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Serum inflammatory factors such as MMP-9 are associated with post-percutaneous transluminal angioplastyacute myocardial infarction in coronary heart disease patients complicated by lower extremity arteriosclerosis obliterans

Changgang Shao[1], Batu Wuren[2], Guoqing Chi[1], Hongcheng Ren[1], Fang Li[3], Wenjie Song[1], Bin Wang[1], Mingchao Ding[4*]

[1] Interventional Vascular Department, Aerospace Center Hospital, Beijing, 100049, China

[2] Vascular Surgery, Horqin Right Front Banner People's Hospital, Inner Mongolia, 137400, China

[3] Interventional Surgery Room, Aerospace Center Hospital, Beijing, 100049, China

[4] Hospital Office, Aerospace Center Hospital, Beijing, 100049, China

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ABSTRACT

This clinical study mainly analyzed the correlation of changes in serum inflammatory factors (IFs), such as matrix metalloproteinase (MMP)-9, hypersensitive C-reactive protein (hs-CRP), tumor necrosis factor (TNF)-α, and interleukin (IL)-6 with post-percutaneous transluminal angioplasty (PTA)acute myocardial infarction (AMI) in coronary heart disease (CHD)patients complicated by lower extremity arteriosclerosis obliterans (ASO). This retrospective study selected sixty ASO+CHD patients (ASO group) who underwent lower limb angioplasty between January 2014 and June 2016, as well as 50 concurrent healthy controls (HCs, HC group). According to the occurrence of AMI after PTA, cases were further subdivided into AMI (n = 18) and non-AMI (n = 42) groups. For all participants, IFs (MMP-9, hs-CRP, TNF-α, and IL-6) were detected on an empty stomach. The correlations of these IFs with the post-PTAAMI risk of ASO + CHD patients were analyzed using Pearson correlation coefficients, and their predictive value for AMI was visualized by receiver operating characteristic (ROC)curves. Finally, the prognostic factors of perioperative AMI in ASO+CHD patients were identified by multivariate analysis using the Cox model. MMP-9, hs-CRP, TNF-α and IL-6 presented statistically higher levels in the AMI group than in non-AMI and HC groups and were positively correlated with AMI. ROC analysis data showed that MMP-9, hs-CRP, TNF-α and IL-6 had better diagnostic performance, sensitivity and specificity for post-PTAAMI in patients with ASO+CHD. According to Cox multivariate analysis, high levels of MMP-9, hs-CRP and IL-6 increased the risk of perioperative AMI in ASO+CHD patients after PTA. This study shows a significant correlation between the changes of serum IFs (MMP-9, hs-CRP, IL-6, and TNF-α) and post-PTA AMI in ASO patients complicated by CHD. Patients with upregulated post-PTA levels of the above Ifs in serum are at an elevated risk of developing AMI, and active and effective control will help to prevent AMI.

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Introduction

As a chronic progressive condition with systemic arteriosclerotic vasculopathy occurring in the lower extremity arteries, lower extremity arteriosclerosis obliterans (ASO) can be clinically manifested as intermittent claudication of the lower limbs, skin temperature decline, pain, and even ulcer or necrosis (1,2). Its pathogenesis is related to arterial stiffness-induced intimal thickening, lumen stenosis or occlusion of the lower extremity blood supply artery, and inadequate blood supply to the diseased limbs (3,4). The disease is easily complicated by coronary heart disease (CHD), a condition that may cause myocardial infarction (MI) due to the gradual blockage of blood vessels and the failure of collateral vessels to sufficiently supply the ischemic site in the early stage (5,6). Minimally invasive interventional therapy is currently the major clinical treatment for ASO, which can effectively boost ASO patients' lower extremity blood supply and greatly lower the risk of amputation (7). Percutaneous transluminal angioplasty (PTA), as a minimally invasive intervention method commonly used in clinical practice, mainly uses a pressurized balloon to compress plaques to rupture and enlarge the arterial lumen to restore blood supply of the affected limb (8). Acute myocardial infarction (AMI), a common perioperative complication of PTA, may occur in patients with ASO + CHD, bringing huge challenges to clinical treatment and rehabilitation (9). Accordingly, exploring predictors of AMI after PTA in ASO + CHD patients has great clinical implications for preventing and reducing the occurrence of AMI in such patients.

