Higher serum angiopoietin 2 levels are independently associated with coronary microvascular dysfunction in patients with angina in the absence of obstructive coronary artery disease

Shao-Min Chen[1], Dan Li[1], Xing Xing[2], Zhao-Ping Li[1]

[1]Department of Cardiology and Institute of Vascular Medicine, Peking University Third Hospital; Key Laboratory of Cardiovascular Molecular Biology and Regulatory Peptides, Ministry of Health; Key Laboratory of Molecular Cardiovascular Science, Ministry of Education; Beijing Key Laboratory of Cardiovascular Receptors Research, Beijing 100191, China;

[2]Department of Cardiovascular Medicine, Beijing First Hospital of Integrated Chinese and Western Medicine, Beijing 100039, China.

Abstract

Background: Angiopoietin-2 (Ang-2) is a type of endothelial growth factor involved in angiogenesis and vascular remodeling. Circulating Ang-2 levels are elevated in patients with obstructive coronary artery disease (CAD). This study aimed to evaluate the association between serum Ang-2 levels and coronary microvascular dysfunction in patients without obstructive CAD.

Methods: A total of 125 patients with angina in the absence of obstructive CAD were included in this cross-sectional study. Coronary flow reserve (CFR) was measured in the distal left anterior descending coronary artery by trans-thoracic Doppler echocardiography. The patients were divided into the following two sub-groups according to CFR: the impaired CFR group with CFR values <2.5 and the preserved CFR group with CFR values ≥2.5. Serum Ang-2 levels were determined using enzyme-linked immunosorbent assay. Independent predictors for impaired CFR were identified by binary logistic regression analysis. The receiver-operating characteristic curve was determined to evaluate the ability of serum Ang-2 in predicting impaired CFR.

Results: We found that age, percentage of female sex, N-terminal pro-B-type natriuretic peptide levels, Ang-2 levels (763.3 ± 264.9 *vs*. 579.7 ± 169.3 pg/mL, $P < 0.001$), and the left atrial volume index were significantly higher in patients with impaired CFR than in patients with preserved CFR. Serum Ang-2 levels were negatively correlated with CFR ($r = -0.386$, $P < 0.001$). Binary logistic regression analysis showed that Ang-2 (odds ratio: 1.004, 95% confidence interval [CI]: 1.001–1.006, $P = 0.003$) and age (odds ratio: 1.088, 95% CI: 1.023–1.156, $P = 0.007$) were independently associated with impaired CFR. Furthermore, Ang-2 was a significant predictor of impaired CFR on the receiver-operating characteristic curve ($P < 0.001$). The area under the curve was 0.712 (95% CI: 0.612–0.813).

Conclusions: High serum Ang-2 levels are independently associated with impaired CFR in patients with angina in the absence of obstructive CAD.

Keywords: Coronary microvascular dysfunction; Angiopoietin 2; Coronary flow reserve

Introduction

Myocardial ischemia and angina are usually caused by flow-limiting atherosclerotic plaques in epicardial coronary arteries. However, 20% to 30% of patients receiving coronary angiography do not have significantly obstructive coronary artery disease (CAD), despite symptoms of angina or an abnormal non-invasive stress test that is suggestive of myocardial ischemia.[1] This condition is usually defined as angina in the absence of obstructive CAD. Of these patients, 40% to 60% may have coronary microvascular dysfunction (CMD).[2] Coronary microvasculature has attracted growing interest from researchers because it affects clinical outcomes.[3]

Myocardial ischemia can provoke angiogenesis, which is a compensatory reaction to improve myocardial perfusion.[4] Angiopoietins are a group of endothelial growth factors involved in angiogenesis and vascular remodeling. Angiopoietin-1 (Ang-1), which is expressed by mesenchymal cells, is the major agonist for the tyrosine kinase receptor Tie-2.[5] Angiopoietin-2 (Ang-2), which is exclusively expressed by endothelial cells, acts as an antagonist for Tie-2.[6] Physiologically, Ang-1 accelerates maturation of blood vessels, while Ang-2 destabilizes vessels and degrades

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Correspondence to: Dr. Zhao-Ping Li, Department of Cardiology and Institute of Vascular Medicine, Peking University Third Hospital, Beijing 100191, China
E-Mail: zhaoping1223@163.com

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the basal lamina.[5,6] Accumulating evidence has shown that circulating Ang-2 levels are significantly elevated in patients with CAD[7-10] and it is a biomarker for cardiovascular diseases.[11-13] However, whether circulating Ang-2 levels are elevated in patients with CMD is still unknown. Therefore, the present study aimed to evaluate the association between serum Ang-2 levels and CMD in patients with angina in the absence of obstructive CAD.

