Influencing factors of vascular endothelial function in patients with non-obstructive coronary atherosclerosis: a 1-year observational study

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Abstract

Background: Endothelial dysfunction may play a key role in non-obstructive coronary artery atherosclerosis. Our study aimed to evaluate the vascular endothelial function and its influencing factors in patients with non-obstructive coronary artery atherosclerosis.

Methods: A total of 131 consecutive patients with non-obstructive coronary artery atherosclerosis were enrolled. Flow-mediated dilatation (FMD) was measured at baseline and 1-year follow-up. Endothelial progenitor cells (EPCs) were counted by staining the fasting venous blood with antibodies against CD34 and vascular endothelial growth factor receptor 2.

Results: Systolic blood pressure, pulse pressure and the levels of HbA1c in participants with baseline FMD < 6% (n = 65) were significantly higher than those with baseline FMD ≥ 6% (n = 66). Baseline FMD was negatively associated with EPC counts (r = −0.199, P < 0.05) and systolic blood pressure (r = −0.315, P < 0.01). The 1-year FMD was significantly increased compared to the baseline FMD [(9.31 ± 5.62)% vs (7.31 ± 5.26)%], P < 0.001]. Independent predictors of FMD improvement included elevated EPC counts (OR = 1.104, 95% CI: 1.047–1.165, P < 0.001) and decreased levels of serum creatinine (OR = 0.915, 95% CI: 0.843–0.993, P = 0.034).

Conclusions: Family history of premature cardiovascular diseases, hypertension, elevated systolic pressure, and HbA1c > 6.5% are independent risk factors for endothelial dysfunction in non-obstructive atherosclerotic patients. Elevated peripheral blood EPC counts and decreased levels of serum creatinine are independent predictors of endothelial function improvement.

Keywords: Non-obstructive coronary atherosclerosis, Endothelial dysfunction, Flow-mediated dilatation, Endothelial progenitor cells

Introduction

Non-obstructive coronary artery atherosclerosis is characterized by coronary artery stenosis less than 50% [1–3]. Vascular endothelial dysfunction, plaque rupture and thrombosis may be the main pathologic mechanism of acute or chronic myocardial ischemia [4, 5]. The vascular endothelium is a multifunctional organ that maintains vascular homeostasis, regulates cell proliferation and angiogenesis and preserves a non-thrombogenic blood-tissue interface. Endothelium dysfunction may play a key role in non-obstructive coronary artery atherosclerosis [6, 7]. Previous studies suggested that impaired endothelial function may be reversed by medicine treatment and lifestyle changes [8, 9].

Endothelial function can be directly evaluated by measuring the changes of arterial diameter in response to vasoactive drugs like nitric oxide that is directly

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atherosclerosis [19, 20]. Elevated EPC counts are thought to represent the ability of endothelial repair and atherosclerosis inhibition [21].

Our study aimed to investigate the influencing factors of vascular endothelial function in patients with non-obstructive coronary artery atherosclerosis.

Methods
Participants
The prospective observation study included 131 consecutive patients with non-obstructive coronary artery atherosclerosis with atypical symptoms and/or non-specific electrocardiogram changes at the Xuanwu Hospital of Capital Medical University. Non-obstructive coronary atherosclerosis was diagnosed using angiography as the absence of obstructive coronary artery disease, i.e., no coronary artery stenosis ≥50% in any coronary artery. This includes patients with normal coronary arteries (no stenosis to stenosis < 30%) or mild coronary atheromatosis (stenosis of 30 to 50%) [PMID: 28158518]. The study was carried out from August 2013 to August 2015. All patients underwent coronary angiography or coronary computed tomographic angiography and had been confirmed with coronary stenosis < 50%.

Patients with the following conditions were excluded: (1) coronary artery stenosis > 50% shown by imaging, or previous history of coronary artery interventional therapy or coronary artery bypass graft; (2) previous positive treadmill test, or transient elevation of ST-segment; (3) previous imaging suggesting myocardial ischemia; (4) previous tests showing levels of troponin I, troponin T, or creatine kinase-MB exceeding the upper limits of normal ranges; (5) patients with symptomatic heart failure, atrial fibrillation, cardiomyopathy or valvular diseases; (6) history of aortic dissection aneurysm, stroke or symptomatic peripheral artery diseases; (7) surgery, trauma or infection within 30 days; (8) renal or liver dysfunction; (9) secondary hypertension, hypertension emergency, or diabetes emergency; (10) rheumatic diseases, cancers, thyroid dysfunction, severe anemia, or use of glucocorticoid; (11) the informed consents were not signed.

