

Value of S100A12 in predicting in-stent restenosis in patients with coronary drug-eluting stent implantation

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Abstract. In-stent restenosis (ISR) after drug-eluting stent (DES) placement has recently emerged as a major concern for cardiologists. Identification of biomarkers to predict ISR may be invaluable for tailored management strategies. The present study aimed to evaluate the prognostic utility of circulating S100 calcium-binding protein A12 (S100A12) for ISR. Out of 2,443 patients with DES-based percutaneous coronary intervention (PCI) and follow-up angiography at ~1 year after DES-based PCI, 258 patients were diagnosed with ISR and 258 patients without ISR were randomly selected as controls. Serum S100A12 levels were determined in the two subsets on admission. The association between ISR and the circulating levels of S100A12 was determined by constructing two multivariate stepwise logistic regression models. In addition, S100A12 was assessed for its ability to predict ISR using receiver operating characteristic (ROC) curve analysis. The serum levels of S100A12 at baseline were significantly elevated in patients in the ISR group compared with those in the non-ISR group ($P<0.001$). In the multivariate logistic regression analysis, after adjusting for conventional cardiovascular risk factors, laboratory parameters and medication after the procedure, the S100A12 level was revealed to be independently associated with ISR. When a cut-off for serum S100A12 levels of 34.75 ng/ml was used, the ROC curve was able to predict ISR with 72.8% sensitivity and 79.1% specificity, and the area under the ROC curve was 0.796 (95% CI: 0.757 to 0.834, $P<0.001$). Furthermore, addition of S100A12 to

established risk factors significantly improved the predictive power of reference models for ISR. S100A12 may serve as an independent marker to predict ISR in patients undergoing coronary DES implantation.

Introduction

Despite dual anti-platelet and intensive statin therapy, in-stent restenosis (ISR) remains the Achilles heel of percutaneous coronary intervention (PCI) in the era of drug-eluting stents (DES); it has been reported that ISR occurs in 12.2-14.6% of patients with a drug-eluting stent, depending on patient-associated factors, as well as lesion and procedure-associated characteristics, including the stenting technique and stent type (1,2). The pathophysiological mechanisms of ISR have remained to be fully elucidated. The major trend of the current viewpoint supports inflammatory mechanisms of adverse responses to DES. It is suggested that the abnormal proliferation and migration of vascular smooth muscle cells (VSMCs), as well as extracellular matrix accumulation activated by various pro-inflammatory mediators and growth factors in response to local vascular injury, result in neointima formation and eventually lumen loss (3-6). Therefore, identifying a specific, sensitive and non-invasive blood biomarker for predicting ISR is of clinical significance in order to select tailored management strategies, including the use of multiple stents or offering more prolonged anti-platelet therapy to patients with coronary artery disease (CAD) with an elevated risk of ISR.

S100 calcium-binding protein A12 (S100A12) is a member of the S100 family of calcium-binding proteins, which are predominantly expressed and secreted by myeloid-derived cells, including neutrophils and monocytes (7). In the past 10 years, the roles of S100A12 in inflammatory diseases have been gradually determined. It has been indicated that the pro-inflammatory effects of S100A12 are mediated through interaction with MOK protein kinase and Toll-like receptor (TLR)4, which have important roles in mediating acute and chronic inflammation and are relevant for atherosclerosis (8). Elevated serum levels of S100A12 have been reported in a number of inflammatory disorders, including chronic active

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inflammatory bowel disease, Kawasaki disease, rheumatoid arthritis, Behcet's disease and glomerulonephritis (9-12).

