

Association between circulating big endothelin-1 and noncalcified or mixed coronary atherosclerotic plaques

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Background Noncalcified plaques (NCPs) and mixed plaques (MPs) are considered as the high-risk coronary plaques. Endothelin-1 (ET-1) is a vasoactive peptide and shows a high expression in vulnerable plaque. The aim of this study is to investigate the relationship between the bigET-1, the precursor of ET-1, and NCPs/MPs in a Chinese population.

Methods and results A total of 513 patients with chest pain and suspected coronary artery disease were collected and divided into three groups with no plaques, calcified plaques, or NCPs/MPs according to the characteristics of all the plaques. It demonstrated that NCPs/MPs were associated with elevated bigET-1 ($P < 0.001$). Moreover, the proportion of NCPs/MPs was significantly increased from 43.3% in bigET-1 tertile 1 to 61.0% in tertile 3 group ($P = 0.001$). Multiple logistic regression analysis further showed that bigET-1 was an independent predictor for

the presence of NCPs/MPs (odds ratio = 1.858; 95% confidence interval: 1.017–3.394; $P = 0.044$).

Conclusion The bigET-1 could be an independent predictor for the presence of NCPs/MPs. *Coron Artery Dis* 30: 461–466 Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

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Keywords: big endothelin-1, coronary atherosclerotic plaques, endothelin-1, mixed plaques, noncalcified plaques

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Introduction

Studies over past two decades have demonstrated that the vulnerable or high-risk plaques are responsible for most acute coronary syndromes (ACS) cases [1–3]. Lipid-enriched noncalcified plaques (NCPs) and mixed plaques (MPs) are considered as the high-risk plaques, because they are prone to rupture, associated with plaque volume progression and increased coronary event rates [4]. In fact, patients with NCPs or MPs (NCPs/MPs) have a higher risk of poor outcomes compared with those without coronary plaque (CP) or those have calcified plaques [5].

Endothelin-1 (ET-1) is a vasoactive peptide and considered as a prognostic parameter in patients with heart failure, stable coronary artery disease (CAD), and hypertrophic cardiomyopathy [6–8]. Moreover, ET-1 plays an important role in the development of atherosclerosis by affecting all cells of the vasculature. The expression of ET-1 and its receptors is upregulated in models of atherosclerosis and in human atherosclerotic lesions [9–11]. The level of ET-1 has been found to be elevated in the

first hour after acute myocardial infarction, and vulnerable plaques show a high content of ET-1 [12,13]. Being a peptide with high biologic activity, ET-1 is rapidly cleared, and most of them may never get into the circulation. However, bigET-1, the precursor of ET-1, circulates in higher concentration, which may open an analytic window [14]. Therefore, the aim of this study is to investigate the relationship between the bigET-1 and the presence of NCPs/MPs in a Chinese population.

Patients and methods

Study design and population

We retrospectively evaluated consecutive patients who underwent coronary computed tomography angiography (CCTA) owing to stable typical or atypical chest pain from July 2015 to July 2016 in Fuwai Hospital (National Center for Cardiovascular Diseases, Beijing, China) and collected their clinical and laboratory data. Exclusion criteria were as follows: (a) patients without a lipid profile and bigET-1 measurements available and (b) other diseases such as congenital heart disease, New York Heart Association functional class III or IV heart failure, stage C or D of the progression of valvular heart disease according to the guideline of 2014 AHA/ACC for the management of patients with valvular heart disease, pulmonary hypertension, hematological disease, cancer, severe renal

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or liver disease. The study protocol complied with the Declaration of Helsinki and was approved by the hospital ethics review board. Written informed consent was obtained from all the participants.

Data acquisition

Scans were performed using a 64-row spiral CT scanner (Light Speed VCT; GE Healthcare, Milwaukee, Wisconsin, USA). Patients were given 25–50 mg of metoprolol (Seloken; AstraZeneca, Zoetermeer, The Netherlands) orally 1 h before scanning according to the heart rates. Contrast medium was injected at different speed during the different stage. The main scanning parameters were as follows: 64 detectors; individual detector width, 0.625 mm; gantry rotation time, 350 ms; tube voltage, 120 kV; ECG modulated tube current; pitch, 0.16–0.22; table feed per rotation, 400 mm; and field of view, 200–250 mm.

