

The association between plasma hyaluronan level and plaque types in ST-Segment–Elevation Myocardial Infarction patients

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Abstract

Background

The metabolism of hyaluronan (HA) is widely known to be involved in the process of acute coronary syndrome, but it is unknown how circulating HA levels change in ST-Segment–Elevation Myocardial Infarction (STEMI) patients and whether HA is associated with plaque morphology, including rupture and erosion.

Objectives

This study focused on the changes in the plasma levels of HA and CD44 in STEMI patients and their relationship with plaque morphology evaluated by optical coherence tomography (OCT).

Methods

We prospectively enrolled 3 cohorts in this study, including 162 patients with STEMI, 34 patients with stable coronary artery disease (SCAD) and 50 healthy controls. Plaque morphology was detected by OCT analysis, and the plasma levels of HA and CD44 were examined by enzyme-linked immunosorbent assay (ELISA). We compared HA and CD44 expression among STEMI patients, SCAD patients and healthy controls, as well as in plaque rupture and plaque erosion.

Results

The plasma levels of HA and CD44 were significantly lower in STEMI patients than in healthy controls ($p = 0.009$ and $p < 0.001$, respectively). In addition, plasma HA expression in plaque erosion was significantly lower than that in plaque rupture ($p = 0.021$), whereas no differences were found in soluble CD44 expression between plaque rupture and erosion.

Conclusions

Low levels of circulating HA and CD44 were independently correlated with STEMI, and low levels of HA were associated with plaque erosion compared with rupture. Moreover, plasma HA might be a useful biomarker for identifying plaque erosion to improve the risk stratification and management of STEMI patients.

Introduction

An increasing number of studies have demonstrated that plaque rupture is not the only cause of ST-Segment–Elevation Myocardial Infarction (STEMI).¹ Nearly one-third of patients with STEMI have plaque erosion in their culprit lesion, which is characterized by a higher concentration of hyaluronan (HA) and versican, with considerably less decorin and biglycan, which exhibit morphological characteristics associated with stability.² In addition, CD44, a cell surface receptor of HA, prominently localizes to eroded plaques more than in ruptured plaques, pointing again to a distinct mechanistic pathway for erosion.^{2,3} However, the mechanism of plaque erosion has not been totally elucidated.

HA is a ubiquitous nonsulfated glycosaminoglycan that exists in nearly all tissues. HA plays an important role in many physiological processes, and it can be activated during pathological conditions such as inflammation and cancer.⁴ It has also been proven that HA modulates the progression of atherosclerotic plaques in cardiovascular disease, including macrophage retention and matrix proliferation.⁵ Recent animal experiments showed that plaque erosion was triggered by disturbed blood flow, which was involved in HA metabolism.⁶ Furthermore, an in vivo experiment found that hyaluronidase 2 (HYAL2) and its receptor mediated the recruitment of polymorphonuclear (PMNc) leukocytes and the formation of thrombi in plaque erosion was induced by flow perturbation.⁷ However, whether systemic HA changes in STEMI is unknown. Moreover, the difference in circulating HA expression between plaque rupture and erosion has not yet been investigated.

Optical coherence tomography (OCT), with an extremely high resolution near infrared light-based intravascular imaging modality, enables an accurate identification of plaque morphology in vivo.⁸ This study not only explores how circulating HA changes in STEMI but also provides potential biomarkers and a clinical risk stratification for plaque erosion determined by OCT.

Methods

Study Population and Design

We prospectively enrolled 3 cohorts for this study. The first cohort comprised sequential patients (age \geq 18 years) who presented with STEMI and underwent emergency procedures at Fuwai Hospital. The culprit lesions of these patients were evaluated using OCT before the interventional procedures. STEMI was defined as continuous chest pain lasting $>$ 30 minutes, ST-Segment–Elevation $>$ 0.1 mV in at least 2 contiguous leads or new left bundle-branch block on the 18-lead electrocardiogram (ECG), and an elevated troponin I level.⁹ Patients with cardiac shock, congestive heart failure, a history of coronary artery bypass graft, liver disease or malignant tumor were excluded. Additionally, those with left main diseases, extremely tortuous or heavily calcified vessels, or chronic total occlusion were excluded owing to the difficulty in performing OCT. Between May 2017 and September 2018, a series of 216 eligible patients with STEMI underwent OCT and were enrolled in our study cohort. The study flow chart is displayed in Fig. 1. The second cohort examined was an independent set of 50 prospectively recruited individuals (age \geq 18 years) without known cardiovascular diseases from health screens to provide

normal HA and CD44 reference interval values. The third cohort includes 34 patients diagnosed as SCAD according to their symptoms and coronary angiographical findings who were matched to patients with STEMI for age, sex, hypertension, hypercholesterolemia and diabetes mellitus. This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Fuwai Hospital. All patients provided written informed consent.

Acquisition of OCT Images

Patients were administered 300 mg of aspirin, 180 mg of ticagrelor, or 600 mg of clopidogrel, and 100 IU/kg of heparin before the interventional procedure. Percutaneous coronary intervention was performed via radial or femoral access. Thrombus aspiration was used to reduce the thrombus burden and restore antegrade coronary flow. OCT images of the culprit lesions were acquired with the frequency domain ILUMIEN OPTIS OCT system and a dragon fly catheter (St. Jude Medical, Westford, MA) after antegrade blood flow was restored, according to the intracoronary imaging technique described previously.