Evidence has shown that the risk of AMI, a target organ ischemia and hypoxia event, is associated with the increase of metabolic activity in atherosclerotic plaques and systemic inflammatory responses (10). Matrix metalloproteinase (MMP)-9 belongs to the MMP family and is strongly linked to cardiovascular disorders such as atherosclerosis, aneurysm, restenosis after PTA and heart failure (11). Na-

* Corresponding author. Email: dingmingchao2022@163.com

kamura et al. (12) pointed out that plasma MMP-9 was abnormally high in ASO patients and decreased markedly after treatment, suggesting that it was closely related to the dynamic development of ASO patients. It also mediates AMI progression and promotes intravascular thrombosis by promoting tissue factor expression, with a close connection with plaque rupture in atherosclerotic lesions (13). High-sensitivity C-reactive protein (hs-CRP), as well as tumor necrosis factor (TNF)-α and interleukin (IL)-6 are considered independent predictors of atherosclerosis development in humans and serum markers of systemic inflammation, with their elevated levels playing a significant role in promoting human atherosclerosis progression (14,15). Moreover, in AMI, hs-CRP is closely related to the risk of death, while IL-6 and TNF-αare strongly linked to the risk of cardiogenic shock (16,17).

By discussing the roles played by MMP-9 and other inflammatory factors (IFs) in predicting perioperative AMI of ASO + CHD patients after PTA, this research intends to provide a better clinical solution for post-PTAAMI in such patients and optimize the evaluation and intervention process.

Materials and Methods

Patient information

In this retrospective study, 60 ASO + CHD patients (ASO group) who underwent PTA in the Aerospace Center Hospital between January 2014 to June 2016 were selected and subdivided into AMI (n=18) and non-AMI (n=42) groups based on the occurrence of post-PTA AMI. Additionally, 50 healthy subjects were selected as the healthy control (HC) group. Cases and controls displayed clinical comparability in general data. A flowchart of the patient selection and experimental procedure can be seen in Figure 1.

The Ethics Committee of the Aerospace Center Hospital approved this research, which was conducted strictly following the Declaration of Helsinki.

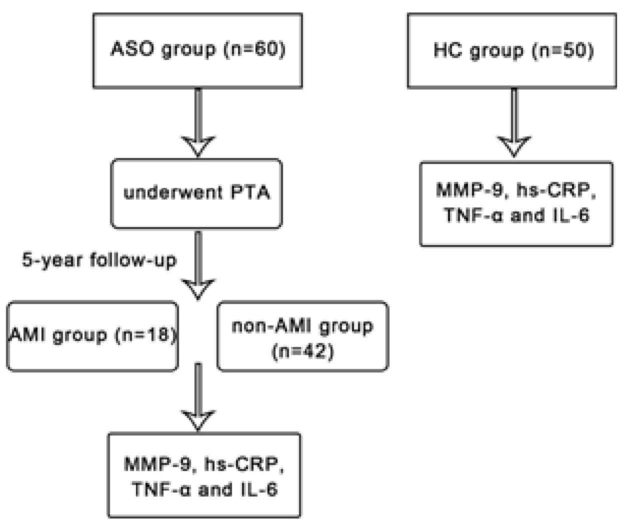

Figure 1. Flow chart of patient selection and experimental process. ASO, arteriosclerosis obliterans; HC, healthy control; PTA, percutaneous transluminal angioplasty; MMP-9, matrix metalloproteinase-9; hs-CRP, hypersensitive C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; AMI, acute myocardial infarction.