Image analysis

The scans were retrospectively analyzed at the workstation (Deep Blue; ADW4.3; GE Healthcare). We classified coronary atherosclerosis plaques as CPs, NCPs, or MPs according to their density. In brief, lesions with attenuation values [measured in Hounsfield Units (HU)] more than 130 HU were classified as CPs and less than 130 HU were defined as NCPs. MPs had the components of both CPs and NCPs. According to whether the participants had coronary atherosclerotic plaques and the characteristics of all the plaques, we divided all participants into three groups with no plaques, calcified plaques (without any NCPs), and NCPs/MPs. The stenosis degree was classified as significant if the patient had more than 50% diameter stenosis on the longitudinal images.

Cardiovascular risk factor assessment

Main cardiovascular risk factors such as diabetes mellitus, hypertension, dyslipidemia, cigarette smoking, alcohol consumption, and family history of CAD were assessed. Diabetes mellitus was defined as fasting glucose of at least 126 mg/dl, nonfasting glucose of at least 200 mg/dl, or receiving hypoglycemic therapy (insulin, oral hypoglycemic therapy, or dietary advice). Hypertension was defined as a previously established diagnosis, systolic blood pressure at least 140 mmHg and diastolic blood pressure at least 90 mmHg, or taking antihypertensive medication. Dyslipidemia was defined according to the medical history or by the current use of lipid-lowering drugs. Smoking status and alcohol consumption were ascertained by the medical history. Family history of CAD was considered as a history of CAD, myocardial infarction, coronary revascularization, or sudden cardiac death before 55 years of age for the father or 65 years of age for the mother.

Laboratory measurements

Venous blood samples were obtained at baseline before undergoing CCTA from each patient. The plasma

bigET-1 was detected using a commercial sandwich enzyme immunoassay (BI-20082H; Biomedica, Wien, Austria). The levels of serum total cholesterol (TC), triglyceride, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), creatinine (CREA), blood urea nitrogen (BUN), uric acid, high-sensitivity C-reactive protein (hsCRP) were measured using an automatic biochemistry analyzer (Olympus Diagnostics, Brea, California, USA) and conventional clinical analytical methods.

Statistical analysis

Statistical analysis was performed with the statistical package SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA). A two-side *P* value of less than 0.05 was considered to be statistically significant. Normally distributed variables were expressed as mean±SD, and non-normally distributed variables are presented as median (interquartile range), which were analyzed by independent-samples *t*-test, one-way analysis of variance, or the Mann-Whitney *U*-test (as appropriate). Categorical variables were expressed as percentages and were assessed by χ^2 or Fisher's exact tests. The correlation between two continuous variables was assessed with Pearson's correlation test or Spearman's correlation test. Multivariate logistic regression analysis was performed to identify the independent risk factors for the presence of NCP/MP. The risk factors were pre-specified on the basis of univariate *P* values of less than 0.05.

Results

Baseline clinical characteristics of study participants

The study population consisted of 513 patients undergoing CCTA. Baseline clinical and laboratory data were shown in Table 1. The participants were divided into three groups based on whether they had atherosclerotic plaques and the characteristics of all the plaques. Most study population (*n*=445, 86.6%) had atherosclerotic plaques and the remaining 68 participants had no CPs. Among the patients with atherosclerotic plaques, 192 patients had CPs and 253 patients had NCPs/MPs. Patients with coronary atherosclerotic plaques were older and had a higher percentage of males than the patients without plaques. In addition, there was a higher proportion of participants with hypertension, diabetes, and dyslipidemia in the CPs and NCPs/MPs groups compared with the NPs group. However, there was no significant difference in smoking, alcohol consumption, and history of CAD among groups.

The laboratory data showed that the levels of TC and LDL-C were lower in the CPs and NCPs/MPs groups compared with the NPs group. The possible explanation was the higher proportion of patients using statins in CPs and NCPs/MPs groups (71.4 and 79.8 vs. 42.6%; *P*<0.001). In addition, hsCRP was higher in the NCPs/MPs groups compared with the NPs and CPs groups.