OCT Image Analysis

All OCT images were anonymously analyzed on a St. Jude OCT Offline Review Workstation by 3 independent investigators who were blinded to the other data¹⁰. The first investigator was primarily responsible for screening suitability for culprit-plaque evaluation. The other two investigators analyzed OCT images. The intra-observer kappa coefficients for plaque rupture and plaque erosion were 0.963 and 0.938, respectively. The inter-observer kappa coefficients for plaque rupture and plaque erosion was 0.950. Inconsistent results were resolved by consensus with investigators who were blinded to the HA and CD44 results. According to previously established criteria,¹¹ plaque rupture was identified by a disrupted fibrous cap with a clear cavity formation (Fig. 2). Plaque erosion was identified by the presence of an attached thrombus overlying an intact and visible plaque, luminal surface irregularity of the plaque in the absence of a thrombus, or attenuation of the underlying plaque by the thrombus without superficial lipid or calcification proximal or distal to the thrombus (Fig. 2).

Laboratory Tests

Blood samples were collected via radial or femoral access before heparinization using vacutainer tubes containing EDTA. Samples were maintained at 4 °C, processed within 3 hours, and then stored at -80 °C until further analysis. Plasma levels of HA and CD44 were determined by enzyme-linked immunosorbent assay (ELISA) using a Quantikine Hyaluronan Immunoassay kit (DHVAL0) (R&D Systems, Abingdon, UK) and Human CD44 Elisa Kit (Abcam, Cambridge UK), in accordance with the protocol supplied by the manufacturer.¹²

Statistical Analysis

Continuous data are presented as the mean \pm SD or median (interquartile range). Student's t-test or a nonparametric test was used for statistical comparisons. Categorical variables are presented as the count (percent); comparisons between groups were made with the χ^2 test or Fisher's exact test. Logistic regression analysis was performed to determine the odds ratio (OR) and 95% confidence interval (CI) for plaque erosion stratified according to HA as a categorical variable. Adjustments were made for traditional risk factors (including age, sex, hypertension, diabetes mellitus, smoking, and low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), triglyceride level), high sensitive C-reactive protein (hs-CRP) level, estimated glomerular filtration rate (eGFR), and body mass index (BMI). The area under the receiver operating characteristic (ROC) curves (AUC), sensitivity, and specificity were calculated to evaluate the predictive ability of HA for plaque erosion. A 2-tailed $P < 0.05$ was considered statistically significant. The statistical analyses were performed using SPSS software, version 25 (IBM, Armonk, NY).

Result:

Patient Characteristics

Of the 216 patients with STEMI who underwent OCT examination, 17 patients were excluded because of massive thrombus ($n = 8$) or poor imaging quality ($n = 9$). The remaining 199 patients were suitable for plaque morphology evaluation; 87 patients had plaque rupture, and 75 had plaque erosion. The baseline patient characteristics are displayed in Table 1 for the entire study cohort and categorized by plaque morphology. The mean age of the cohort was 57.4 years, 85.2% were men, 57.4% had hypertension, and 29.6% had diabetes mellitus. Patients with plaque erosion were more likely to be younger and have better renal function than patients with plaque rupture. In addition, patients with plaque erosion had higher levels of LDL-C. There were no significant differences in the other clinical variables between the plaque rupture and plaque erosion groups.

We first performed a cross-sectional comparison of the HA levels among the whole STEMI cohort, an independent set of 50 prospectively recruited individuals without known cardiovascular disease and 34 patients diagnosed as SCAD. We observed that plasma HA levels were significantly lower in the patients with STEMI than in the healthy controls (30.1 ng/ml [17.6–47.7] versus 39.3 ng/ml [26.0–53.7] $p = 0.009$), but there is no significant difference of HA levels between patients with STEMI and SCAD (Fig. 3). When we distributed patients with STEMI into plaque rupture (PR) and plaque erosion (PE), we found HA levels of PE is significantly lower than PR and SCAD (25.1 ng/ml [15.4–41.4] versus 36.2 ng/ml [18.9–51.9] $p = 0.021$; 25.1 ng/ml [15.4–41.4] versus 36.5 ng/ml [18.3–67.8] $p = 0.027$, respectively) (Fig. 4). Then we compared plasma CD44 concentration among these three groups. We found that CD44 levels in patients with STEMI is significantly lower than healthy controls and patients with SCAD (143.7 ng/ml [126.2–162.8] versus 172.1 ng/ml [156.4–197.4] $p < 0.001$; 143.7 ng/ml [126.2–162.8] versus 178.7 ng/ml [160.1–191.3] $p < 0.001$, respectively) (Fig. 5). However, subgroup analysis showed that plasma CD44 levels are not significantly different between patients with PR and those with PE (142.5 ng/ml (129.1–162.6) versus 144.5 ng/ml (123.4–164.7), $p = 0.975$) (Table 1).

According to the ROC analysis, the AUC of HA levels in discriminating plaque erosion from rupture was 0.605 (95% CI 0.518 to 0.692, $p = 0.021$) (Fig. 6). The optimal cutoff value was 29.6 ng/ml. In logistic regression analysis, HA was transformed to categorical variables through cutoff value (29.6 ng/ml). After adjustment of age, sex, BMI, history of diabetes mellitus, hypertension, hyperlipidemia, smoking, LDL-C, HDL-C, triglyceride, eGFR and hs-CRP, HA levels were independently associated with plaque types (Table 2).