Methods

Ethical approval

The study was performed in accordance with the *Declaration of Helsinki*. This study was approved by the ethics review board of Peking University Third Hospital (No. 2012094), and written informed consent was obtained from each patient.

Selection of patients

Adult patients with typical or atypical angina in the absence of obstructive CAD were included in this cross-sectional study. Typical angina was defined as having three of the following characteristics: sub-sternal chest discomfort, precipitated by physical exertion or emotion, and relieved by rest or nitroglycerin. Atypical angina was defined as having two of these characteristics. All of the patients received invasive coronary angiography or coronary computed tomography angiography (CTA). The absence of obstructive CAD was defined as <50% stenosis in any epicardial coronary artery on invasive coronary angiography or CTA. Exclusion criteria were as follows: (1) the presence of acute coronary syndrome; (2) a history of percutaneous coronary intervention or coronary artery bypass grafting; (3) the presence of hypertrophic cardiomyopathy or valvular heart disease, which could probably cause angina; (4) chronic heart failure; (5) contraindications for adenosine administration, including high-degree atrioventricular block, and an allergic reaction to the medicine; (6) renal insufficiency (serum creatinine levels >133 μmol/L); (7) peripheral vascular disease; (8) chronic inflammatory diseases; and (9) a tumor. Demographic information, comorbidities, body mass index, blood pressure, heart rate, and stress testing results were collected by reviewing medical records.

Coronary flow reserve and echocardiography

Trans-thoracic echocardiography was performed using a Vivid E9 (GE, USA) device and a 5-MHz transducer. Parameters were measured in the parasternal and apical views in the left lateral decubitus position. As described in detail in our previous study,[14] coronary flow velocity was measured in the distal left anterior descending coronary artery (LAD) using a modified apical two-chamber view that scanned the anterior interventricular sulcus. The LAD flow velocity profile was recorded using pulsed wave Doppler at baseline and after adenosine infusion (140 $g \cdot kg^{-1} \cdot min^{-1}$ for 2 min). Coronary flow reserve (CFR) was calculated via the following formula: CFR = peak coronary flow velocity during hyperemia/peak coronary flow velocity at rest.[15] A CFR ≥2.5 was considered normal, and the patients were divided into two sub-groups according to CFR values.[15] All participants abstained from caffeine and long-acting nitroglycerin 12 h before examinations. All images were recorded digitally and later analyzed off-line by observers who were blinded for all clinical variables. The intra-observer and inter-observer variability of repeated off-line CFR measurements were 3.8% ($n = 10$) and 5.5% ($n = 30$), respectively.

Laboratory assays

Venous blood samples for serum Ang-2 level measurement were collected in vacuum blood collection tubes with a clot activator and were immediately placed in 4°C refrigerators. Within 30 minutes after collection, samples were centrifuged (1500 × *g* for 10 min) at 4°C, divided into aliquots, and frozen at −80°C. Repeated freeze-thaw cycles were avoided. Serum Ang-2 levels were determined using enzyme-linked immunosorbent assay kits (R&D Systems, USA). The minimal detection limit was 156 pg/mL. These assays were performed by an investigator who was blinded for all clinical variables. Data on other laboratory parameters, including the white blood cell count, serum levels of creatinine, glucose, and high-sensitivity C-reactive protein (hs-CRP), lipid profiles, and N-terminal pro-B-type natriuretic peptide (NT-pro BNP) levels were collected by reviewing medical records.