Table 1 Baseline data of the study population according to the characteristics of plaques

Variables	NP (n=68)	CP (n=192)	NCP and MP (n=253)	P
Age (years)	50.7±9.1	62.3±9.0	58.2±10.9	<0.001
BMI (kg/m ²)	25.4±3.5	25.6±3.6	25.7±3.8	0.813
Male [n (%)]	34 (50.0)	100 (52.1)	175 (69.2)	<0.001
Hypertension [n (%)]	27 (39.7)	140 (72.9)	177 (70.0)	<0.001
Diabetes [n (%)]	9 (13.2)	36 (18.8)	68 (26.9)	0.021
Dyslipidemia [n (%)]	37 (54.4)	157 (81.8)	220 (87.0)	<0.001
Smoking [n (%)]	16 (23.5)	47 (24.5)	83 (32.8)	0.098
Alcohol consumption [n (%)]	20 (29.4)	56 (29.2)	82 (32.4)	0.737
History of CAD [n (%)]	8 (11.8)	16 (8.3)	28 (11.1)	0.570
Biochemical parameters				
TC (mmol/l)	5.0 (4.4, 5.6)	4.4 (3.8, 5.3)	4.5 (3.8, 5.3)	0.005
TG (mmol/l)	1.5 (1.2, 2.1)	1.5 (1.1, 2.1)	1.6 (1.1, 2.2)	0.802
HDL-C (mmol/l)	1.2±0.4	1.2±0.3	1.1±0.3	<0.001
LDL-C (mmol/l)	3.1±0.8	2.7±0.9	2.8±0.9	0.011
hsCRP (mg/l)	1.4 (0.8, 2.4)	1.5 (0.8, 2.8)	1.8 (1.0, 3.7)	0.042
CREA	65.7±13.9	72.8±14.6	77.2±18.9	<0.001
BUN	5.0 (4.5, 5.8)	5.8 (4.9, 6.7)	5.7 (4.9, 6.9)	0.003
URIC	320.0±80.9	344.6±89.9	345.6±97.5	0.113
BigET-1	0.22 (0.17, 0.31)	0.30 (0.22, 0.48)	0.32 (0.22, 0.56)	<0.001
Medications [n (%)]				
Statins	29 (42.6)	137 (71.4)	202 (79.8)	<0.001
Aspirin	37 (54.4)	147 (76.6)	208 (82.2)	<0.001
Calcium antagonists	12 (17.6)	88 (45.8)	93 (36.8)	<0.001
ARB/ACEI	17 (25.0)	86 (44.8)	123 (48.6)	0.002
β-Blockers	29 (42.6)	100 (52.1)	147 (58.1)	0.063

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; BigET-1, big endothelin-1; BUN, blood urea nitrogen; CAD, coronary artery disease; CP, calcified plaque; CREA, creatinine; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MP, mixed plaque; NCP, noncalcified plaque; NP, no plaque; TC, total cholesterol; TG, triglycerides; URIC, uric acid.

Table 2 Clinical and demographic characteristics according to big endothelin-1 tertiles

Variables	Tertile 1 (n=178)	Tertile 2 (n=163)	Tertile 3 (n=172)	P
Age (years)	55.1±8.9	60.0±11.0	61.4±10.9	<0.001
BMI (kg/m ²)	25.8±3.7	25.4±3.3	25.6±4.0	0.682
Male [n (%)]	109 (61.2)	89 (54.6)	111 (64.5)	0.168
Biochemical parameters				
TC (mmol/l)	4.7 (3.9, 5.4)	4.7 (4.0, 5.4)	4.2 (3.6, 4.9)	0.007
TG (mmol/l)	1.6 (1.2, 2.2)	1.5 (1.1, 2.1)	1.4 (1.0, 2.2)	0.930
HDL-C (mmol/l)	1.2±0.3	1.2±0.3	1.1±0.3	0.043
LDL-C (mmol/l)	2.9±0.9	2.9±0.9	2.6±0.8	0.002
Coronary plaques [n (%)]				
NP	39 (21.9)	21 (12.9)	8 (4.7)	<0.001
CP	62 (34.8)	71 (43.6)	59 (34.3)	0.146
NCP and MP	77 (43.3)	71 (43.6)	105 (61.0)	0.001
Stenosis [n (%)]				
≤50	103 (57.9)	92 (71.0)	67 (39.0)	<0.001
>50	75 (42.1)	71 (46.3)	105 (61.0)	

CP, calcified plaque; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MP, mixed plaque; NCP, noncalcified plaque; NP, no plaque; TC, total cholesterol; TG, triglycerides.