Discussion:

Currently, with the development of new technologies for imaging and pathology, we have further insight into the mechanism of myocardial infarction. Myocardial infarction can be divided into different plaque types according to OCT examination, and it is important to distinguish plaque rupture from erosion because patients with different plaque types have different risks and prognoses. Patients with erosion may benefit from pharmacological therapy rather than mechanical revascularization.¹³ It is more convenient to determine the types of plaque by biomarkers rather than OCT, which is more expensive for patients. Our previous study revealed that plasma trimethylamine N-oxide (TMAO) can be a useful biomarker to predict plaque rupture.¹⁴ However, although the mechanism of plaque erosion has been partly studied, there is still no acknowledged biomarker for predicting plaque erosion. In this study, we first reported the distinction of plasma HA and CD44 between patients with STEMI and healthy people. Additionally, we found that plasma HA levels in erosion patients were significantly lower than in rupture, SCAD patients and healthy subjects, which meant that plasma HA levels can be used as a potential biomarker to predict plaque erosion.

HA plays a crucial role in the progression of cardiovascular disease and is involved in several important phases of coronary artery disease (CAD), such as inflammation and angiogenesis.¹⁵ HA exists in plasma in two forms: high molecular weight (HMW)-HA and low molecular weight (LMW)-HA. At homeostasis, HA is predominantly in its high molecular mass form of over 1,000 kDa. However, in tissue injury or inflammation, HYAL2 and HYAL1 are upregulated to break down HA to 20 kDa, which binds to the receptors of immune cells.¹⁶ It has been reported that HYAL2-deficient mice display a significant increase in plasma HA levels.¹⁷ HYAL2 and CD44 exist in many kinds of components in blood, such as platelets and monocytes, all of which contribute to HMW-HA degradation when inflammation occurs. Furthermore, the metabolism of HA is influenced by not only inflammation but also disturbed flow, which is related to plaque erosion according to the current study.¹⁸ Several in vivo and in vitro mechanistic studies indicated that disturbed flow might be the initial factor inducing erosion, which is related to an alteration of HA metabolism. Additionally, an increasing number of clinical investigations have found that HA is correlated with the pathogenesis of plaque erosion, and pathological observations have shown that eroded plaques have few inflammatory cells but abundant proteoglycan and glycosaminoglycans, including HA and its receptor CD44.¹⁹ A recent clinical study revealed that the expression of HYAL2 and CD44 in peripheral blood mononuclear cells (PBMCs) increased in acute coronary syndrome (ACS), especially in patients with plaque erosion compared with stable CAD and healthy people.⁷

However, most previous studies focused on HA inside the plaque, not in circulation. Interestingly, our study found that plasma HA levels decreased in STEMI patients, particularly in plaque erosion. This may indicate that regional disturbed flow and eroded plaques might influence the systemic change in HA levels in STEMI patients. HA experiences many pathophysiological processes in plaque erosion, including Toll-like receptor 2 (TLR2) stimulation, endothelial activation and neutrophil accumulation. Based on a previous study, we hypothesized that plasma HA depletion is induced by plaque erosion in three phases. The first hit is mediated by TLR2. Previous studies found that TLR2 is widely expressed on the surface of eroded plaques in the zone of flow perturbation.²⁰ As one of the endogenous ligands, HA activate TLR2 which contributes to endothelial cell detachment and apoptosis.²¹ The second hit of HA is binding to immune cells, such as macrophages and neutrophils, and then eventually being degraded via the HYAL2 and CD44 pathways. In plaque erosion, overexpression of CD44 induces the adhesion of neutrophils to HA.⁷ HA fragmentation can result from degradation by the reactive oxygen species (ROS) that are produced by neutrophils.²² On the other hand, there is evidence that macrophages are involved in HA uptake and the removal of HA fragments in inflammation.²³ The third hit is the degradation of HA by platelets. In the condition of platelet aggregation, HYAL2 becomes expressed on the cell surface but is stored into α -granules in rest.²⁴ In particular, activated platelets have higher hyaluronidase activity than nonactivated platelets and can reduce the concentration of free HA in plasma. This phenomenon is more obvious in plaque erosion than plaque rupture because platelet-rich thrombi usually occur in eroded plaques. Furthermore, platelets also express CD44, which can bind to free HA in plasma when it is activated.²⁵

Furthermore, in our study, the plasma level of CD44 was also decreased in STEMI patients compared with healthy controls. This result mirrors the combination of HA and soluble CD44 in circulation. In reference with a previous study, we deduced that plasma HA binds to the N-terminal hyaluronan binding domain (HABD) of CD44 in an inflammatory state to consume free CD44 in plasma.²⁶ Although the mRNA expression of CD44v1 and CD44v6 in PBMCs has been reported to be different between plaque rupture and erosion in the past,⁷ there was no difference in soluble CD44 between plaque erosion and rupture in our study. Soluble CD44 is not only regulated by HA but also other factors, such as cytokines and shedding from immune cells.²⁷ The metabolism of soluble CD44 in STEMI patients needs further investigation.

It has been reported that the inhibition of HA synthesis accelerates the process of atherosclerosis because HA provides a protective effect on blood vessels.²⁸ Therefore, the relationship between HA reduction and atherosclerosis may exist as a circle of positive feedback. However, the continuous production of HA has a compensatory function. HA is produced by stromal cells via hyaluronan synthases (HAS1-3).⁴ A recently published study found that the gene expression of CD44 and HYAL2 are different between plaque rupture and erosion, but they can return to baseline after 1 year of follow-up.⁷ This finding implies that HA has a compensatory effect but that this effect does not occur rapidly. In the

acute phase of ischemia and inflammation, the level of HA decreased, but whether it can recover to normal needs further investigation.

Taken together, these findings suggest that plasma HA is consumed by an enhanced expression of HYAL2 in monocytes and platelets, a combination of TLR2 on endothelial cells and the uptake by macrophages mediated with the CD44 receptor (central illustration). This model derives from both the existing experimental and clinical investigations about plaque erosion and the data emerging from our study.