Recent studies suggest the biological and clinical involvement of S100A12 in atherosclerosis. S100A12 was also indicated to participate in atherosclerosis by mast cell and monocyte recruitment as well as mast cell activation (13). Animal experiments in transgenic mice with smooth muscle cell-targeted expression of S100A12 suggested more severe atherosclerosis, an increased necrotic core, calcified plaque area and reduced extracellular matrix in apolipoprotein E (ApoE)^{-/-} mice (14). In fact, strong immunostaining for S100A12 has been detected in advanced atherosclerosis, particularly at the rupture sites of atherosclerotic plaques of subjects with sudden cardiac death, suggesting a potential association of locally expressed S100A12 with plaque instability (15). Mahajan *et al* (16) indicated that S100A12 exacerbated atherosclerosis by promoting pro-inflammatory cytokines in an autocrine, paracrine and endocrine manner at the site of atherosclerotic lesions via activation of RAGE signaling. Ligthart's study demonstrated the predictive value of serum S100A12 for future myocardial infarction (MI) and CAD-associated mortality in 839 healthy participants over a 10.6-year follow-up after adjusting for inflammatory markers and traditional risk factors (17). Another cross-sectional study analyzed 652 patients with stable CAD who underwent PCI, revealing that the serum levels of S100A12 were an independent factor for predicting major adverse cardiovascular events, defined as a composite of events of congestive heart failure, recurrence of angina pectoris, acute myocardial infarction, stroke, critical arrhythmia, intervention on peripheral arteries and cardiac death (18).

Given the pro-inflammatory characteristics of S100A12 and its potential implications in the initiation and progression of atherosclerosis and CAD, it was speculated that increased serum levels of S100A12 at baseline may contribute to restenosis in patients with stent implantation. To test this hypothesis, the serum levels of S100A12 were compared between consecutive patients with angiographically documented ISR and patients with no ISR after DES-based PCI, and the predictive value of circulating S100A12 levels for ISR was also assessed.

Patients and methods

Patient population. All of the patients (n=2,443) who underwent coronary angiography (CAG) and subsequent DES-based PCI of *de novo* lesions in native coronary arteries between October 2014 and June 2018 at the Department of Cardiology, Affiliated Provincial Hospital, Shandong University (Jinan, China) were retrospectively screened. Each patient was routinely treated with dual anti-platelet therapy (aspirin for an indefinite period and a P2Y₁₂ inhibitor for at least 1 month and up to 12 months) after stent deployment. At ~1 year after the procedure, 642 patients were subjected to follow-up CAG due to recurrent symptoms of abnormal non-invasive test results for angina (either treadmill exercise tests or myocardial perfusion scintigraphy), and ISR was detected in 308 patients. Of these 308 patients, those who had concomitant valvular disease (n=8), systematic inflammatory disease (n=16), malignant tumor (n=10), severe liver disease (n=6), moderate to severe chronic renal insufficiency [estimated glomerular filtration

rate (eGFR) <60 ml/min/1.73 m²; n=10] were excluded. The remaining 258 patients with ISR constituted the population of the present study. In addition, 258 age- and sex-matched patients who had no ISR at the follow-up for CAG within the same study period were randomly selected as the control group. The protocol was approved by the institutional review board of Shandong Provincial Hospital (Jinan, China).

CAG study. Coronary angiography was performed according to standard Judkins techniques. Coronary cineangiography was interpreted by two cardiologists who were blinded to each patient's clinical characteristics and laboratory test results. Using the outer diameter of the contrast-filled catheter as the calibration standard, the minimal lumen diameter was measured in diastolic frames from orthogonal projections. The reference vessel diameter was averaged from user-defined, 5-mm, angiographically normal segments proximal and distal to the lesion but between any major side branches. The lesion was stented using a normal-to-normal technique, usually including 5-mm, angiographically normal segments proximal and distal to the lesion. A successful PCI was considered as achieving a minimum stenosis diameter reduction to <30% in the absence of any branch loss, dissection or thrombus formation. ISR was defined as recurrence of luminal diameter narrowing by >50% in a vessel of otherwise normal diameter, including 5 mm proximal and distal to the stent edge.

Evaluation of risk factors. Demographic and clinical characteristics were obtained by reviewing the hospital records of patients. Age, sex, body mass index (BMI), family history of CAD, smoking status, prevalence of concomitant disease, including hypertension, type 2 diabetes, hypercholesterolemia, as well as laboratory data, including fasting blood glucose, cholesterol, high-/low-density lipoprotein cholesterol and cardiac troponin T, were all recorded. Hypertension, type 2 diabetes and hypercholesterolemia were diagnosed in accordance with the European Society of Hypertension/European Society of Cardiology guidelines for the management of arterial hypertension (19), the criteria of the American Diabetes Association and the Third Report of The National Cholesterol Education Program (20,21), respectively. Family history of CAD was defined as the presence of CAD or sudden cardiac death in a first-degree relative prior to the age of 55 years for males and prior to the age of 65 years for females.