Association of big endothelin-1 with noncalcified plaques/mixed plaques

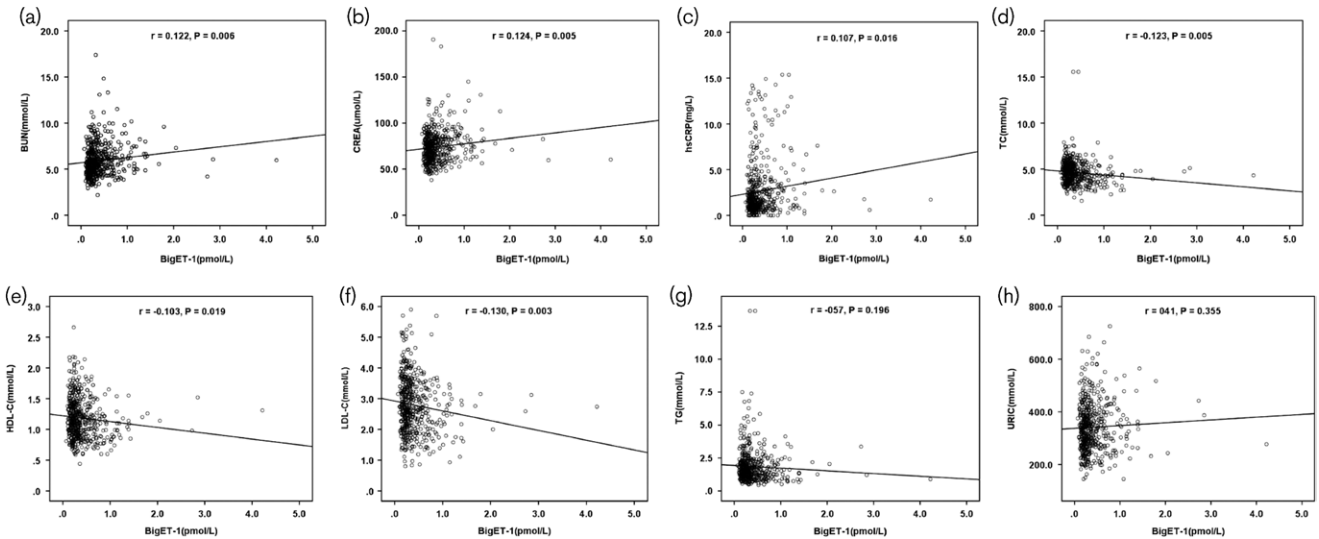
To investigate the relationship between the bigET-1 and NCPs/MPs, we divided the participants into three groups according to bigET-1 tertiles. As shown in Table 2, the prevalence of NCPs/MPs increased significantly from 43.3% in tertile 1 to 61.0% in tertile 3 group ($P=0.001$) and patients with NPs decreased gradually from 21.9% in tertile 1 to 4.7% in tertile 3 ($P<0.001$). However, there was no significant difference in the proportion of patients with CPs among the three groups ($P=0.146$). In addition, the prevalence of stenosis more than 50% was higher in tertile 3, whereas the patients with stenosis up to 50% were more likely to be in tertile 1 and tertile 2 ($P<0.001$).

Further analysis showed that bigET-1 level was positively correlated with BUN CREA and hsCRP ($r=0.122$, $P=0.006$; $r=0.124$, $P=0.005$; $r=0.107$, $P=0.016$) but inversely correlated with TC, HDL-C and LDL-C ($r=-0.123$, $P=0.005$; $r=-0.103$, $P=0.019$; $r=-0.130$, $P=0.003$) and not correlated with triglyceride and uric acid ($r=0.057$, $P=0.196$; $r=0.041$, $P=0.355$) (Fig. 1).