Statistical analysis

Comparisons between the two groups were performed by the Chi-squared test, Student unpaired *t* test, or Mann-Whitney *U* test. Correlations between numerical parameters were analyzed by the Spearman or Pearson correlation test. Independent predictors for impaired CFR were identified by binary logistic regression analysis (forward stepwise), including potential confounders (comorbidities including hypertension, diabetes mellitus, current smoker, and dyslipidemia) and variables with a value of $P < 0.10$ by univariate analysis. The receiver-operating characteristic curve was determined to evaluate the ability of serum Ang-2 in predicting impaired CFR. The area under the curve was calculated. Statistical significance was defined as $P < 0.05$. All analyses were performed with SPSS for Windows version 18.0 (SPSS, Chicago, IL, USA).

Results

Clinical characteristics

A total of 125 patients with typical or atypical angina in the absence of obstructive CAD were included in the study from October 2012 to May 2016. Among these patients, 82 underwent invasive coronary angiography and 43 completed coronary CTA. The patients were divided into the following two sub-groups according to CFR: the impaired CFR group with CFR values <2.5 and the preserved CFR group with CFR values ≥2.5.

Tables 1 and 2 show the clinical characteristics, laboratory findings, and echocardiographic parameters of the patients with impaired or preserved CFR. The factors of age (66.5 ± 7.6 *vs.* 58.9 ± 8.8 years, $P < 0.001$), percentage

of female sex (77.1% *vs.* 56.7%, $P = 0.034$), NT-pro BNP levels (66.5 [36.7–109.0] *vs.* 46.0 [22.2–75.0] pg/mL, $P = 0.046$), Ang-2 levels (763.3 ± 264.9 *vs.* 579.7 ± 169.3 pg/mL, $P < 0.001$), and left atrial volume index (LAVI) (29.6 ± 5.0 *vs.* 26.4 ± 5.9 cm^3/m^2, $P = 0.017$) were significantly higher in patients with impaired CFR than in patients with preserved CFR. Patients with impaired CFR were more likely to have typical angina (54.3% *vs.* 31.1%, $P = 0.016$) and ischemia in a stress test (71.4% *vs.* 35.6%, $P = 0.001$) than those with preserved CFR. There were no significant differences in concomitant illnesses, body mass index, blood pressure, heart rate, methods of assessment of epicardial coronary artery stenosis, white blood cell count, creatinine levels, glucose levels, hs-CRP levels, lipid profiles, medications, and echocardiographic parameters besides the LAVI and CFR between the two groups.

Association between serum Ang-2 levels and CFR

Serum Ang-2 levels were negatively correlated with CFR ($r = -0.386$, $P < 0.001$) [Figure 1]. Variables with a value of $P < 0.10$ by univariate analysis (age, sex, Ang-2, NT-pro BNP, and LAVI) and potential confounders (comorbidities including hypertension, diabetes mellitus, current smoker, and dyslipidemia) were included in binary logistic regression analysis. Ang-2 levels (odds ratio: 1.004, 95% confidence interval [CI]: 1.001–1.006, $P = 0.003$) and age (odds ratio: 1.088, 95% CI: 1.023–1.156, $P = 0.007$) were independently associated with impaired CFR.

Table 1: Clinical characteristics and laboratory findings of patients.