Data collection and follow-up
Patient general information such as gender, age, smoking, height and weight were collected. The family history of premature cardiovascular disease was defined as having a first-degree male relative aged < 55 years and/or a first-degree female relative aged < 65 years.

Blood pressure was measured using a mercury sphygmomanometer for three times with intervals of 2 min [22]. The mean blood pressure and the pulse pressure were calculated. Fasting blood was drawn from the median cubital vein in the morning. Blood routine was performed by a hematology analyzer (Sysmex XE-2100). Serum levels of glycated hemoglobin (HbA1c), homocysteine and high-sensitivity C-reactive protein were measured by a biochemical analyzer (HITACHI 7600). To exclude myocardial injury/infarction, levels of cardiac troponin I were measured by a triage quantitative myocardial infarction/heart failure diagnostic device (Biosite, USA).

Patients were followed 1 year ±30 days later from the time of enrollment. The following events during the year were registered: myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, heart failure, angina, hospitalization related to cardiovascular diseases, stroke (ischemic and/or hemorrhagic), transient ischemia attack, cardiac death and all-cause death.

Evaluation of vascular endothelial function
Vascular endothelial function was evaluated using the flow-mediated dilatation (FMD) on the day after admission and on the morning of follow-up visit [23]. Participants were required to fasted for 8 h and to avoided exercising, smoking, drinking, coffee, tea, and high-fat food (at least 12 h). Medicines that contain vasoactive agents were stopped for at least 24 h. The evaluation was performed by one investigator blinded to the study design in a quiet room after rest for 20 min. Color Doppler ultrasonography of the brachial artery was performed using a L12–3 transducer (10–13 MHz, Philips IE33). Electrocardiogram was recorded synchronously. FMD was calculated using the following formula: FMD = (D1 - D0) / D0 × 100%. D1 was the inner diameter of the brachial artery at the end of diastole. D0 was the basal inner diameter of the brachial artery. FMD < 6% was considered abnormal [24]. ΔFMD was calculated using the formula: ΔFMD = (1-year FMD - baseline FMD) / baseline FMD × 100%.

EPC counting
EPCs in the whole blood were identified and counted by detecting the expression of CD34 and vascular endothelial
growth factor receptor 2 (VEGFR2) using flow cytometry (BD Biosciences, USA) [25–27]. The data were processed by the BD FACS Diva software (BD Biosciences, USA).

Statistical analysis
Data normality was examined using the Kolmogorov-Smirnov test. Quantitative variables with a normal distribution were presented as means ± standard deviations. Variables with a skewed distribution were presented as medians (interquartile ranges). Categorical variables were shown as numbers and percentage values.

One-way analysis of variance test or Kruskal-Wallis test was used to compare the continuous variables. Chi-square test or Fisher’s exact test was used for comparing the categorical variables. Spearman or Pearson correlation coefficient was used to represent the relationships between the variables.

Multivariate logistic regression was used to analyze the influencing factors of FMD. The dependent variable was FMD, and the independent variables were age > 65, male gender, smoking, hypertension, family history of premature cardiovascular diseases, HbA1c ≥ 6.5%, total cholesterol, low-density lipoprotein cholesterol, systolic blood pressure, pulse pressure and EPC count.

Differences were considered statistically significant if the two-sided P < 0.05. All analyses were performed with SPSS statistical software version 19.0 (SPSS, Chicago, IL).

Results
Our study included 131 consecutive patients with non-obstructive coronary artery atherosclerosis (Tables 1). The male to female ratio was 1.1:1. Age, year 60.15 ± 11.17, Male, n (%) 62 (47.3), Body mass index, kg/m² 25.64 ± 3.52, Heart rate, beats/min 71.87 ± 10.49, Systolic blood pressure, mmHg 134.54 ± 16.72, Diastolic blood pressure, mmHg 75.32 ± 10.55, Pulse pressure, mmHg 58.62 ± 16.28, Homocysteine, μmol/L 14.01 ± 8.27, Glucose, mmol/L 5.54 ± 1.49, HbA1c, % 6.16 ± 0.85, Serum creatinine, μmol/L 66.59 ± 15.88, Uric acid, μmol/L 334.27 ± 93.42, Total cholesterol, mmol/L 4.19 ± 0.84, Triglyceride, mmol/L 1.77 ± 1.00, High-density lipoprotein cholesterol, mmol/L 1.33 ± 0.34, Low-density lipoprotein cholesterol, mmol/L 2.39 ± 0.68, Flow-mediated dilatation, % 7.31 ± 5.259, Hypertension 68 (51.9), Diabetes mellitus 30 (23.3), Smoking 28 (21.4), Family history of premature cardiovascular disease 27 (20.6), Antiplatelet therapy 35 (26.7), β-blocker 28 (21.4), Calcium antagonist 35 (26.7), Angiotensin-converting-enzyme inhibitors / angiotensin II receptor blockers 35 (26.7), Diuretics 10 (7.6), Statins 28 (21.4), Hypoglycemic agents 22 (16.8).