Blood sampling and measurements of serum S100A12. Peripheral blood samples from all patients had been obtained upon admission prior to coronary angiography after overnight fasting. Blood samples for measurement of S100A12 were collected in tubes containing potassium EDTA and then centrifuged for 20 min at 670.8 x g and 4°C and stored at -80°C until analysis. S100A12 levels in serum were determined using the Human S100A12 Platinum ELISA (eBioscience) according to the manufacturer's protocol. The overall intra- and inter-assay coefficient of variation were 5.5 and 11.9%, respectively. All measurements were performed in a blinded manner in duplicate.

Statistical analysis. The Kolmogorov-Smirnov test was used to assess the distribution pattern of data. Continuous variables

Table I. Baseline clinical, biochemical and angiographic characteristics of the study population.

Parameter	ISR(-) (n=258)	ISR(+) (n=258)	P-value
Age (years)	59.1 ± 10.4	61.8 ± 10.1	0.569
Sex (male/female)	178/80	183/75	0.631
Body mass index (kg/m ²)	26.82 ± 3.96	29.19 ± 3.41	0.001
Systolic blood pressure (mmHg)	137.6 ± 18.2	139.8 ± 20.7	0.329
Diastolic blood pressure (mmHg)	85.1 ± 10.1	84.7 ± 12.8	0.239
Family history of CAD	33 (12.8)	68 (26.4)	<0.001
Cardiovascular risk factors			
Hypertension	192 (74.4)	181 (70.2)	0.279
Diabetes mellitus	70 (27.1)	91 (35.3)	0.046
Hyperlipidemia	78 (29.7)	97 (40.8)	0.033
Smoking	76 (27.5)	133 (51.6)	<0.001
Biochemical measurements			
Total cholesterol (mmol/l)	4.85 ± 1.09	4.96 ± 1.13	<0.005
LDL-cholesterol (mmol/l)	2.77 ± 0.83	2.91 ± 1.54	<0.001
HDL-cholesterol (mmol/l)	1.25 ± 0.31	1.13 ± 0.28	<0.001
Triglyceride (mmol/l)	1.93 ± 1.51	1.96 ± 1.75	0.326
White blood cells (x10 ⁹ /l)	7.82 ± 1.51	7.22 ± 2.08	0.369
Fibrinogen (g/l)	3.11 ± 1.17	3.22 ± 0.91	0.129
Fasting glucose (mmol/l)	5.72 ± 1.42	5.92 ± 1.96	0.251
eGFR (ml/min/1.73 m ²)	83.74 ± 26.04	86.01 ± 22.06	0.170
hsCRP (mg/l)	5.33 ± 2.55	5.47 ± 6.02	0.087
S100A12 (ng/ml)	32.61 ± 5.82	39.84 ± 9.15	<0.001
LVEF (%)	64.6 ± 3.7	58.6 ± 5.3	0.005
Coronary angiography Gensini score	62.4 ± 31.16	62.2 ± 36.9	0.129
Lesion characteristics			
CTO	21 (8.1)	55 (21.3)	<0.001
Bifurcation lesions	53 (20.5)	83 (32.2)	0.003
LM stenosis	16 (6.2)	16 (6.2)	1.0
Stent characteristics			
Number of stents	1.91 ± 1.1	2.1 ± 1.3	<0.001
Diameter (mm)	3.24 ± 0.34	3.18 ± 0.39	<0.001
Length (mm)	34.2 ± 22.3	42.8 ± 27.8	0.008
Stent type			
Sirolimus-eluting stent	169	176 (68.2)	0.513
Zotarolimus-eluting stent	53	48 (18.6)	0.579
Everolimus-eluting stent	20	16 (6.2)	0.489
Tacrolimus-eluting stent	16	18 (7.0)	0.814
Cardiac medication after PCI			
Dual anti-platelet therapy	253 (98.1)	255 (98.8)	0.476
β-blockers	205 (79.5)	220 (85.3)	0.083
ACEI/ARB	130 (50.4)	152 (58.9)	0.052
Calcium antagonists	105 (40.7)	102 (39.5)	0.778
Statins	245 (95)	255 (98.8)	0.011