Eligibility criteria

Patients who were diagnosed with ASO + CHD and met PTA indications (18), with clinical presentations of intermittent claudication and resting pain, normal cognitive and communication skills, and informed consent provided were included. In addition, patients with post-PTA AMI all met the diagnostic criteria for AMI (19).

Those meeting any of the following criteria were excluded: malignant tumors; serious diseases of vital organs (heart, brain, kidneys, etc.); blood and immune system dysfunction; incomplete medical records; refusal to participate in this clinical experiment; hematological or infectious diseases; pregnant or lactating patients.

Treatment methods

PTA: retrograde puncture of the femoral artery of the affected limb was performed with the Seldinger technique. The catheter was placed into the lower segment of the diseased vessel along the guide wire after the insertion of the arterial sheath. After peripheral injection of heparin for routine anticoagulation, a catheter was used to guide the guide wire through the site of vascular stenosis. Following the successful placement of the guide wire, a dilated balloon was introduced, the pressure of which was controlled between normal pressure and bursting pressure and maintained at 180s. Lower limb arteriography was performed again after dilation, and stent placement was performed if necessary. Techniques such as atherectomy and thrombus aspiration could also be used in the surgical process. Finally, the actual situation of the patient's vascular reconstruction was comprehensively evaluated to confirm the success of the reconstruction.

Follow-up

All cases were followed up quarterly during the 5 years through telephone visits and medical records queries, and the occurrence of AMI in the ASO group was recorded.

Detection methods

Peripheral venous blood (five milliliters), collected on an empty stomach during morning hours, was centrifuged after anticoagulation treatment to separate plasma and serum for cryopreservation. Among them, serum samples of ASO patients were collected one day before PTA. Enzyme-linked immunosorbent assays (ELISAs) were then performed to quantify serum MMP-9, hs-CRP, TNF-α, and IL-6 levels in strict accordance with the corresponding human ELISA kit recommendations (Shanghai Guangrui Biological Technology Co., Ltd., Cat. Nos. 716, 1497, R-1389, and 1401).

Statistical methods

This study used SPSS25.0 for statistical analysis and $P < 0.05$ as the significance threshold. The number of cases/percentage (n/%) was utilized to statistically describe the enumeration data and Mean ± SEM to represent the quantitative data. For data comparisons, the χ^2 test was used for enumeration data and variance analysis (multi-group comparison) and t-test (inter-group comparison) for quantitative data. The correlations of hs-CRP, MMP-9, TNF-α, and IL-6 with perioperative AMI in PTA-treated ASO + CHD patients were analyzed by Spearman correlation coefficients, and the predictive value of these IFs for AMI was determined using the receiver operating characteristic curve (ROC). Finally, independent prognostic factors for perioperative AMI in ASO + CHD patients after PTA were analyzed by Cox multivariate analysis.

Results

Patient general data

Cases and controls exhibited non-significant differences in general data (sex, average age, disease course, lesion location, hyperlipidemia, hypertension, diabetes, residence, etc.), as presented in Table 1 (P>0.05).

Comparison of IFs among AMI, non-AMI and HC groups

We detected IF (MMP-9, hs-CRP, TNF-α and IL-6) levels in three groups to analyze abnormal inflammatory expression in ASO + CHD patients with post-PTA AMI (Figure 2). The 5-year follow-up results demonstrated 18 cases of postoperative AMI among the 60 ASO + CHD patients. The levels of these Ifs were the highest in the AMI group and the lowest in the HC group, while those in the non-AMI group were in between, with statistical differences among groups (P<0.05).

Correlation analysis

We analyzed the correlation of these IFs with post-PTA AMI in ASO + CHD patients by Spearman correlation coefficients (Figure 3). We set the presence of post-PTA AMI as 1 and the absence of post-PTAAMI as 2. The results showed identified a positive correlation of MMP-9, hs-CRP, TNF-α, and IL-6 with post-PTA AMI (r=0.569, 0.575, 0.475, and 0.545, respectively, all P<0.01).