Characteristics	CFR <2.5 ($n = 35$)	CFR ≥2.5 ($n = 90$)	Statistics	P
Age (years)	66.5 ± 7.6	58.9 ± 8.8	4.897*	<0.001
Female	27 (77.1)	51 (56.7)	4.503†	0.034
Hypertension	29 (82.9)	66 (73.3)	1.253†	0.263
Diabetes mellitus	6 (17.1)	16 (17.8)	0.007†	0.933
Current smoker	7 (20.0)	22 (24.4)	0.279†	0.597
Dyslipidemia	25 (71.4)	54 (60.0)	1.415†	0.234
BMI (kg/m^2)	24.8 ± 3.9	25.5 ± 3.2	−1.305*	0.355
Systolic blood pressure (mmHg)	131.4 ± 12.2	128.4 ± 13.2	1.111*	0.269
Diastolic blood pressure (mmHg)	72.5 ± 8.0	75.0 ± 9.5	−1.302*	0.196
Heart rate (beats/min)	64.5 ± 10.7	64.5 ± 8.2	−0.047*	0.963
Chest pain characteristics				
Typical	19 (54.3)	28 (31.1)	5.768†	0.016
Atypical	16 (45.7)	62 (68.9)		
Stress testing results				
No test	4 (11.4)	17 (18.9)	13.357†	0.001
Normal	6 (17.1)	41 (45.6)		
Ischemic	25 (71.4)	32 (35.6)		
Assessment of epicardial coronary artery stenosis				
Angiography	24 (68.6)	58 (64.4)	0.190†	0.663
CTA	11 (31.4)	32 (35.6)		
WBC count (10^9/L)	7.4 ± 1.1	7.6 ± 1.3	−0.234*	0.843
Creatinine (μmol/L)	73.6 ± 16.9	77.6 ± 13.1	−1.427*	0.156
Glucose (mmol/L)	5.5 ± 1.2	5.4 ± 1.5	0.342*	0.733
Hs-CRP (mg/L)	0.7 (0.5, 2.6)	1.2 (0.5, 2.5)	−0.468‡	0.640
TC (mmol/L)	4.3 ± 0.8	4.5 ± 0.9	−0.904*	0.367
TG (mmol/L)	1.4 ± 0.7	1.7 ± 1.1	−1.583*	0.116
HDL-C (mmol/L)	1.3 ± 0.3	1.2 ± 0.3	1.164*	0.247
LDL-C (mmol/L)	2.5 ± 0.7	2.6 ± 0.8	−1.170*	0.244
NT-pro BNP (pg/mL)	66.5 (36.7, 109.0)	46.0 (22.2, 75.0)	−1.994‡	0.046
Serum Ang-2 (pg/mL)	763.3 ± 264.9	579.7 ± 169.3	4.601*	<0.001
Medication				
Anti-platelets	30 (85.7)	71 (78.9)	0.757†	0.384
Nitrites	9 (25.7)	17 (18.9)	0.713†	0.399
ACE inhibitors/ARB	17 (48.6)	36 (40.0)	0.758†	0.384
β-Blockers	13 (37.1)	44 (48.9)	1.402†	0.236
CCB	15 (42.9)	30 (33.3)	0.992†	0.319
Diuretics	2 (5.7)	6 (6.7)	0.038†	0.845
Statins	28 (80.0)	72 (80.0)	0.000†	1.000

Data are represented as mean ± standard deviation, median (25%, 75%), or *n* (%). 1 mmHg = 0.133 kPa. * *t* value. † χ^2 value. ‡ *Z* value. ACE: Angiotensin converting enzyme; Ang-2: Angiopoietin 2; ARB: Angiotensin receptor blocker; BMI: Body mass index; CCB: Calcium channel blocker; CFR: Coronary flow reserve; CTA: Coronary tomography angiography; HDL-C: High-density lipoprotein cholesterol; Hs-CRP: High-sensitive C reaction protein; LDL-C: Low-density lipoprotein cholesterol; NT-pro BNP: N-terminal pro-B-type natriuretic peptide; TC: Total cholesterol; TG: Triglyceride; WBC: White blood cell.

Table 2: Echocardiographic parameters of patients.

Parameters	CFR <2.5 ($n=35$)	CFR ≥2.5 ($n=90$)	Statistics	P
LAVI (cm^3/m^2)	29.6 ± 5.0	26.4 ± 5.9	2.437*	0.017
LVEDD (mm)	46.8 ± 4.6	46.7 ± 4.3	0.088*	0.930
LVMI (g/m^2)	79.1 (70.4, 91.9)	76.4 (63.3, 87.2)	−0.742†	0.458
LVEF (%)	69.8 ± 4.4	70.3 ± 4.7	−0.955*	0.602
E/A	0.8 ± 0.3	0.9 ± 0.3	−0.556*	0.579
s′ (cm/s)	9.7 ± 1.5	10.2 ± 2.0	−1.382*	0.170
e′ (cm/s)	9.5 ± 1.7	10.2 ± 2.4	−1.553*	0.123
E/e′	7.6 ± 3.0	7.1 ± 2.6	1.547*	0.124
CFR	2.2 ± 0.2	3.1 ± 0.5	−12.020*	<0.001

Data are represented as mean ± standard deviation or median (25% percentile, 75% percentile). * *t* value. † *Z* value. CFR: Coronary flow reserve; E/A: Early diastolic mitral inflow velocity/late diastolic mitral inflow velocity; e′: Early diastolic mitral annular velocity; E/e′: Early diastolic mitral inflow velocity/early diastolic mitral annular velocity; LAVI: Left atrial volume index; LVEDD: Left ventricular end diastolic diameter; LVEF: Left ventricular ejection fraction; LVMI: Left ventricular mass index; s′: Early systolic mitral annular velocity.