Values are expressed as n (%) or mean ± standard deviation. ISR, in-stent restenosis; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; hsCRP, hypersensitive C-reactive protein; LVEF, left ventricular ejection fraction; CTO, chronic total occlusion; LM stenosis, left main coronary artery stenosis; PCI, percutaneous coronary intervention; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; H/LDL, high-/low-density lipoprotein; S100A12, S100 calcium-binding protein A12.

were expressed as the mean ± standard deviation or median with interquartile range and were compared by analysis of one-way ANOVA or unpaired Student's t-test. Categorical variables were expressed as counts and percentages and were

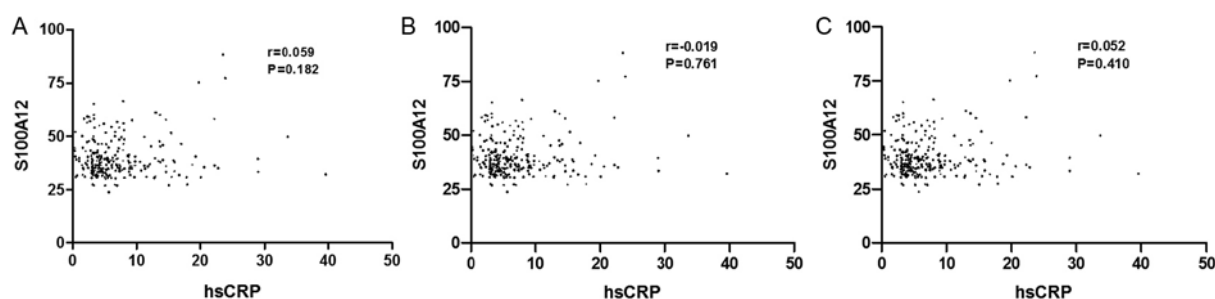


Figure 1. Scatter plots showing correlation between S100A12 and hsCRP in (A) all enrolled patients, (B) patients with ISR, and (C) patients without ISR. ISR, in-stent restenosis; S100A12, S100 calcium-binding protein A12; hsCRP, hypersensitive C-reactive protein.

compared by using the χ^2 test. Patients with ISR were categorized into 4 groups according to the quartile distribution of serum S100A12 levels ($\mu\text{g/ml}$). Spearman's correlation coefficient was determined to evaluate the association between two continuous variables.

Two multivariate stepwise logistic regression models were constructed to determine the association between ISR and circulating levels of S100A12. In Model 1, age, sex, BMI, conventional risk factors, biochemical parameters, medication, angiographic and procedure-associated features, were included. In model 2, the analysis was additionally adjusted for the serum levels of S100A12. The two models were tested for their ability to predict ISR using receiver operating characteristic (ROC) analysis, and the areas under the ROC curve (C statistics) were compared using MedCalc software for Windows (version 5.0; MedCalc Software) to evaluate whether S100A12 levels have an incremental predictive value for ISR. Net reclassification improvements (NRI) and integrated discrimination improvements (IDI) were also calculated to assess the improvement of the regression model with further inclusion of the circulating S100A12 concentration. The calibration of the model was checked using the Hosmer-Lemeshow χ^2 test. All tests were 2-tailed and $P < 0.05$ was considered to indicate statistical significance. All analyses were performed using SPSS version 19.0 for Windows (IBM Corp.).

Results

Clinical characteristics. A total of 516 patients were enrolled in the present study. The mean age in the study population was 61.2 ± 10.2 years and 361 (69.9%) subjects were males. The baseline clinical, biochemical and angiographic characteristics of patients with and without ISR are detailed in Table I. The prevalence rates of hypertension was similar in the two groups. Patients with ISR were more likely to have a family history of CAD ($P < 0.001$), to smoke ($P < 0.001$) and to have hyperlipidemia ($P = 0.033$) and diabetes mellitus ($P = 0.046$) compared with those without ISR, and they had a higher BMI ($P = 0.033$).

As for the angiography results, despite the similar degree of stenosis prior to PCI, left main coronary artery stenosis and type of DES implanted, patients in the ISR group were observed to more frequently have severe coronary lesions, including chronic total occlusion and bifurcation lesions. Furthermore, patients with ISR received smaller in diameter but longer stents and they had a higher percentage of statin

therapy after the procedure as compared to patients without ISR.