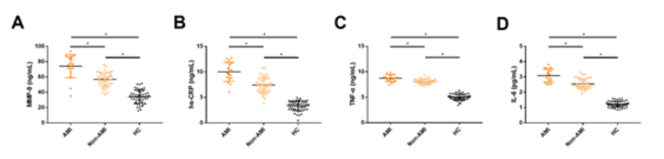

Figure 2. Comparison of inflammatory factors among AMI, non-AMI, and HC groups. (A) MMP-9 in AMI, non-AMI, and HC groups. (B) hs-CRP in AMI, non-AMI, and HC groups. (C) TNF-α in AMI, non-AMI, and HC groups. (D) IL-6 in AMI, non-AMI, and HC groups. Note: Inter-group comparisons, * P<0.05. The inter-group comparison quantitative was performed by the t-test. AMI, acute myocardial infarction; HC, healthy control; MMP-9, matrix metalloproteinase-9; hs-CRP, hypersensitive C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; AMI, acute myocardial infarction.

ROC analysis

We drew the ROC curves of preoperative serum Ifs in ASO + CHD patients to differentiate post-PTA-AMI. The AUC of MMP-9 in differentiating post-PTA AMI was 0.859 (95% CI: 0.720-0.997), the cut-off value was 69.810, the sensitivity (SEN) was 95.24%, and the specificity (SPE) was 77.78%; the AUC of hs-CRP in differentiating post-PTA AMI was 0.862 (95% CI: 0.754-0.971), and the cut-off, SEN, and SPE were 8.655, 80.95%, and 83.33%, respectively; while for TNF-α, the AUC, cut-off, SEN, and SPE were 0.799 (95% CI: 0.664-0.934), 8.160, 69.05%, and 83.33%, respectively; and the corresponding data of IL-6 in differentiating post-PTA AMI were 0.843

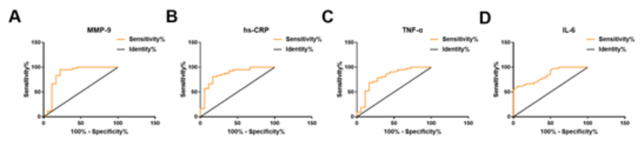

Figure 3. ROC analysis. (A) ROC curve of MMP-9 in differentiating post-PTA AMI. (B) ROC curve of hs-CRP in differentiating post-PTA AMI. (C) ROC curve of TNF-α in differentiating post-PTA AMI. (D) ROC curve of IL-6 in differentiating post-PTA AMI. Note: The predictive ability and retrograde analyses of MMP-9 and other indicators for post-PTA were performed using the ROC curve and its related data (AUC, Cut-off, Sensitivity, Specificity). ROC, receiver operating characteristic curve; PTA, percutaneous transluminal angioplasty; MMP-9, matrix metalloproteinase-9; hs-CRP, hypersensitive C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; AMI, acute myocardial infarction.

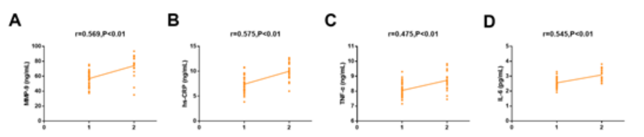

Figure 4. Correlation analysis. (A) The correlation between MMP-9 and post-PTA AMI (r=0.569, P<0.01). (B) The relationship between hs-CRP and post-PTA AMI (r=0.575, P<0.01). (C) The correlation between TNF-α and post-PTA AMI (r=0.475, P<0.01). (D) The correlation between IL-6 and post-PTA AMI (r=0.545, P<0.01). Note: The correlation of MMP-9 and other indicators with the occurrence of post-PTA AMI was analyzed by Spearman correlation coefficients. PTA, percutaneous transluminal angioplasty; MMP-9, matrix metalloproteinase-9; hs-CRP, hypersensitive C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; AMI, acute myocardial infarction.

Table 1. Patient general data [n, mean±SEM].