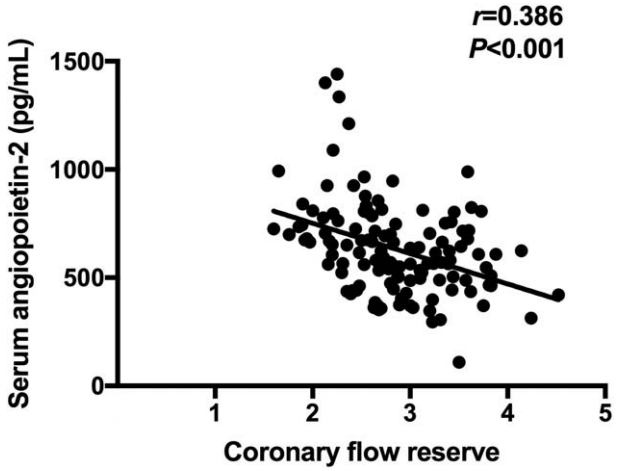

Figure 1: Relationship between serum angiopoietin-2 levels and coronary flow reserve.

Ang-2 levels were a significant predictor of impaired CFR on the receiver-operating characteristic curve ($P < 0.001$). The area under the curve was 0.712 (95% CI: 0.612–0.813) [Figure 2]. The best cut-off value of Ang-2 levels to predict impaired CFR was 648 pg/mL, with a sensitivity of 71.4%, a specificity of 67.8%, a positive predictive value of 68.9%, and a negative predictive value of 70.3%.

Associations between serum Ang-2 levels and other variables

Female patients (661.8 ± 227.3 *vs.* 580.2 ± 187.3 pg/mL, $P = 0.040$) and hypertensive patients (656.3 ± 213.5 *vs.* 551.2 ± 207.6 pg/mL, $P = 0.020$) showed significantly higher Ang-2 levels. Serum Ang-2 levels were positively correlated with age ($r = 0.259$, $P = 0.003$) and the early diastolic mitral inflow velocity/early diastolic mitral annular velocity ratio (E/e′) ($r = 0.251$, $P = 0.005$) [Figure 3]. No significant associations were found between serum Ang-2 levels and a history of diabetes mellitus, dyslipidemia, smoking status, blood pressure, heart rate, medications, laboratory findings, and echocardiographic parameters other than the early diastolic mitral inflow velocity/early diastolic mitral annular velocity ratio and CFR (data not shown).

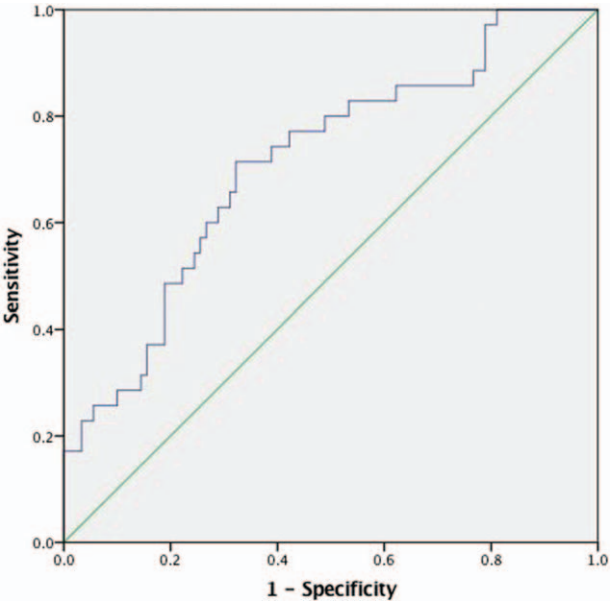

Figure 2: Receiver-operating characteristics curve analysis of serum angiopoietin-2 for impaired coronary flow reserve. Area under the curve = 0.712, $P < 0.001$, and impaired CFR was defined as CFR values <2.5. CFR: Coronary flow reserve.