Table 1 General information of the participants

| Participants (n = 131) |  |
|------------------------|------------------------|
| Age, year | 60.15 ± 11.17 |
| Male, n (%) | 62 (47.3) |
| Body mass index, kg/m² | 25.64 ± 3.52 |
| Heart rate, beats/min | 71.87 ± 10.49 |
| Systolic blood pressure, mmHg | 134.54 ± 16.72 |
| Diastolic blood pressure, mmHg | 75.32 ± 10.55 |
| Pulse pressure, mmHg | 58.62 ± 16.28 |
| Homocysteine, μmol/L | 14.01 ± 8.27 |
| Glucose, mmol/L | 5.54 ± 1.49 |
| HbA1c, % | 6.16 ± 0.85 |
| Serum creatinine, μmol/L | 66.59 ± 15.88 |
| Uric acid, μmol/L | 334.27 ± 93.42 |
| Total cholesterol, mmol/L | 4.19 ± 0.84 |
| Triglyceride, mmol/L | 1.77 ± 1.00 |
| High-density lipoprotein cholesterol, mmol/L | 1.33 ± 0.34 |
| Low-density lipoprotein cholesterol, mmol/L | 2.39 ± 0.68 |
| Ejection fraction, % | 65.69 ± 7.14 |
| Flow-mediated dilatation, % | 7.31 ± 5.259 |
| Hypertension | 68 (51.9) |
| Diabetes mellitus | 30 (23.3) |
| Smoking | 28 (21.4) |
| Family history of premature cardiovascular disease | 27 (20.6) |
| Antiplatelet therapy | 35 (26.7) |
| β-blocker | 28 (21.4) |
| Calcium antagonist | 35 (26.7) |
| Angiotensin-converting-enzyme inhibitors / angiotensin II receptor blockers | 35 (26.7) |
| Diuretics | 10 (7.6) |
| Statins | 28 (21.4) |
| Hypoglycemic agents | 22 (16.8) |

was noticed between EPC count and systolic blood pressure/heart rate/white blood cell count, FMD and heart rate/white blood cell count, and systolic blood pressure and heart rate/white blood cell count.

Multivariate logistic regression showed that hypertension (odds ratio [OR] = 24.335, 95% confidence interval [CI]: 2.467–240.048), family history of premature cardiovascular (OR = 0.068, 95% CI 0.006–0.720), HbA1c ≥ 6.5% (OR = 0.059, 95% CI 0.007–0.485) and elevated
systolic blood pressure (OR = 0.902, 95% CI: 0.821–0.990) were independently related to FMD decline at 1-year follow-up (Table 4).

Five participants were lost to follow-up (3.82%). The 1-year FMD was significantly improved from the baseline [(9.31 ± 5.62) % vs (7.31 ± 5.26) %, P < 0.001). The use of antiplatelet therapy, angiotensin-converting enzyme inhibitor / angiotensin II receptor blockers, β-blockers and statins were significantly higher at 1-year follow-up than that at baseline (Table 5).

Participants with ΔFMD ≥10% had significantly higher proportions of hypertension, elevated systolic blood pressure, elevated pulse pressure and lower baseline FMD than those ΔFMD < 10%. Participants with ΔFMD > 10% had significantly more patients with diabetes and hypoglycemic therapy (biguanides, sulfonylureas, glinides and alpha-glucosidase inhibitors) than those with ΔFMD ≤10% (Table 6). EPC counts in participants with ΔFMD ≥10% was significantly higher than those with ΔFMD < 10% (59.14 ± 24.36 per 10^6 cells vs 36.11 ± 15.16 per 10^6 cells) at baseline (Table 6).

Multivariate logistic regression analysis showed that elevated EPC counts (OR = 1.104, 95% CI: 1.047–1.165) and decreased levels of serum creatinine (OR = 0.915, 95% CI: 0.843–0.993) were independently associated with FMD improvement at 1-year follow-up (Table 7).