Limitation: This study has several potential limitations. First, patients with cardiac shock, congestive heart failure, a history of coronary artery bypass graft, left main diseases, extremely tortuous or heavily calcified vessels, or chronic total occlusion were not enrolled in our study. In addition, patients with massive thrombi have poor image quality. Therefore, selection bias cannot be excluded. Second, there is no independent cohort to validate the predictive value of HA in discriminating between plaque morphologies, which we aim to include in future studies. Third, hyaluronan ELISA kit (DHYALO, R&D Systems, Abingdon, UK) is only able to test the hyaluronan > 35 kDa, and the circulating level of LMW-HA which is lower than 35 kDa is unknown. In the future, we hope to use mass spectrometer to separate different molecular weight of HA. Finally, the sensitivity and specificity of plasma HA levels to predict erosion is not very high, which may be related to the small sample size. We hope to expand the number of enrolled patients in our further investigation.

Conclusion

To the best of our knowledge, this study is the first to demonstrate an independent association of low plasma HA levels with plaque erosion using OCT in patients with STEMI, which may become a potential biomarker or provide novel therapeutic targets for plaque erosion.

Abbreviations

HA: hyaluronan; STEMI: ST-Segment–Elevation Myocardial Infarction; OCT: optical coherence tomography; SCAD: stable coronary artery disease; ELISA: enzyme-linked immunosorbent assay; PMNc: polymorphonuclear; HYAL2: hyaluronidase 2; ECG: electrocardiogram; LDL-C: low-density lipoprotein-cholesterol; HDL-C: high-density lipoprotein-cholesterol; hs-CRP: high sensitive C-reactive protein; eGFR: estimated glomerular filtration rate; BMI: body mass index; ROC: receiver operating characteristic; AUC: area under curves; PR: plaque rupture; PE: plaque erosion; TMAO: trimethylamine N-oxide; CAD: coronary artery disease; HMW: high molecular weight; LMW: low molecular weight; PBMCs: peripheral blood mononuclear cells; ACS: acute coronary syndrome; TLR2: Toll-like receptor 2; ROS: reactive oxygen species; HABD: N-terminal hyaluronan binding domain; HAS1-3: hyaluronan synthases.

Declarations

Ethics approval and consent to participate

The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Fuwai Hospital. All patients provided written informed consent.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions

Study conception and design: Jiannan Li, Yu Tan, Hongbing Yan. Data acquisition, data analysis and interpretation: Zhaoxue Sheng, Runzhen Chen, Peng Zhou, Chen Liu. Drafting and revising the article: Jiannan Li, Yi Chen, Li Song, Hanjun Zhao. Final approval of the version to be published: All authors.

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Tables

Table 1: Baseline characteristics of subjects stratified by plaque types

Variables	Total (n = 162)	Plaque type		p-value
		Rupture (n = 87)	Erosion (n = 75)	
Age (years)	57.4 ± 11.5	59.4 ± 12.2	55.1 ± 10.2	0.018
Males	138(85.2%)	76(87.4%)	62(82.7%)	0.402
Body mass index (kg/m ²)	26.5 ± 4.0	26.4 ± 4.5	26.5 ± 3.4	0.948
Diabetes mellitus	48(29.6%)	27(31%)	21(28%)	0.673
Hypertension	93(57.4%)	54(62.1%)	39(52%)	0.196
Hyperlipidemia	132(81.5%)	75(86.2%)	57(76%)	0.095
Ischemic stroke	12(7.4%)	8(9.2%)	4(5.3%)	0.349
Smoker	113(69.8%)	61(70.1%)	52(69.3%)	0.914
Hs-CRP (mg/L)	6.3(2.6–11.0)	7.2(2.6–11.1)	5.3(2.6–10.9)	0.593
eGFR (ml/min/1.732 m ²)	87.9 ± 22.3	82.4 ± 22.6	94.3 ± 20.2	0.001
Glycosylated hemoglobin	6.6 ± 1.7	6.5 ± 1.7	6.8 ± 1.8	0.412
Triglyceride (mmol/L)	1.4(0.8-2.0)	1.2(0.8–1.9)	1.5(0.9–2.1)	0.193
LDL-C (mmol/L)	2.7 ± 0.9	2.6 ± 0.9	2.9 ± 0.8	0.045
HDL-C (mmol/L)	1.1(0.9–1.2)	1.1(0.9–1.2)	1.1(1.0-1.3)	0.117
Plasma hyaluronan (ng/ml)	30.1(17.6–47.7)	36.2(18.9–51.9)	25.1(15.4–41.4)	0.021
Plasma CD44 (ng/ml)	143.7(90.2-427.4)	142.5(129.1-162.6)	144.5(123.4-164.7)	0.975
Continuous data are presented as mean ± SD or median (interquartile range), categorical variables are presented as %. eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; hs-CRP, high sensitive C-reactive protein				

Table 2: Logistic Regression Analyses of Plasma HA Levels in Patients with Plaque rupture and erosion

Variables	Univariate		Multivariate	
	OR(95%CI)	p value	OR(95%CI)	p value
Age(years)	1.034(1.005–1.063)	0.020	1.010(0.973–1.048)	0.606
Sex(male)	0.690(0.289–1.648)	0.404	0.883(0.292–2.668)	0.826
Body mass index (kg/m ²)	0.997(0.924–1.077)	0.947	0.965(0.878–1.061)	0.466
Smoking	1.038(0.530–2.032)	0.914	0.861(0.363–2.042)	0.734
Hypertension	1.510(0.807–2.827)	0.197	1.042(0.485–2.237)	0.916
Diabetes	1.157(0.587–2.281)	0.673	0.947(0.421–2.129)	0.894
Hyperlipidemia	1.947(0.868–4.369)	0.106	2.599(0.954–7.080)	0.062
LDL-C (mmol/L)	0.694(0.483–0.997)	0.048	0.886(0.577–1.361)	0.580
HDL-C (mmol/L)	0.251(0.072–0.872)	0.030	0.215(0.043–1.091)	0.064
Triglyceride (mmol/L)	0.989(0.793–1.234)	0.923	0.964(0.727–1.278)	0.800
Hs-CRP (mg/L)	1.025(0.957–1.097)	0.479	1.011(0.934–1.094)	0.790
eGFR	0.973(0.958–0.989)	0.001	0.979(0.961–0.998)	0.029
HA (ng/ml)	2.596(1.375–4.899)	0.003	2.673(1.269–5.627)	0.010

LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; hs-CRP, high sensitive C-reactive protein; eGFR, estimated glomerular filtration rate. HA, hyaluronan; HA was transformed to categorical variables through cutoff value (29.6 ng/ml).

Figures

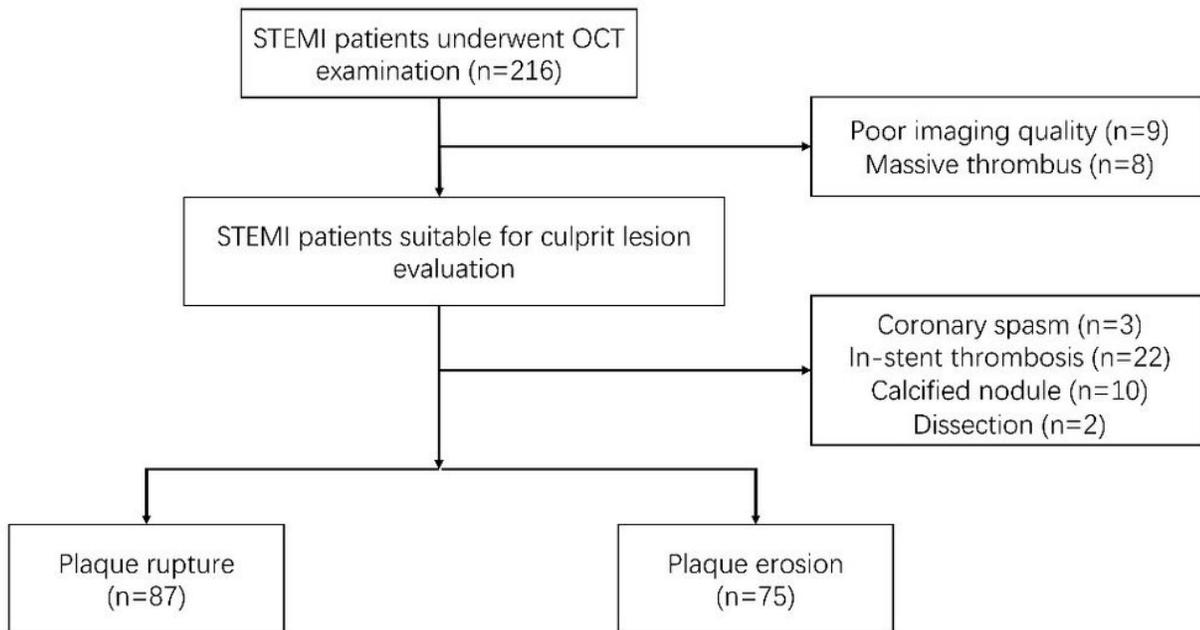


Figure 1

Study flow chart. OCT=optical coherence tomography; STEMI=ST-Segment–Elevation Myocardial Infarction.