Factors	ASO group (n=60)	HC group (n=50)	χ^2/t	P
Sex (male/female)	33/27	29/21	0.100	0.752
Average age (years old)	56.94±7.66	57.81±8.94	0.548	0.585
Disease course (years)	25.93±3.44	26.11±3.68	0.267	0.790
Lesion site (iliac artery/middle and superficial segment of superficial femoral artery/inferior segment of superficial femoral artery)	28/21/11	24/20/6	0.901	0.637
Hyperlipidemia (yes/no)	35/25	25/25	0.764	0.382
Hypertension (yes/no)	41/19	30/20	0.828	0.363
Diabetes mellitus (yes/no)	23/37	22/28	0.362	0.547
Residence (urban/rural)	45/15	31/19	2.158	0.142

Note: ASO, arteriosclerosis obliterans; HC, healthy control.

Table 2. ROC analysis.

Factors	AUC	95%CI	S.E	Cut-off	Sensitivity (%)	Specificity (%)
MMP-9	0.859	0.720-0.997	0.071	69.810	95.24	77.78
hs-CRP	0.862	0.754-0.971	0.055	8.655	80.95	83.33
TNF-α	0.799	0.664-0.934	0.069	8.160	69.05	83.33
IL-6	0.843	0.744-0.943	0.051	2.520	61.90	94.44

Note: ROC, receiver operating characteristic curve; AUC, the area under the curve; MMP-9, matrix metalloproteinase-9; hs-CRP, hypersensitive C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.

Table 3. Cox multivariate analysis of perioperative AMI in patients with ASO complicated with CHD undergoing PTA.

Factor	β	S.E	Wald	P	OR (95% CI)
MMP-9	0.047	0.023	4.161	0.041	1.048 (1.002-1.097)
hs-CRP	0.366	0.139	6.904	0.009	1.442 (1.097-1.895)
TNF-α	0.625	0.384	2.654	0.103	1.869 (0.881-3.967)
IL-6	1.751	0.678	6.675	0.010	5.761 (1.526-21.747)

Note: CHD, coronary heart disease; ASO, arteriosclerosis obliterans; PTA, percutaneous transluminal angioplasty; AMI, acute myocardial infarction; MMP-9, matrix metalloproteinase-9; hs-CRP, hypersensitive C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.

(95% CI: 0.744-0.943), 2.520, 61.90%, and 94.44%, respectively (Table 2, Figure 4).

Cox analysis

Multivariate Cox regression analysis of the IFs that differed between cases with and without post-PTA AMI showed that MMP-9 (P=0.041), hs-CRP (P=0.009), and IL-6 (P=0.010) were independent prognostic factors affecting the perioperative AMI in ASO + CHD patients, but not TNF-α (P=0.103). ASO + CHD patients with enhanced MMP-9, hs-CRP, and IL-6 levels were at increased risk of perioperative AMI after PTA. See Table 3 for details.

Discussion

Our results indicated that the AMI group had the highest concentrations of four IFs, namely, MMP-9, hs-CRP, TNF-α, and IL-6, followed in descending order by the non-AMI group and HC group, suggesting a close correlation of these IFs with post-PTA AMI in ASO + CHD patients. We then carried out validation through correlation analysis. In addition, a positive correlation of these four Ifs in serums with post-PTA AMI was identified in ASO + CHD patients. As a member of the zinc and calcium-dependent endopeptidase family, MMP-9, or gelatinase B, is primarily responsible for elastin and collagen IV degradation, which can induce the destruction of vascular EEM in advanced atherosclerosis (20). Previous research has revealed abnormally high MMP-9 levels in patients with severe lower limb ischemia, similar to our findings (21). It pointed out that MMP-9 was involved in the pathogenic mechanism of AMI, with its high level strongly related to myocardial no-reflow in ST-segment elevation MI (STEMI) patients, which can serve as a predictor of myocardial no-reflow in AMI patients undergoing PTA. By promoting endothelial and vascular smooth muscle cell growth and metastasis, MMP-9 has also been shown to affect restenosis and occlusion of venous artery bypass graft after PTA (22-24). IFs, like hs-CRP, TNF-α and IL-6 studied in this research, all show a significant correlation with cardiovascular events in atherosclerosis, and are also strongly linked to systemic inflammatory responses after PTA (25,26). Post-PTA systemic inflammatory responses not only mediate cardiac remodeling, but are also closely related to myocardial infarction size (27,28). Furthermore, AMI will exacerbate the imbalance of the body's inflammatory microenvironment, resulting in increased serum inflammatory factor levels, which will lead to vascular endothelial injury and elevated risk of AMI progression and recurrence (29).