### Table 2: Comparison between patients with FMD < 6% and those with FMD ≥6%

|                         | FMD < 6% (n = 65) | FMD ≥ 6% (n = 66) | P-value |
|-------------------------|-------------------|-------------------|---------|
| FMD, %                  | 3.11 ± 1.69       | 11.45 ± 4.17      | < 0.001 |
| Age, year               | 60.74 ± 11.41     | 59.56 ± 10.99     | 0.548   |
| Male, n (%)             | 30 (46.2)         | 32 (48.5)         | 0.771   |
| Body mass index, kg/m²  | 25.82 ± 3.86      | 25.46 ± 3.16      | 0.584   |
| Smoking, n (%)          | 13 (20.3)         | 15 (23.1)         | 0.689   |
| Diabetes mellitus, n (%)| 19 (29.7)         | 11 (16.9)         | 0.121   |
| Family history of premature cardiovascular disease, n (%) | 13 (20.0)         | 14 (21.2)         | 0.839   |
| Hypertension, n (%)     | 33 (50.8)         | 35 (53.0)         | 0.778   |
| Statins, n (%)          | 10 (15.4)         | 18 (27.3)         | 0.106   |
| β-blocker, n (%)        | 16 (24.6)         | 12 (18.8)         | 0.419   |
| Calcium channel blockers, n (%) | 16 (24.6) | 19 (28.8)         | 0.589   |
| Angiotensin-converting enzyme inhibitors / angiotensin II receptor blockers, n (%) | 19 (29.2)         | 16 (24.2)         | 0.519   |
| Heart rate, beats/min   | 70.98 ± 11.42     | 72.73 ± 9.52      | 0.351   |
| Systolic blood pressure, mmHg | 138.38 ± 16.41   | 130.69 ± 16.25   | 0.008   |
| Diastolic blood pressure, mmHg | 74.75 ± 10.72    | 75.88 ± 10.43    | 0.546   |
| Pulse pressure, mmHg    | 63.75 ± 16.29     | 54.89 ± 14.75     | 0.001   |
| Serum creatinine, μmol/L| 65.64 ± 17.08     | 67.53 ± 14.02     | 0.497   |
| Uric acid, mmol/L       | 338.73 ± 109.08   | 329.95 ± 75.89    | 0.600   |
| Glucose, mmol/L         | 5.68 ± 1.60       | 5.40 ± 1.37       | 0.290   |
| HbA1c, %                | 6.49 ± 0.94       | 5.79 ± 0.53       | < 0.001 |
| Total cholesterol, mmol/L| 1.79 ± 1.19       | 1.75 ± 0.78       | 0.822   |
| Triglyceride, mmol/L    | 4.04 ± 0.76       | 4.33 ± 0.89       | 0.058   |
| High-density lipoprotein cholesterol, mmol/L | 1.30 ± 0.36       | 1.35 ± 0.32       | 0.493   |
| Low-density lipoprotein cholesterol, mmol/L | 2.26 ± 0.62       | 2.51 ± 0.70       | 0.035   |
| Homocysteine, μmol/L    | 15.11 ± 12.04     | 13.22 ± 3.92      | 0.439   |
| Ejection fraction, %    | 66.90 ± 6.58      | 64.46 ± 7.52      | 0.093   |
| White blood cell, ×10^9/L | 6.27 ± 1.71       | 6.87 ± 1.94       | 0.068   |
| Neutrophil, ×10^9/L     | 3.55 ± 1.49       | 3.97 ± 1.68       | 0.140   |
| Neutrophil-to-lymphocyte ratio | 2.08 ± 1.25   | 1.97 ± 1.12       | 0.618   |
| Red blood cell distribution width, % | 12.73 ± 2.76       | 12.93 ± 0.73       | 0.818   |
| High-sensitivity C-reactive protein, mg/L | 3.37 ± 4.13       | 7.87 ± 14.31       | 0.160   |
| Endothelial progenitor cells per 10^6 cells | 52.00 ± 22.13       | 46.59 ± 24.78       | 0.254   |

FMD flow-mediated dilatation
### Table 3: Comparison between patient with lower EPC counts and those with higher EPC counts