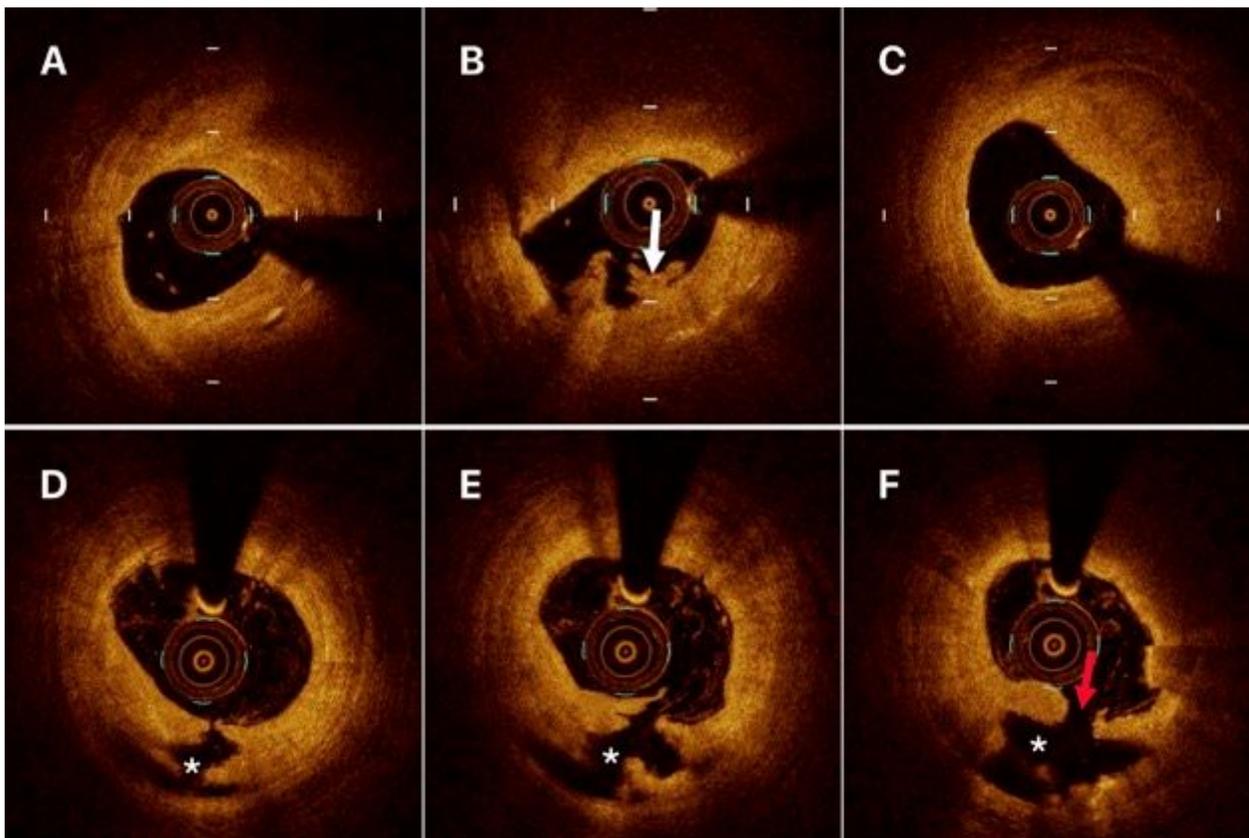


Figure 2

Representative optical coherence tomography images for plaque erosion and rupture. A–C, Plaque erosion defined by residual white thrombus (white arrow) underlying a fibrous plaque without evidence of fibrous cap disruption. D–F, Plaque rupture identified by the presence of disrupted fibrous cap (red arrow) and cavity formation (asterisk).

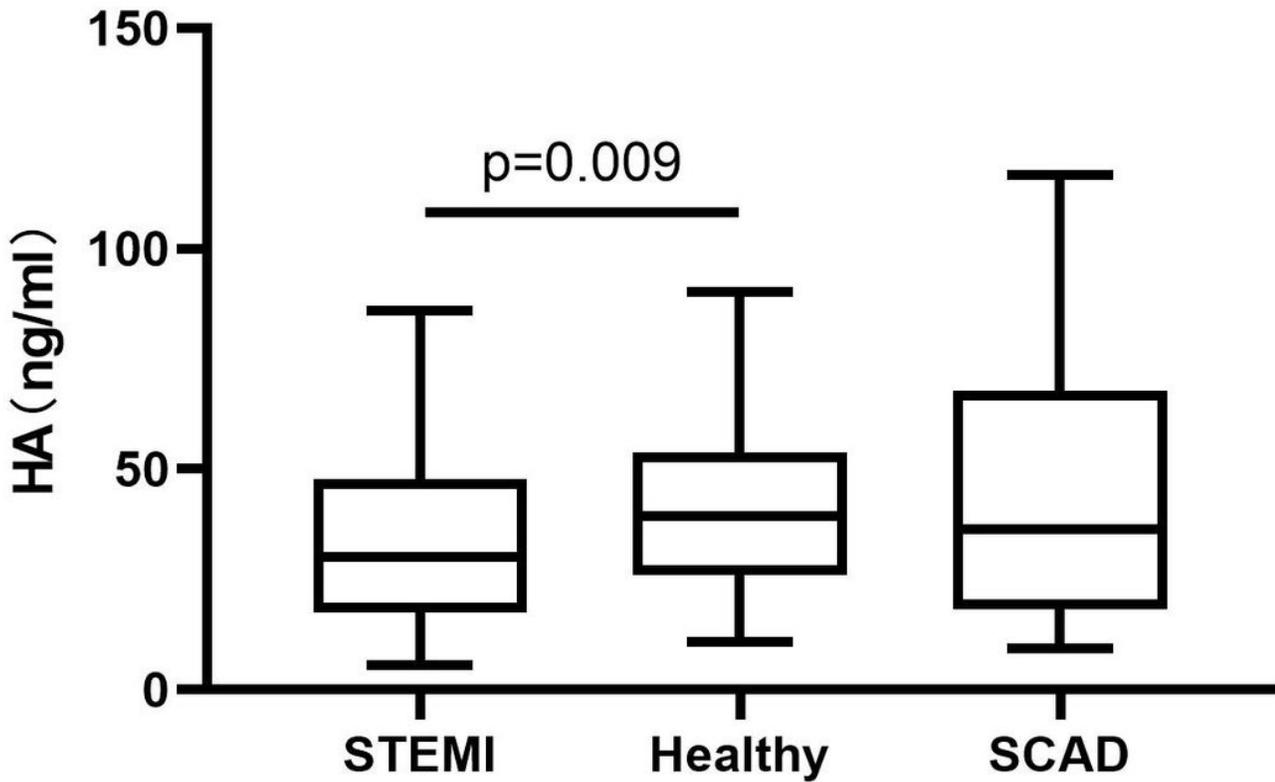


Figure 3

Relations of plasma HA levels among patients with STEMI, SCAD and healthy subjects. HA=hyaluronan; STEMI=ST-Segment–Elevation Myocardial Infarction; SCAD=Stable Coronary Artery Disease

