O, Călmăc L, Vasiljevic Z, et al. The no-reflow phenomenon in the young and in the elderly. Int J Cardiol. 2016;222:1122-1128. doi: 10.1016/j.ijcard.2016.07.209.
6. Brosh D, Assali AR, Mager A, Porter A, Hasdai D, Teplitsky I, et al. Effect of no-reflow during primary percutaneous coronary intervention for acute myocardial infarction on six-month mortality. Am J Cardiol. 2007;99:442-445. doi: 10.1016/j.amjcard.2006.08.054.
7. Iwakura K. Modulation of individual susceptibility to the no-reflow phenomenon after acute myocardial infarction. Curr Pharm Des. 2013;19:4519-4528.
8. Song R, Chou YI, Kong J, Li J, Pan B, Cui M, et al. Association of endothelial microparticle with NO, eNOS, ET-1, and fractional flow reserve in patients with coronary intermediate lesions. Biomarkers. 2015;20:429-435. doi: 10.3109/01423561.2015.1094140.
9. Rodríguez-Pascual F, Busnagade O, Lagares D, Lamas S. Role of endothelin in the cardiovascular system. Pharmacol Res. 2011;63:463-472. doi: 10.1016/j.phrs.2011.01.014.
10. Spinella MJ, Malik AB, Everitt J, Andersen TT. Design and synthesis of a specific endothelin 1 antagonist: effects on pulmonary vasconstriction. Proc Natl Acad Sci USA. 1991;88:7443-7446.
11. Böhm F, FernoW J. The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. Cardiovasc Res. 2002;56(1):18-36. doi: 10.1016/s0008-6261(02)01202-0.
12. Rodríguez-Pascual F, Busnagade O, Lagares D, Lamas S. Role of endothelin in the cardiovascular system. Pharmacol Res. 2011;63:463-472. doi: 10.1016/j.phrs.2011.01.014.
13. Spinella MJ, Malik AB, Everitt J, Andersen TT. Design and synthesis of a specific endothelin 1 antagonist: effects on pulmonary vasconstriction. Proc Natl Acad Sci USA. 1991;88:7443-7446.
14. Iwakura K. Modulation of individual susceptibility to the no-reflow phenomenon after acute myocardial infarction. Curr Pharm Des. 2013;19:4519-4528.
15. Song R, Chou YI, Kong J, Li J, Pan B, Cui M, et al. Association of endothelial microparticle with NO, eNOS, ET-1, and fractional flow reserve in patients with coronary intermediate lesions. Biomarkers. 2015;20:429-435. doi: 10.3109/01423561.2015.1094140.
16. Rodríguez-Pascual F, Busnagade O, Lagares D, Lamas S. Role of endothelin in the cardiovascular system. Pharmacol Res. 2011;63:463-472. doi: 10.1016/j.phrs.2011.01.014.
17. Spinella MJ, Malik AB, Everitt J, Andersen TT. Design and synthesis of a specific endothelin 1 antagonist: effects on pulmonary vasconstriction. Proc Natl Acad Sci USA. 1991;88:7443-7446.
18. Böhm F, FernoW J. The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. Cardiovasc Res. 2002;56(1):18-36. doi: 10.1016/s0008-6261(02)01202-0.
19. Rodríguez-Pascual F, Busnagade O, Lagares D, Lamas S. Role of endothelin in the cardiovascular system. Pharmacol Res. 2011;63:463-472. doi: 10.1016/j.phrs.2011.01.014.
20. Pflug BR, Zheng H, Udan MS, D’Antonio JM, Marshall FF, Brooks JD, et al. Endothelin-1 promotes cell survival in renal cell carcinoma through the ET(A) receptor. Cancer Lett. 2007;246:139-148. doi: 10.1016/j.canlet.2006.02.007.
21. Abis M, Ward DT. Increased endothelin-1 responsiveness in human coronary artery smooth muscle cells exposed to 1,25-dihydroxyvitamin D(3). Am J Physiol Cell Physiol. 2013;304:C666-C672. doi: 10.1152/ajpcell.00349.2012.
22. El Safoory OS, Ezzat M, Abdelhamid MF, Shoukry N, Badawy E. The Evaluation of the Impact of Age, Skin Tags, Metabolic Syndrome, Body Mass Index, and Smoking on Homocysteine, Endothelin-1, High-sensitive C-reactive Protein, and on the Heart. Indian J Dermatol. 2013;58:C666-C672. doi: 10.1152/ajpcell.00349.2012.
23. Zhu HJ, Lu S, Su W, Gong SY, Zhang ZB, Li P, et al. Effects of lian dog qingmai recipe (see text) on endothelin-1, nitric oxide, interleukin-6 and interleukin-10 levels in patients with coronary heart disease. J Tradit Chin Med. 2011;31:173-177.
24. Ding SK, Wang LX, Guo LS. [Effect of percutaneous coronary intervention on coronary microcirculation instable angina patients. J Med Forum. 2016;37:81-82.

**Author’s Contribution:**

**ML, TL, QZ, LL & ZW:** Data collection and analysis.

**PZ:** Study design and manuscript writing.