|                          | EPC counts < 43 cells per 10⁶ cells (n = 65) | EPC counts ≥ 43 cells per 10⁶ cells (n = 66) | P-value |
|--------------------------|---------------------------------------------|---------------------------------------------|---------|
| Flow-mediated dilatation, % | 9.16 ± 5.86                                | 6.47 ± 4.33                                 | 0.009   |
| Age, year                | 59.38 ± 11.04                               | 59.98 ± 11.02                               | 0.784   |
| Male, n (%)              | 29 (44.6)                                   | 33 (50.0)                                   | 0.537   |
| Body mass index, kg/m²   | 25.47 ± 3.62                                | 25.60 ± 3.40                                | 0.858   |
| Smoking, n (%)           | 16 (24.6)                                   | 12 (18.2)                                   | 0.369   |
| Diabetes mellitus, n (%) | 16 (24.6)                                   | 14 (21.2)                                   | 0.643   |
| Family history of premature cardiovascular disease, n (%) | 13 (20.0) | 14 (21.2) | 0.864   |
| Heart rate, beats/min    | 69.04 ± 9.11                                | 74.14 ± 10.64                               | 0.012   |
| Systolic blood pressure, mmHg | 130.16 ± 16.55   | 137.29 ± 15.14                             | 0.026   |
| Diastolic blood pressure, mmHg | 75.14 ± 8.53   | 74.31 ± 10.81                              | 0.669   |
| Pulse pressure, mmHg     | 54.41 ± 15.08                                | 61.92 ± 16.53                               | 0.020   |
| Serum creatinine, μmol/L | 66.76 ± 13.97                                | 65.23 ± 17.01                               | 0.627   |
| Uric acid, mmol/L        | 313.33 ± 86.46                               | 337.66 ± 95.91                              | 0.188   |
| Glucose, mmol/L          | 5.40 ± 1.12                                  | 5.54 ± 1.67                                 | 0.608   |
| HbA1c, %                 | 6.00 ± 0.55                                  | 6.29 ± 1.11                                 | 0.148   |
| Total cholesterol, mmol/L | 1.68 ± 0.69                                 | 1.70 ± 0.85                                 | 0.882   |
| Triglyceride, mmol/L     | 4.20 ± 0.90                                  | 4.25 ± 0.87                                 | 0.805   |
| High-density lipoprotein cholesterol, mmol/L | 1.31 ± 0.35 | 1.35 ± 0.34 | 0.589   |
| Low-density lipoprotein cholesterol, mmol/L | 2.44 ± 0.72 | 2.44 ± 0.67 | 0.952   |
| Homocysteine, μmol/L     | 13.24 ± 4.01                                 | 13.32 ± 3.99                                | 0.439   |
| Ejection fraction, %     | 65.54 ± 6.27                                 | 64.82 ± 7.55                                | 0.957   |
| White blood cell, ×10⁹/L | 6.13 ± 2.34                                  | 6.75 ± 2.17                                 | 0.118   |
| Neutrophil, × 10⁹/L      | 3.51 ± 1.58                                  | 3.88 ± 1.86                                 | 0.222   |
| Neutrophil-to-lymphocyte ratio | 2.08 ± 1.25 | 1.97 ± 1.12 | 0.057   |
| Red blood cell distribution width, % | 12.78 ± 0.76 | 12.96 ± 0.79 | 0.270   |
| High-sensitivity C-reactive protein, mg/L | 5.24 ± 11.52 | 6.33 ± 11.62 | 0.802   |

**EPC** endothelial progenitor cell

### Table 4: Multivariate logistic regression analysis of influencing factors of FMD decline at 1-year follow-up

|                          | B     | S.E.  | Wald  | P    | Exp (B) | 95% CI     |
|--------------------------|-------|-------|-------|------|---------|------------|
| Age ≥ 65 years           | −1.369| 1.198 | 1.306 | 0.253| 0.254   | 0.024−2.661|
| Male gender              | −0.619| 0.844 | 0.539 | 0.463| 0.538   | 0.103−2.815|
| Hypertension             | 3.192 | 1.168 | 7.470 | 0.006| 24.335  | 2.467−240.048|
| Family history of premature cardiovascular diseases | −2.685 | 1.202 | 4.987 | 0.026| 0.068   | 0.006−0.720|
| Smoking                  | 0.412 | 1.179 | 0.122 | 0.727| 1.510   | 0.150−15.231|
| Hba1c ≥ 6.5%             | −2.829| 1.075 | 6.934 | 0.008| 0.059   | 0.007−0.485|
| Total cholesterol        | 1.078 | 1.190 | 0.821 | 0.365| 2.939   | 0.285−30.277|
| Low-density lipoprotein cholesterol | −0.582 | 1.566 | 0.138 | 0.710| 0.559   | 0.026−12.030|
| Systolic blood pressure  | −0.103| 0.048 | 4.668 | 0.031| 0.902   | 0.821−0.990|
| Pulse pressure           | −0.029| 0.040 | 0.526 | 0.468| 0.971   | 0.898−1.051|
| Endothelial progenitor cells | 0.001 | 0.014 | 0.006 | 0.941| 1.001   | 0.975−1.028|
| Constant                 | 15.174| 5.853 | 6.722 | 0.010| 3,889,333.926 |