Multivariate analysis

Multivariable binary logistic regression analysis was performed to identify the predictors for the presence of NCPs/MPs. Variables, which had a P value less than 0.05 in univariate analysis, were included in the model of multivariate analysis including sex, smoking, diabetes

Fig. 1



The correlation of bigET-1 with BUN, CREA, hsCRP, TC, HDL-C, LDL-C TG and URIC. BigET-1 level was positively correlated with BUN, CREA and hsCRP (a-c) but inversely correlated with TC, HDL-C and LDL-C (d-f) and not correlated with TG and URIC (g,h). BigET-1, Big Endothelin-1; BUN, blood urea nitrogen; CREA, creatinine; hsCRP, high sensitivity C-reactive protein; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; URIC, uric acid.

Table 3 Multivariate logistic regression analysis for prediction of the presence of noncalcified plaque and mixed plaque

Models	BigET-1		hsCRP	
	OR (95% CI)	P	OR (95% CI)	P
Model 1	2.385 (1.314–4.329)	0.004	1.083 (1.019–1.152)	0.010
Model 2	1.977 (1.071–3.651)	0.029	1.079 (1.010–1.153)	0.025
Model 3	1.858 (1.017–3.394)	0.044	–	–

Model 1: Unadjusted.

Model 2: Adjusted for sex, diabetes, dyslipidemia, smoking, statins, aspirin, ARB/ACEI, HDL-C, CREA, and BUN.

Model 3: Adjusted for sex, diabetes, dyslipidemia, smoking, statins, aspirin, ARB/ACEI, HDL-C, CREA, BUN, and hsCRP.

ARB, angiotensin II receptor blockers; BigET-1, big endothelin-1; BUN, blood urea nitrogen; CI, confidence interval; CREA, creatinine; HDL-C, high-density lipoprotein-cholesterol; hsCRP, high-sensitivity C-reactive protein; OR, odds ratio.

mellitus, dyslipidemia, statin, aspirin, angiotensin II receptor blockers/angiotensin-converting enzyme inhibitor, HDL-C, CREA, BUN, hsCRP, and bigET-1. As shown in Table 3, multivariate analysis showed that both bigET-1 (odds ratio=1.977; 95% confidence interval: 1.071–3.651; $P=0.029$) and hsCRP (odds ratio=1.079; 95% confidence interval: 1.010–1.153; $P=0.025$) were the independent predictors for the presence of NCPs/MPs. Moreover, bigET-1 was independent of hsCRP. Furthermore, the bigET-1 tertile was also independently associated with an increased prevalence of NCPs/MPs (Table 4).

Discussion

This is the first study to show the correlation between the bigET-1 and NCPs/MPs in patients with chest pain

Table 4 Regression analysis to assess the presence of plaques according to big endothelin-1 and big endothelin-1 tertiles^a

Variables	OR	95% CI	P
Presence of atherosclerosis plaques			
BigET-1	39.820	5.294–299.533	<0.001
BigET-1 tertiles	–	–	–
Tertile 1	1	–	–
Tertile 2	2.003	1.047–3.831	0.036
Tertile 3	5.802	2.446–13.765	<0.001
The presence of CP			
BigET-1	0.972	0.578–1.636	0.915
BigET-1 tertiles	–	–	–
Tertile 1	1	–	–
Tertile 2	1.465	0.934–2.296	0.096
Tertile 3	1.088	0.683–1.735	0.722
Presence of NCP and MP			
BigET-1	1.858	1.017–3.394	0.044
BigET-1 tertiles	–	–	–
Tertile 1	1	–	–
Tertile 2	1.037	0.658–1.635	0.875
Tertile 3	1.855	1.164–2.954	0.009

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; BigET-1, big endothelin-1; BUN, blood urea nitrogen; CI, confidence interval; CP, calcified plaque; CREA, creatinine; HDL-C, high-density lipoprotein-cholesterol; hsCRP, high-sensitivity C-reactive protein; MP, mixed plaque; NCP, noncalcified plaque; OR, odds ratio.

^aAdjusted for sex, diabetes, dyslipidemia, smoking, statins, aspirin, ARB/ACEI, HDL-C, CREA, BUN, and hsCRP.

who had suspected CAD undergoing CCTA. BigET-1 was significantly associated with the presence of NCPs/MPs and was an independent predictor for the presence of NCPs/MPs.