Association between serum S100A12 and ISR. The serum levels of S100A12 were significantly elevated in patients in the ISR group compared with those in the non-ISR group ($P < 0.001$). However, no significant correlation was observed between S100A12 and hsCRP (Fig. 1). The patients were further categorized into four groups according to the quartile distribution of S100A12. The incidence of ISR increased gradually across the quartiles of S100A12 ($P < 0.001$; Fig. 2). This association remained significant in subgroups of males ($P = 0.023$), older patients (age, > 65 years; $P = 0.036$) and smokers ($P < 0.001$; Fig. 3).

In the multivariate logistic regression analysis (Table II), after adjusting for conventional cardiovascular risk factors, laboratory parameters, medication after the procedure, angiographic and procedural features, it was determined that smoking, LVEF, bifurcation lesion and stent diameter were independent risk factors for ISR (Model 1). When S100A12 was further included in model 2, in addition to the factors in model 1, they all (including S100A12) remained independently associated with ISR.

In the study population, addition of S100A12 to the model enhanced the predictive value for ISR with an increase of the C statistic of 0.027 (95% CI, 0.011-0.043, $P = 0.026$). At the same time, the values of NRI and IDI achieved by inclusion of S100A12 were also significantly improved (6.1% for NRI, $P = 0.003$; 5.3% for IDI, $P < 0.0001$, respectively; Table III).

ROC analysis revealed that when a cut-off for the serum levels of S100A12 of 34.75 ng/ml was used, it was possible to predict ISR with 72.8% sensitivity and 79.1% specificity, and the area under the ROC curve was 0.796 (95% CI: 0.757-0.834; $P < 0.001$; Fig. 4).

Discussion

The major results of the present study are as follows: i) Serum levels of S100A12 were significantly elevated in patients with ISR compared with those in patients without ISR. ii) Multivariate logistic regression analysis further revealed that S100A12 was an independent risk factor for IRS, beyond the predictive capacity of traditional risk factors, biochemical measurements, angiographic and procedural characteristics. iii) Baseline levels of S100A12 provide incremental predictive value for ISR in patients undergoing coronary DES implantation.

Table II. Multivariate logistic regression analysis for the risk of in-stent restenosis.

Factor	Model 1		Model 2	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Smoking	7.853 (2.597-23.743)	0.001	4.952 (1.893-12.954)	<0.001
LVEF	0.765 (0.674-0.868)	<0.001	0.739 (0.644-0.848)	<0.001
Bifurcation lesion	2.021 (1.018-5.332)	0.021	1.912 (1.236-6.398)	0.046
Stent diameter	0.48 (0.11-0.818)	<0.001	0.201 (0.102-0.763)	<0.001
S100A12	NA	NA	1.228 (1.152-1.309)	<0.001

In model 1, adjusted covariates include conventional cardiovascular risk factors, laboratory parameters, medication after the procedure, as well as angiographic and procedural features. In model 2, S100A12 was included in addition to the factors in model 1. OR, odds ratio; LVEF, left ventricular ejection fraction. NA, not applicable.

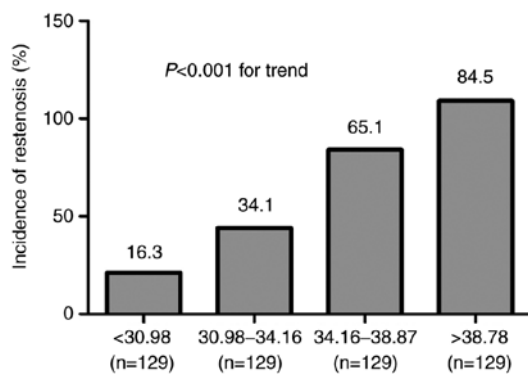


Figure 2. Incidence of IRS according to the quartiles of the S100A12 concentration (ng/ml). The incidence of IRS across the quartiles of S100A12 was compared using the χ^2 test. ISR, in-stent restenosis; S100A12, S100 calcium-binding protein A12.