**FMD** flow-mediated dilatation
permeability [28, 29]. Our study found that systolic

tensive patients can significantly affect endothelial

increased blood flow-associated shear stress in hyper-

Table 5 Medications at baseline and 1-year follow-up [n (%)]

| Medications                  | Baseline (n = 131) | 1-year follow-up (n = 126) | P-value |
|------------------------------|--------------------|----------------------------|---------|
| Antiplatelet therapy         | 35 (26.7)          | 85 (67.5)                  | <0.001  |
| β-blocker                    | 28 (21.4)          | 68 (54.0)                  | <0.001  |
| Calcium channel blockers     | 35 (26.7)          | 28 (22.2)                  | 0.312   |
| ACE-I/ARB                    | 35 (26.7)          | 56 (44.4)                  | 0.003   |
| Diuretics                    | 10 (7.6)           | 10 (7.9)                   | 1       |
| Statins                      | 28 (21.4)          | 86 (68.3)                  | <0.001  |
| Hypoglycemic agents          | 22 (16.8)          | 29 (23.0)                  | 0.215   |

ACE-I/ARB angiotensin-converting enzyme inhibitors / angiotensin II receptor blockers

Discussion

Increased blood flow-associated shear stress in hypertensive patients can significantly affect endothelial permeability [28, 29]. Our study found that systolic blood pressure and pulse pressure were significantly higher in the participants with FMD < 6% than those with FMD ≥ 6%. We also found that hypertension, systolic blood pressure and pulse pressure were independent risk factors in predicting endothelial dysfunction. It has been suggested that oxidative stress and endothelial dysfunction are associated with impaired vasodilatory function. It has been suggested that oxidative stress and endothelial dysfunction are associated with impaired vasodilatory function. In addition, endothelial dysfunction is also associated with increased pulse pressure and hypertension in type 1 diabetes [PMID: 29101422].

Our study included 30 participants with diabetes and found elevated HbA1c levels were an independent influencing factor of endothelial dysfunction, suggesting diabetes may be associated with endothelial dysfunction. Hyperglycemia in diabetes is associated with inflammation and oxidative stress, which can result in endothelial dysfunction [PMID: 26781070, 30,274,207].

It has been shown that the phenotypic EPCs are independently associated with the severity of coronary artery lesion and carotid intima-media thickness and can be used as an independent predictor of cardiovascular outcomes [30, 31]. Our study found that the CD34 + VEGFR2+ EPC count was associated with the baseline FMD. Heart rate, systolic blood pressure and pulse pressure in participants with higher EPC counts were significantly higher than that in those with lower EPC counts. These results suggest that elevated systolic blood pressure and pulse pressure were more likely to be associated with differentiation and release of bone marrow-derived EPCs into the blood in comparison with other risk factors of endothelial dysfunction. However, multivariate logistic regression analysis did not find independent association between EPC counts and baseline FMD.

A previous study found that high-sensitivity C-reactive protein was an independent risk factor for coronary heart disease and its level was significantly associated with the risk of future cardiovascular events, such as sudden death, acute myocardial infarction, and peripheral vascular disease [32, 33]. Another study showed that neutrophil-to-lymphocyte ratio was significantly associated with urinary albumin-to-creatinine ratio in asymptomatic stable coronary heart disease populations and was an independent predictor of systemic endothelial dysfunction [34, 35]. Neutrophil-to-lymphocyte ratio was independently associated to endothelial dysfunction and could predict composite cardiovascular endpoints [36, 37]. However, our study found that high-sensitivity C-reactive protein, white blood cell count and neutrophil-to-lymphocyte ratio were not significantly associated with FMD, suggesting that these inflammatory factors have no definite diagnostic value in low-risk patients with non-obstructive coronary atherosclerosis.

All our participants received intensive blood-pressure control, antiplatelet and statins therapy. No major cardiovascular events occurred during the 1-year follow-up. We found participants with worse baseline endothelial function had greater increase in 1-year FMD. Our study suggests that patients with hypertension, elevated systolic blood pressure and elevated pulse pressure at baseline are more likely to benefit from antihypertensive treatment. It has been shown that aliskiren, a direct renin inhibitor, can improve endothelial function and arterial stiffness when being used as an antihypertensive agent [PMID: 24994608, 24,708,382]. Another study showed that bisoprolol improved endothelial function in patients with hypertension and stable angina [PMID: 23609363]. Further research is needed to illustrate the detailed mechanisms between antihypertensive treatment and endothelial dysfunction.