Several CCTA studies have demonstrated that NCPs were more frequently associated with the presence of ACS and predicted increased future ACS events compared with low-risk CP [15–17]. Pundziute *et al.* [18] investigated

the characteristics of CPs using both multi-slice CT (MSCT) and virtual histology intravascular ultrasound in patients with ACS and stable CAD and found that NCPs and MPs were more prevalent in patients with ACS compared with those with stable CAD. Moreover, thin cap fibroatheromas as detected by virtual histology intravascular ultrasound were most frequently observed in MPs on MSCT [18]. Another study reported by Hou *et al.* [5] showed that patients with NCPs and MPs had three times higher risk of 3-year major adverse cardiac events in outpatients. So, both NCPs and MPs determined by CCTA could be classified as high-risk plaques. However, CCTA is not suitable for large-scale screening to identify these high-risk plaques in China owing to high cost and complicated procedure. Therefore, preliminary screening by testing circulating biomarker, which is cost effective and easy, might be an alternative and provide valuable information.

ET-1, the first member of the endothelin peptide family identified in 1988, is the most abundant isoform in human cardiovascular system [19]. Beyond its function as a potent vasoconstrictor, many studies have proposed that ET-1 plays a crucial role in the process of atherosclerosis. ET-1 in both peripheral blood and coronary circulation has been found to increase in patients with atherosclerosis and coronary endothelial dysfunction [11,20]. Long-term endothelin receptor antagonism improves endothelial function and attenuates plaque progression in patients with early atherosclerosis [21,22]. More importantly, plasma ET-1 level has been reported to elevate in the first hours after acute myocardial infarction [12] and active coronary lesions exhibited a high immunoreactivity of ET-1 compared with nonactive lesions [13,23]. Taken together, these findings suggest a possible role for ET-1 in the plaque vulnerability. In the present study, we found that the bigET-1 was an independent predictor for the presence of NCPs/MPs, which not only suggested that bigET-1 could be a potential new circulating biomarker of unstable plaques but provided new evidence for ET-1 involved in the formation of active coronary atherosclerotic plaque. However, recently Ibrahim *et al.* [24] showed that ET-1 was not associated with presence or severity of CAD, which was inconsistent with our study. The reason might be owing to the difference of the analyte. ET-1 was a biologically active peptide and had a short half-life *in vivo*, which could lead to high variability. However, bigET-1 was the precursor protein of ET-1 and thought to be more stable in circulation. Therefore, it is possible that the variability of ET-1 was too high to show association with CAD in the study of Ibrahim *et al.* [24].

In addition, our data showed that hsCRP was also an independent risk factor for the presence of NCPs/MPs, which was consistent with previous studies. Both Zhang *et al.* [25] and Sarlon-Bartoli *et al.* [26] reported that an elevation of hsCRP was related to plaque instability and

rupture. Moreover, we found that hsCRP was positively correlated with the bigET-1, which suggested the possible role of ET-1 in the development of inflammation, a key feature of atherosclerosis. In fact, it has been shown that ET-1 was a proinflammatory factor and implicated in the development of atherosclerosis. ET-1 was not only produced by endothelial cells but also by macrophages and polymorphonuclear leukocytes [27,28]. ET-1 stimulated neutrophil to adhere to coronary artery endothelial cells and accumulate in the heart by promoting the expression of adhesive molecules on the neutrophil surface [29,30]. In human monocytes, ET-1 also activated nuclear factor- κ B, a key transcription factor of the inflammation-cascade [31]. Furthermore, receptor activator of nuclear factor- κ B ligand was found to be upregulated in vulnerable rupture-prone atherosclerotic plaques and supposed to contribute to the transition from a stable to an unstable plaque phenotype in both human and murine atherosclerosis [32]. Therefore, all these findings, together with our results, suggested a role of ET-1 in plaque destabilization.

Several limitations need to be mentioned. First, it was a cross-sectional study, which could not predict future cardiovascular events. Second, the size of the study population was small for clinical outcome assessment. Third, bigET-1 was measured at one time point, and serial measurements of bigET-1 and CCTA would be useful to explore the dynamic correlation between them. So further study including a larger population and dynamic measurement of bigET-1 is necessary in the future.

Conclusion

BigET-1 was independently associated with the presence of NCPs/MPs. The finding highlights the potential value of this assay in predicting the characteristics of plaques in patients with suspected or known CAD.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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