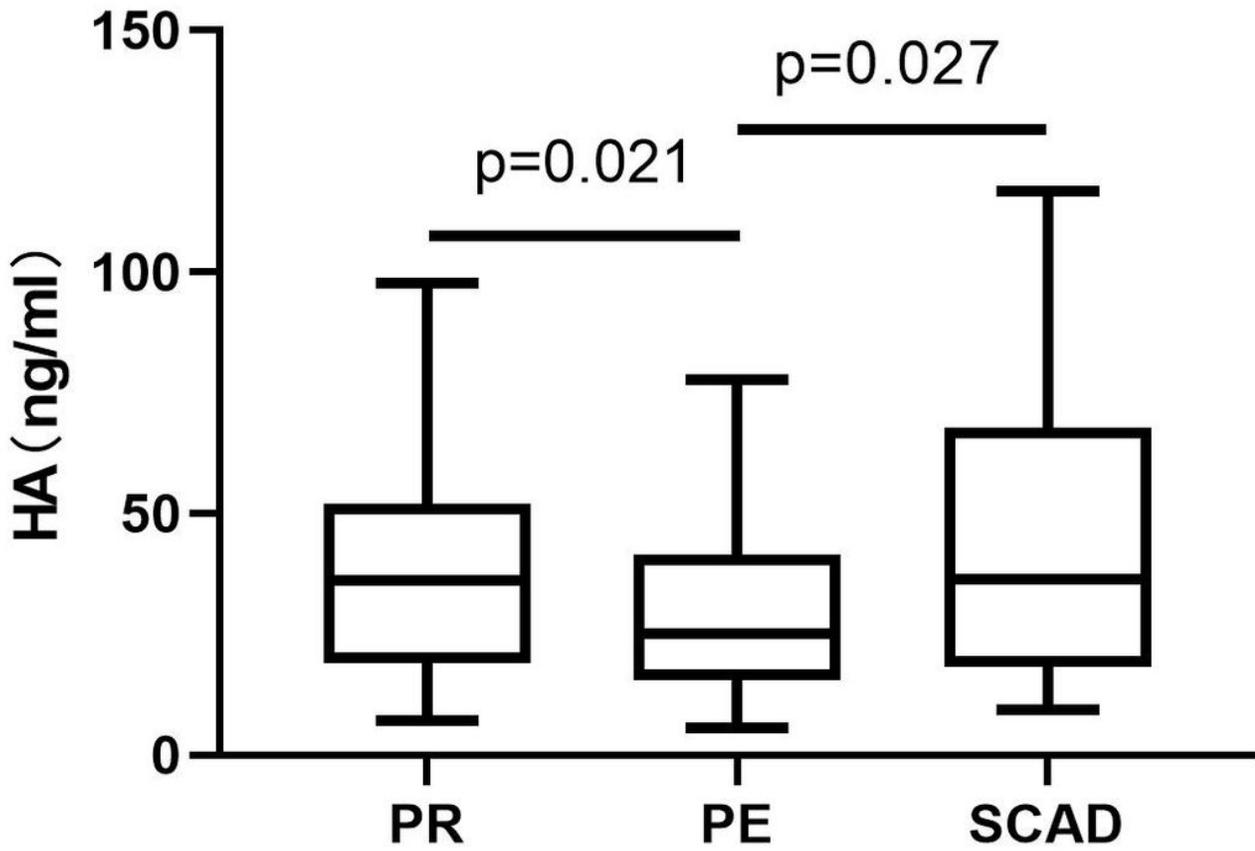


Figure 4

Relations of plasma HA levels among patients with PR, PE and SCAD. HA=hyaluronan; PR=Plaque Rupture; PE=Plaque Erosion; SCAD=Stable Coronary Artery Disease