In our study, participants with diabetes and elevated HbA1c did not show FMD improvements despite the same antihypertensive, antiplatelet and antihyperlipidemic treatments with the non-diabetic participants. We speculate the conventional antidiabetic medications have limited protective effect for vascular endothelial function. Diabetes can induce endothelial dysfunction, leading to increased risks of cardiovascular diseases [PMID: 25084409]. In addition, hyperglycemia is associated with EPCs dysfunction and endothelial dysfunction [PMID: 28718318]. Dapagliflozin is used to treat type 2 diabetes and showed improvements in endothelial function and arterial stiffness [PMID: 29061124]. New antidiabetic drugs, such as dipeptidyl peptidase-4 inhibitors, sodium-glucose cotransporter-2 inhibitors and glucagon-like peptide-1 receptor agonists also showed differentia effect on endothelial function and arterial stiffness [PMID: 30622967].
Chronic kidney disease and cardiovascular disease share similar risk factors. It has been shown that vascular endothelial function and FMD decreased significantly in patients with end-stage renal disease [38, 39]. Similarly, our study found that elevated levels of serum creatinine were associated with continuous endothelial injury. We speculate that major cardiovascular risk factors such as hypertension, diabetes and renal dysfunction can aggravate atherosclerosis partly by impairing the endothelial function. Our findings suggest that vascular endothelial dysfunction induced by hypertension is can be

|                     | ΔFMD < 10% (n = 55) | ΔFMD ≥10% (n = 71) | P-value |
|---------------------|---------------------|---------------------|---------|
| Baseline FMD, %     | 9.92 ± 5.48         | 6.11 ± 4.51         | < 0.001 |
| 1-year FMD, %       | 9.35 ± 5.71         | 9.28 ± 5.61         | 0.951   |
| Age, years          | 58.62 ± 10.74       | 60.51 ± 11.19       | 0.387   |
| Male, n (%)         | 27 (49.1)           | 35 (49.3)           | 0.944   |
| Body mass index, kg/m² | 25.60 ± 3.69       | 25.50 ± 3.37        | 0.891   |
| Smoking, n (%)      | 14 (25.5)           | 14 (19.7)           | 0.803   |
| Diabetes mellitus, n (%) | 19 (34.5)       | 11 (15.5)           | 0.017   |
| Family history of premature cardiovascular diseases, n (%) | 14 (25.5) | 13 (18.3) | 0.644 |
| Hypertension, n (%) | 23 (41.8)           | 45 (63.4)           | 0.012   |
| Heart rate, beats/min | 71.66 ± 10.40    | 71.58 ± 10.12       | 0.970   |
| Systolic blood pressure, mmHg | 129.14 ± 16.95 | 137.46 ± 14.66 | 0.010   |
| Diastolic blood pressure, mmHg | 75.09 ± 8.66 | 74.64 ± 10.87 | 0.733   |
| Pulse pressure, mmHg | 54.05 ± 14.60     | 63.04 ± 16.04       | 0.005   |
| Serum creatinine, μmol/L | 69.02 ± 14.41 | 63.55 ± 16.07      | 0.081   |
| Uric acid, mmol/L   | 330.23 ± 80.96     | 321.93 ± 100.05     | 0.657   |
| Glucose, mmol/L     | 5.41 ± 1.63        | 5.52 ± 1.21         | 0.715   |
| HbA1c, %            | 6.06 ± 1.07        | 6.21 ± 0.70         | 0.468   |
| Total cholesterol, mmol/L | 1.72 ± 0.69   | 1.68 ± 0.84         | 0.795   |
| Triglyceride, mmol/L | 4.24 ± 0.88       | 4.21 ± 0.89         | 0.884   |
| High-density lipoprotein cholesterol, mmol/L | 1.27 ± 0.31 | 1.38 ± 0.37       | 0.116   |
| Low-density lipoprotein cholesterol, mmol/L | 2.50 ± 0.74 | 2.39 ± 0.06       | 0.448   |
| Homocysteine, μmol/L | 12.28 ± 2.89     | 14.53 ± 5.69        | 0.132   |
| Ejection fraction, % | 64.26 ± 6.52      | 65.95 ± 7.24        | 0.291   |
| Medications         | ΔFMD < 10% (n = 55) | ΔFMD ≥10% (n = 71) | P-value |
| Antiplatelet therapy | 37 (67.2)         | 48 (67.6)           | 0.879   |
| ACE-I/ARB           | 21 (38.2)          | 35 (49.3)           | 0.193   |
| β-blockers          | 27 (49.1)          | 41 (57.7)           | 0.291   |
| Calcium channel blockers | 11 (20.0)     | 17 (23.9)           | 0.549   |
| Diuretics           | 4 (7.3)            | 6 (8.5)             | 0.777   |
| Statins             | 37 (67.3)          | 49 (69.0)           | 0.462   |
| Hypoglycemic agents | 18 (32.7)          | 11 (15.5)           | 0.017   |
| White blood cell, x10⁹/L | 6.35 ± 1.42   | 6.87 ± 1.94         | 0.542   |
| Neutrophil, x10⁹/L  | 3.53 ± 1.37        | 3.83 ± 1.70         | 0.352   |
| Lymphocytes, x10⁹/L | 2.23 ± 0.90        | 2.02 ± 0.62         | 0.169   |
| Neutrophil-to-lymphocyte ratio | 1.92 ± 1.35 | 2.00 ± 0.92         | 0.736   |
| Red blood cell distribution width, % | 12.76 ± 0.63 | 12.96 ± 0.87 | 0.191   |
| High-sensitivity C-reactive protein, mg/L | 5.65 ± 11.42 | 5.95 ± 11.74 | 0.944   |
| Endothelial progenitor cells per 10⁶ cells | 36.11 ± 15.16 | 59.14 ± 24.36 | < 0.001 |