PCI with DES has revolutionized the treatment of patients with CAD over the past decade; however, its benefits are overshadowed by the occurrence of in-stent restenosis (1,2). Vascular inflammation has a pathogenetic role in the development of ISR after deployment of DES (3,5). It is generally accepted that the vascular inflammatory status prior to the intervention and the severity of the inflammatory reaction after the procedure are determinants of ISR (22). In parallel, due to the involvement of the inflammatory process that follows DES implantation, the use of non-invasive biomarkers for the identification of patients at an increased risk of ISR appears suitable.

Due to its pro-inflammatory features, S100A12 has been indicated to predict outcomes in different subsets of patients, including those with chronic heart failure, acute coronary syndrome (ACS) and stable coronary artery disease (SCAD), as well as in the general population (11,16,23,24). Furthermore, the predictive value of S100A12 for ISR after DES has remained elusive. Of note, as an important supplement to previous studies, the present study demonstrated the independent and incremental predictive value of the circulating S100A12 concentration for ISR following deployment of DES.

Intimal and medial injury during PCI provokes a perivascular inflammatory response (22,25). The vascular inflammatory status prior to intervention and the severity of inflammatory

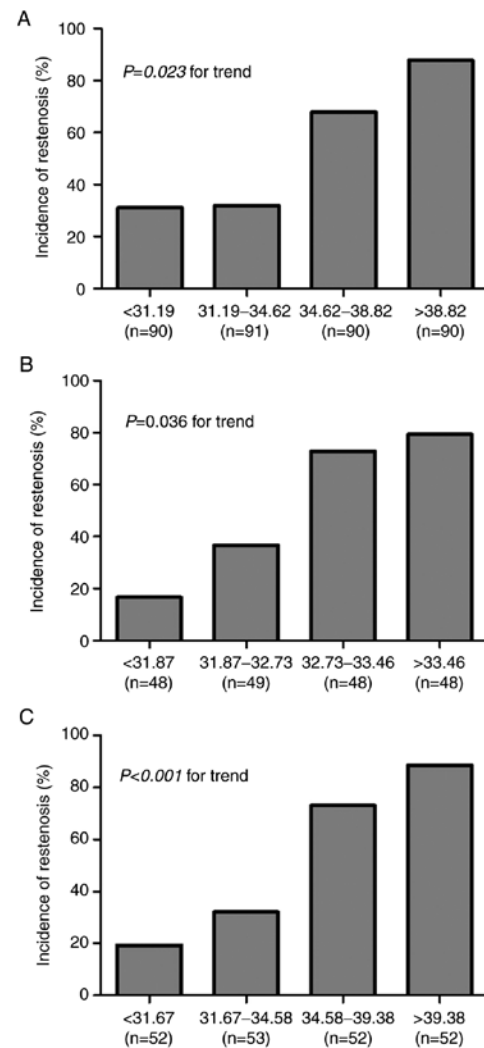


Figure 3. Incidence of IRS according to the quartiles of S100A12 concentration (ng/ml) in (A) male patients, (B) subjects aged >65 years and (C) patients who were smokers. The incidence of IRS across the quartiles of S100A12 was compared using the χ^2 test. ISR, in-stent restenosis; S100A12, S100 calcium-binding protein A12.

reaction after the procedure appear to be the determinants of ISR. The pro-inflammatory and atherogenic properties of local S100A12 expression have been well elucidated. Consistent with

Table III. Incremental predictive value of S100 calcium-binding protein A12 level for in-stent restenosis calculated by AUC difference, NDR and IDI.

	AUC	P-value	NRI	P-value	IDI	P-value
Model 1	0.916	<0.0001	Reference	Reference	Reference	Reference
Model 2	0.943	<0.0001	6.1%	0.003	5.3%	<0.0001
$AUC_{Model\ 2} - AUC_{Model\ 1}$	0.027	0.026				

AUC, area under the ROC curve; NRI, net reclassification improvement; IDI, integrated discrimination improvement.