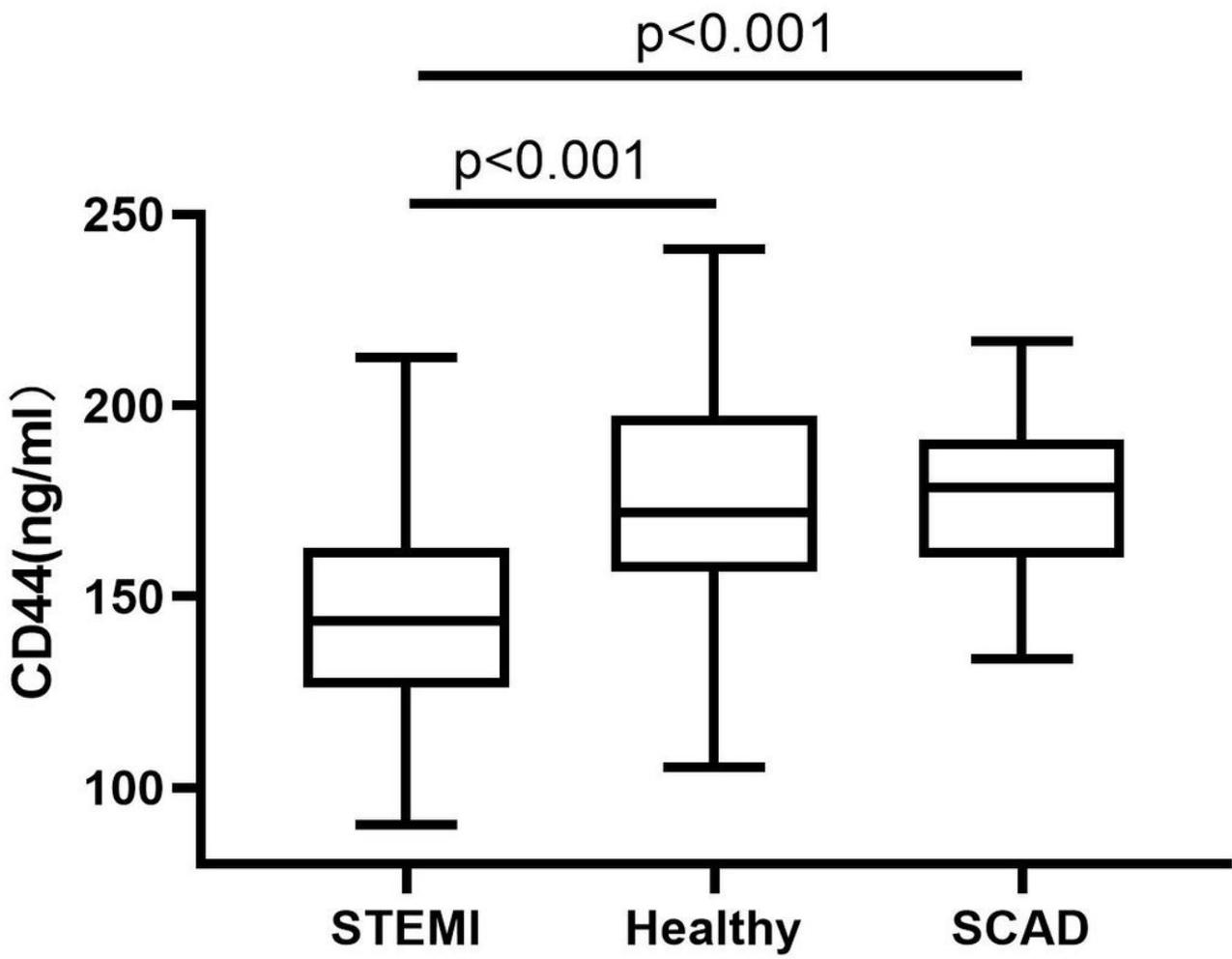


Figure 5

Relations of plasma CD44 levels among patients with STEMI, SCAD and healthy subjects.
HA=hyaluronan; STEMI=ST-Segment-Elevation Myocardial Infarction; SCAD=Stable Coronary Artery Disease

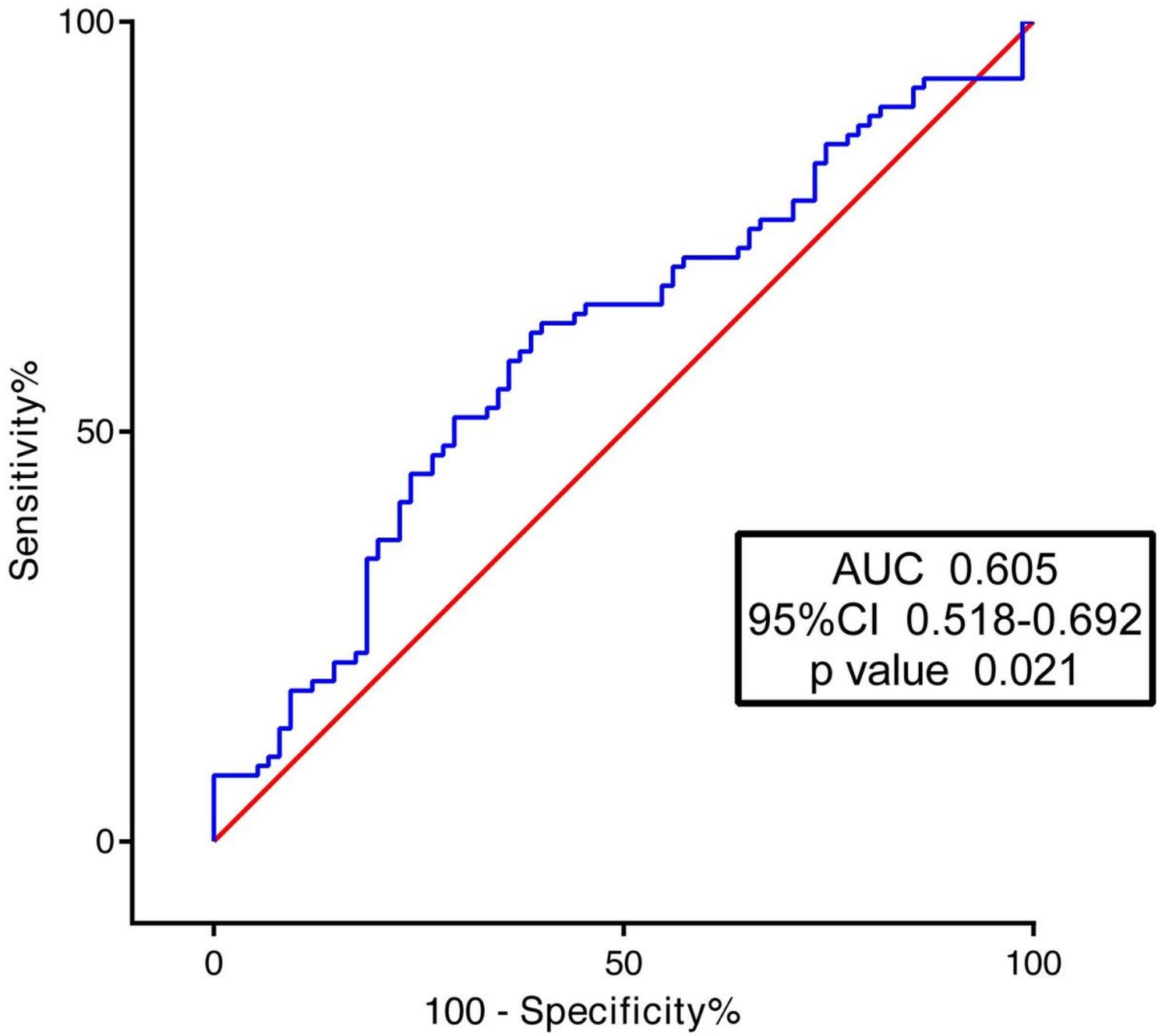


Figure 6

Receiver operating characteristic curves of hyaluronan (HA) for predicting plaque erosion.