Discussion

The present study evaluated the association between serum Ang-2 levels and CFR in patients with angina in the absence of obstructive CAD using trans-thoracic Doppler echocardiography for determining CFR. The main findings of this study were that Ang-2 levels were significantly higher in patients with impaired CFR than in patients with preserved CFR. Additionally, binary logistic regression analysis showed that Ang-2 levels and age were independently associated with CFR. To the best of our knowledge, this is the first study to investigate the relationship between serum Ang-2 levels and CFR.

Numerous studies have shown that circulating Ang-2 levels are increased in patients with CAD.[7-10] Experimental studies have shown that hypoxia or ischemia directly up-regulates Ang-2 expression in endothelial cells.[16] Therefore, the increase in Ang-2 levels in patients

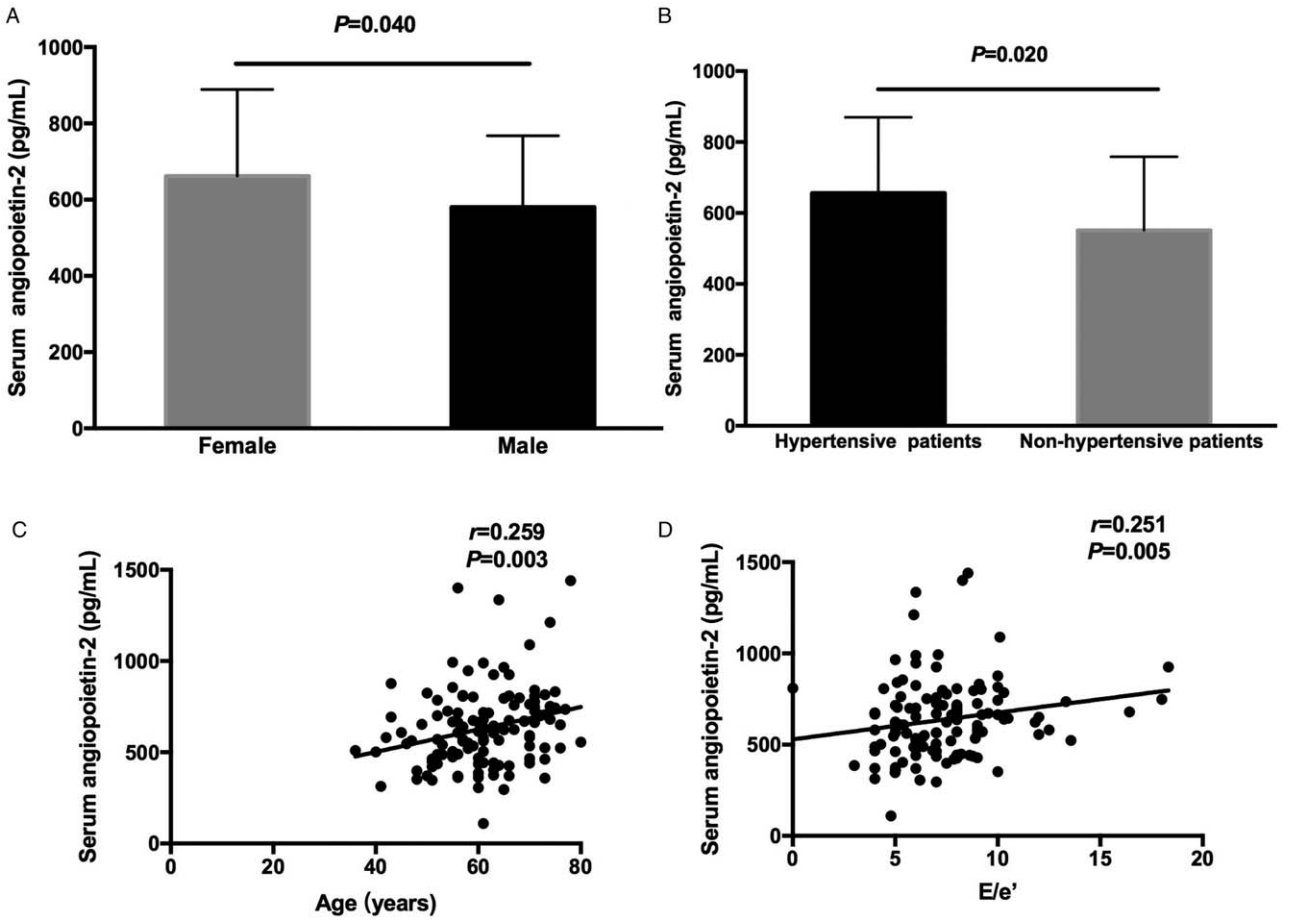

Figure 3: Association of serum angiopoietin-2 with gender, hypertension, age, and E/e′. (A) Serum angiopoietin-2 levels were significantly higher in females than in males. (B) Serum angiopoietin-2 levels were significantly higher in hypertensive patients than in non-hypertensive patients (B). (C, D) Serum Ang-2 levels were positively correlated with age and E/e′, respectively. Ang-2: Angiopoietin 2; E/e′: Early diastolic mitral inflow velocity/early diastolic mitral annular velocity.

with impaired CFR is likely to be caused by a response to ischemia. Increased circulating Ang-2 levels have also been reported in several types of diseases, including hypertension, chronic kidney disease, and diabetes mellitus.[17-19] Furthermore, accumulating evidence suggests that Ang-2 is a biomarker of cardiovascular risk in these patients,[11,13] and even in the general population.[12]

In recent years, there has been wide acceptance that CMD plays an important role in angina pectoris.[20] CMD leads to myocardial ischemia by impairing the ability of the coronary microvasculature to increase coronary blood flow during stress.[20] CFR reflects an increase in coronary blood flow, which is affected by the epicardial coronary artery and the coronary microvasculature. In the absence of obstructive CAD, impaired CFR is a good marker of CMD.[21,22] CFR can be investigated invasively during angiography, and non-invasively using positron-emission tomography, cardiac magnetic resonance imaging, and trans-thoracic Doppler echocardiography.[20] CFR as measured by trans-thoracic Doppler echocardiography of the LAD is highly feasible with good reproducibility.[23] The cut-off value of impaired CFR is usually <2.0 or <2.5.[15,20]