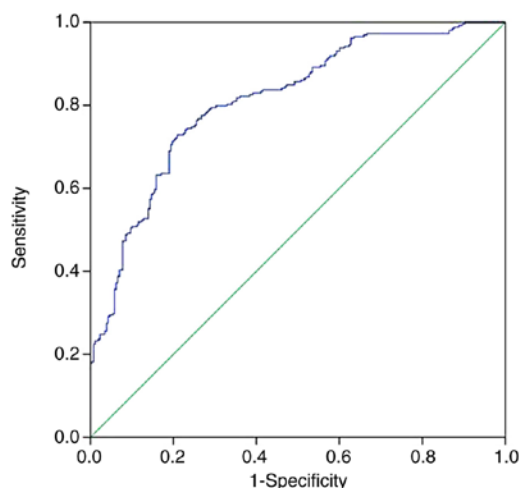


Figure 4. Receiver operating characteristics curve for the prediction of in-stent restenosis by the circulating levels of S100 calcium-binding protein A12.

the results of basic studies, S100A12 levels have been demonstrated to be a reliable and independent marker of increased cardiovascular risk in different subgroups of patients, including those with recent ACS and SCAD, as well as in healthy individuals (11,18,26). S100A12 concentrations have also been reported to be associated with the extent of atherosclerosis in coronary arteries and the presence of complex lesion morphology as detected by coronary angiogram (24,27). The levels of S100A12 were reported to be elevated in ACS patients as compared with those in patients with SCAD and healthy controls, and to have a favorable prognostic value for ACS (23), suggesting a significant link between plaque vulnerability and S100A12. All of this suggests that the circulating levels of S100A12 may be capable of reflecting the inflammatory status in the vascular wall, considering the consistency between the local expression and the circulation level. Furthermore, patients with more extensive vascular inflammation after PCI may be more vulnerable to recurrent lumen narrowing; thus, the higher pre-procedural S100A12 values are bound to aggravate the extent and susceptibility of the inflammation in response to vascular injury, including hypertrophic vascular remodeling and excessive neointima formation, which are hallmarks of ISR.

One of the possible mechanisms for the involvement of S100A12 in ISR after PCI is its affinity to bind to MOK protein kinase and TLR4. The interaction of S100A12 with MOK protein kinase or TLR4 triggers activation of NF- κ B, which results in the production of pro-inflammatory cytokines,

including TNF- α and IL-1 β (8,28,29). All of these cytokines are known to have a pathogenetic role in the development of atherosclerosis and restenosis after PCI (30). The chemotactic effect of S100A12 may be another mechanism underlying the association of S100A12 with ISR. S100A12 was reported to enhance the expression of adhesion molecules, including intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, contributing to monocyte recruitment as well as migration (8,31). This is important, as monocytes/macrophages are directly involved in ISR by secretion of pro-inflammatory cytokines (32). Furthermore, the pivotal role of augmented reactive oxygen species (ROS) in the pathogenesis of ISR has been well established (33). Tardif *et al* (34) indicated that ROS were involved in the secretion of S100A12 by neutrophils. It may be speculated that ROS-mediated S100A12 production, as observed in the serum of patients with ISR, also provides a possible mechanistic explanation for the strong positive link between elevated levels of S100A12 and increased risk for ISR.

The present study has several limitations that should be addressed. First, the observations of the present cross-sectional study should be further evaluated in large-scale studies prior to establishing a causal association between S100A12 and ISR following DES implantation. The present study was a single-center and non-randomized study and the sample size was relatively small. This may limit the interpretation and application of the present results to a certain extent. In addition, the inclusion of patients may have been biased due to symptom-driven coronary angiography at follow-up, which may limit the reliability of the results.

In conclusion, the present study was the first, to the best of our knowledge, to indicate that the elevated level of S100A12 at baseline is independently associated with an increased risk for restenosis after DES-based PCI. The present results support a prognostic utility of S100A12 in ISR. Although the utility of serum S100A12 as a single surrogate biomarker for predicting ISR is limited, it may be useful as part of a multi-marker panel for improved identification of patients with increased risk of ISR after DES implantation.

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Availability of data and materials

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

Authors' contributions

LMC designed the study. YHL and YQC reviewed the medical records and collected the data. HRB, HL, PCY and LMC performed data analysis. YHL and YQC wrote the manuscript. LMC and YQC was responsible for manuscript revisions and all the authors reviewed the manuscript.

Ethics approval and consent to participate

The protocol was approved by the institutional review board of Shandong Provincial Hospital (Jinan, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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