FMD flow-mediated dilatation; ACE-I/ARB, ACE-I/ARB angiotensin-converting-enzyme inhibitors / angiotensin II receptor blockers
improved with antihypertensive treatment, while that with diabetes and renal dysfunction is more difficult to reverse.

Our study has limitations. Our study excluded patients with non-obstructive coronary atherosclerosis who present with symptoms of typical myocardial ischemia and acute coronary syndrome, suggesting worse endothelial dysfunction. This may underestimate the incidence and severity of endothelial dysfunction in patients with early atherosclerosis. At the 1-year follow-up, blood pressure, low-density lipoprotein cholesterol and HbA1c were not included in the analysis, making it difficult to assess the effect of anti-atherosclerosis treatment on vascular endothelial function.

Conclusion
Family history of premature cardiovascular diseases, hypertension, elevated systolic pressure, and HbA1c > 6.5% are independent risk factors for endothelial dysfunction in non-obstructive atherosclerotic patients. Elevated circulating EPC counts and decreased levels of serum creatinine are independent predictors of endothelial function improvement. Our findings may help to facilitate the risk stratification of patients with mild coronary atherosclerosis, and to explore intervention methods to repair vascular endothelial function.

Abbreviations
EPCs: Endothelial progenitor cells; FMD: Flow-mediated dilatation; VEGFR2: Vascular endothelial growth factor receptor 2

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Authors’ contributions
YPL, ZXF, QH and JL contributed to the design of the study. YPL, ZXF, JG, JS, XBZ and ZL contributed to the interpretation of data. YPL and ZXF drafted the manuscript. All authors revised and approved the final manuscript.

Table 7 Multivariate logistic regression analysis of influencing factors of FMD improvement at 1-year follow-up

|                              | B    | S.E.  | Wald  | P     | Exp (B) | 95% Cl          |
|------------------------------|------|-------|-------|-------|---------|-----------------|
| Male gender                  | −1.126 | 1.163 | 0.937 | 0.333 | 0.324   | 0.033–3.171     |
| Age ≥ 65 years               | 1.798 | 0.938 | 3.673 | 0.055 | 6.038   | 0.960–37.974    |
| Family history of premature cardiovascular diseases | −0.691 | 0.849 | 0.661 | 0.416 | 0.501   | 0.095–2.648     |
| Smoking                      | −0.176 | 0.975 | 0.033 | 0.856 | 0.838   | 0.124–5.666     |
| Systolic blood pressure      | 0.041 | 0.025 | 2.793 | 0.095 | 1.042   | 0.993–1.094     |
| Low-density lipoprotein cholesterol | −0.471 | 0.687 | 0.470 | 0.493 | 0.625   | 0.163–2.400     |
| Serum creatinine             | −0.089 | 0.042 | 4.479 | 0.034 | 0.915   | 0.843–0.993     |
| HbA1c ≥ 6.5%                 | −0.686 | 0.518 | 1.754 | 0.185 | 0.503   | 0.182–1.390     |
| Endothelial progenitor cells | 0.099 | 0.027 | 13.295| 0.000 | 1.104   | 1.047–1.165     |
| Constant                     | 3.208 | 5.772 | 0.309 | 0.578 | 24.725  |                 |

FMD flow-mediated dilatation

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Availability of data and materials
The datasets used and/or analyzed during the current study will be available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Our study was approved by the ethics committee of the Xuanwu Hospital of Capital Medical University. All participants have signed the informed consents.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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