In order to explore the potential value of these four serum IFs in predicting post-PTA AMI in ASO + CHD patients, we further delved into them by ROC and Cox analyses. The data showed that the AUCofMMP-9 (AUC: 0.859) and hs-CRP (AUC: 0.862) in differentiating post-PTA AMI were all over 0.850, the AUC of IL-6 (AUC: 0.843) was over 0.800, and the AUC of TNF-α (AUC: 0.799) was nearly 0.800. In terms of SEN and SPE, MMP-9 had the highest SEN (95.24%) in identifying AMI after PTA, IL-6 had the highest SPE (94.44%), while the SEN and SPE of hs-CRP and TNF-α were all over 80.00%. The above results indicate the good diagnostic performance of the four IFs in predicting post-PTA AMI and can thus be used as auxiliary indicators for the prediction of post-PTA AMI in such patients. Finally, Cox multivariate analysis identified that MMP-9, hs-CRP, and IL-6 were independent prognostic factors affecting perioperative AMI in ASO + CHD patients after PTA and their high levels would increase the risk of post-PTA AMI in such patients. In AMI patients undergoing emergency percutaneous coronary intervention, MMP-9 has also been indicated to be an independent predictor of in-hospital mortality (30). Schoos et al. (31) reported that hs-CRP can not only independently predict STEMI patients' outcomes during the 36-month follow-up, but also provide clinical guidance for the selection of stent types in percutaneous coronary intervention. TNF-αcan be a predictor of impaired peak leg blood flow in chronic heart failure patients, as it can reduce the peak leg blood flow (32). While IL-6 has been indicated to be a reliable predictor of 6-month in-stent restenosis after femoral-popliteal artery stenting (33). The preceding studies all indicate the close correlation of the four Ifs studied in this research with postoperative adverse events in the setting of cardiovascular disease.

The major contribution of the present paper is to confirm the strong relationship between four serum IFs (MMP-9, hs-CRP, TNF-α and IL-6) and post-PTA AMI in patients with ASO + CHD and their great potential to predict the occurrence of post-PTAAMI, which can provide guidance for ASO + CHD patients after PTA. There are some limitations in this research, which need further consideration. First of all, there are too few samples included, with only 60 ASO patients and 50 healthy controls. Increasing the sample size would be beneficial to improve the universality and accuracy of the results. Second, since this is a single-center study, it may be helpful to avoid the problem of information collection bias if multi-center data can be included in the analysis. Third, if a more comprehensive analysis of risk factors for post-PTA AMI in ASO + CHD patients can be supplemented, the surgical management of such patients can be further improved to reduce the risk of AMI after PTA as much as possible. Supplementary analysis will be carried out from these aspects in the future.

To sum up, we propose for the first time that serum IFs MMP-9, hs-CRP, TNF-α, and IL-6 have favorable predictive value for AMI after PTA and can be predictors of post-PTA AMI in ASO patients complicated by CHD given their significant positive correlation with AMI after PTA, providing new evidence and choices for judging the condition of such patients and lowering the risk of AMI.

Conflict of interest

The author declares no competing interests.

Funding Statement

Serum inflammatory factors such as MMP-9 are associated with acute myocardial infarction in patients with coronary heart disease complicated with lower extremity arteriosclerosis obliterans after percutaneous transluminal angioplasty*Supported by the Foundation of Aerospace Center Hospital (No. YN202210).

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