Several pathophysiologic mechanisms have been proposed in the development of CMD, including endothelial dysfunction and inflammation.[24] Although adenosine is a non-endothelium-dependent vasodilator, intact endothelial function is required for achieving maximum hyperemic flow during measurement of CFR. A previous study showed that CFR was associated with peripheral endothelial function in patients with chest pain and in healthy volunteers.[25] Additionally, CMD is associated with low-level systemic inflammation. A series of studies showed that CFR was correlated with markers of systemic inflammation, such as CRP, the neutrophil count, and interleukin-6.[26-28] Circulating Ang-2 levels are a marker of endothelial dysfunction[29,30] and systemic inflammation.[31,32] Experimental studies have shown that Ang-2 can cause destabilization of blood vessels by antagonizing Ang-1 binding to Tie-2. This leads to sensitivity of endothelial cells to the effects of pro-inflammatory cytokines and other endothelial growth factors, resulting in an increase in vascular activation and inflammation.[33,34] Observational studies have shown that plasma Ang-2 levels are independently associated with peripheral endothelial function in children with obesity and obstructive sleep apnea,[30] as well as in patients with systemic lupus erythematosus.[29] Elevated Ang-2 levels are also predictive of prognosis in sepsis.[32]

Consistent with previous studies,[2] the present study showed that age was independently associated with

impaired CFR. We also found that women have a higher incidence of CMD than men, which is in agreement with the findings of previous studies.[35] However, sex was not significantly associated with impaired CFR in the multivariable model in this study. Although diabetes mellitus,[36] hypertension,[37] and obesity[38] may cause CMD, none of these factors were associated with impaired CFR in the current study.

This study has some limitations. This was a cross-sectional study. Therefore, we could not determine the causal relationship between Ang-2 levels and CMD. Additionally, the sample size of this study was small. Therefore, our data need confirmation in future studies. Further investigation is warranted to determine the role of Ang-2 as a predictor of cardiovascular events in patients with CMD.

In conclusion, higher serum Ang-2 levels are independently associated with impaired CFR in patients with angina in the absence of obstructive CAD. Increased Ang-2 levels may be a biomarker of CMD in patients without obstructive CAD.

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Conflicts of interest

None.

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