Increased Serum Level of Soluble Vascular Endothelial Growth Factor Receptor-1 Is Associated With Poor Coronary Collateralization in Patients With Stable Coronary Artery Disease

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Background: The present study investigated whether serum levels of soluble vascular endothelial growth factor receptor (sVEGFR)-1, -2 and -3 are related to poor coronary collateralization in patients with stable coronary artery disease (CAD).

Methods and Results: Serum levels of sVEGFR-1, -2, -3, VEGF, and placental growth factor (PLGF) were determined in 403 consecutive patients with angiographic total or subtotal occlusion of at least 1 major coronary artery. The degree of collateralization was graded according to the Rentrop scoring system. Low (Rentrop score of 0 or 1) and high (Rentrop score of 2 or 3) coronary collateralization occurred in 161 and 242 patients, respectively. Serum levels of sVEGFR-1 and -2 were significantly elevated, in contrast, VEGF and PLGF levels were remarkably decreased in patients with low collateralization than in those with high collateralization (all P<0.05). Significant differences in sVEGFR-1, VEGF and PLGF levels was consistently detected between the low and high collateralization subgroups for patients with and without type 2 diabetes mellitus (DM) (for all comparisons, P<0.01). Multivariable regression analysis revealed that DM, dyslipidemia, elevated sVEGFR-1, and reduced VEGF and PLGF in serum were independently associated with a low degree of coronary collateralization.

Conclusions: Increased serum sVEGFR-1 level is associated with poor coronary collateralization in patients with stable CAD. Type 2 DM is a predominant factor affecting collateral growth in these patients.  

Key Words: Collateral growth; Coronary artery disease; Diabetes mellitus; Soluble vascular endothelial growth factor receptor-1
results jointly suggest that the VEGF–sVEGFR-1 mechanism is crucial to the physiological homeostasis of vasculature and modulation of pro- and anti-angiogenesis. Till now, it has been unknown whether the sVEGFR-1 level differs in patients with CAD and good or poor collateral growth. Therefore, in the present study we analyzed the serum levels of sVEGFR-1, -2 and -3 in patients with chronic total or subtotal coronary artery occlusion and low or high collateralization according to the Rentrop scoring system. Serum levels of high-sensitivity C-reactive protein (hs-CRP), VEGF and placental growth factor (PLGF) were also measured to compare inflammatory and pro-angiogenic factors in these patients.

Methods

Study Population

Based on a previous report that a severe coronary artery obstruction was a prerequisite for spontaneous collateral recruitment, a total of 1,264 consecutive patients with a total or subtotal occlusion (≥90%) of at least 1 major epicardial coronary artery between January 2009 and December 2011 were selected from the database of Shanghai Rui Jin Hospital Percutaneous Coronary Intervention (PCI) Outcomes Program. This program uses clinical and angiographic information to estimate risk-adjusted outcomes. Stable angina was diagnosed according to the criteria recommended by the American College of Cardiology/American Heart Association. The diagnosis of type 2 diabetes mellitus (DM) was made according to the criteria of American Diabetes Association, including symptoms of DM plus casual plasma glucose concentration >200 mg/dl (11.1 mmol/L), or an increased fasting (126 mg/dl [7.0 mmol/L]) or 2-h postprandial (200 mg/dl [11.1 mmol/L]) during an oral glucose tolerance test) glucose level. Hyperlipidemia was defined according to the guidelines of the American Diabetes Association. For the purpose of this study and to avoid confounding data, patients undergoing PCI within 1 month of acute myocardial infarction (n=183) and non-ST-elevation acute coronary syndrome (n=332) were excluded. Patients with type 1 DM were excluded by measurement of C-peptide (n=75). We also excluded those with chronic bacterial infection, heart failure, renal failure requiring hemodialysis, pulmonary heart disease, malignant tumor or immune system disorders (n=271). The remaining 403 eligible patients with stable CAD and total or subtotal occlusions (308 men and 95 women, aged 44–76 years) were enrolled in this study (Figure 1).

The study protocol was approved by the Hospital Ethics Committee and written informed consent was given by all patients.

Coronary Angiography

Coronary angiography was performed through the femoral or radial approach. Intracoronary administration of nitroglycerin was encouraged prior to angiography. Coronary angiograms were reviewed by 2 experienced cardiologists who were blinded to the study protocol and biochemical measurements, and any difference in interpretation was resolved by a third reviewer. Significant CAD was diagnosed if there was ≥70% diameter stenosis in at least 1 major epicardial coronary artery, and left main coronary artery narrowing ≥50% was considered as 2- vessel disease. The severity of CAD was determined by the number of significantly diseased coronary arteries. The presence and number of collaterals supplying the distal aspect of a total or subtotal (≥90%) coronary occlusion were graded on a 4-point scale from 0 to 3 according to the Rentrop scoring system: zero = no collateral vessels; 1 = threadlike, poorly opacified collaterals with faint visualization of the distal vessel; 2 = moderately opacified collateral channels; 3 = large, brightly filled collateral channels with immediate visualization of the entire distal vessel >10 mm. Patients were then classified as having low (Rentrop score of 0 and 1) and high (Rentrop score of 2 and 3) coronary collateralization, as in previous studies. In patients with more than 1 total or subtotal coronary occlusion, the vessel with the highest grade of collateralization was chosen for analysis.

Biochemical Measurement

Blood samples were collected after an overnight fasting. All samples were stored at −80°C until analysis. Serum glucose, glycated hemoglobin (HbA1c), blood urea nitrogen, creatinine, uric acid, and lipid profiles were measured with standard laboratory techniques on a Hitachi 912 Analyzer (Roche...
Table 1. Baseline Characteristics and Biochemical Measurements of the Patients With Coronary Artery Disease

| Variable                        | Low collateralization (n=161) | High collateralization (n=242) | P value |
|---------------------------------|-------------------------------|--------------------------------|---------|
| Male sex (%)                    | 124 (77.0)                   | 184 (76.0)                    | 0.819   |
| Age (years)                     | 66±10                        | 65±11                         | 0.795   |
| Cigarette smoking (%)           | 56 (34.8)                    | 86 (35.5)                     | 0.877   |
| Hypertension (%)                | 111 (68.9)                   | 192 (79.3)                    | 0.018   |
| Systolic BP (mmHg)              | 141±21                       | 146±22                        | 0.023   |
| Diastolic BP (mmHg)             | 86±12                        | 89±12                         | 0.014   |
| Type 2 diabetes                 | 84 (52.2)                    | 61 (25.2)                     | <0.001  |
| Duration of CAD (years)         | 9.8±5.4                      | 6.7±4.8                       | <0.01   |
| Carotid artery stenosis (%)*    | 20 (12.4)                    | 23 (9.5)                      | 0.263   |
| Dyslipidemia history (%)        | 99 (61.5)                    | 116 (47.9)                    | 0.008   |
| Total cholesterol (mmol/L)      | 4.39±1.45                    | 4.15±1.13                     | 0.062   |
| HDL-cholesterol (mmol/L)        | 0.99±0.26                    | 1.04±0.27                     | 0.105   |
| LDL-cholesterol (mmol/L)        | 2.7±1.12                     | 2.56±0.95                     | 0.194   |
| Triglycerides (mmol/L)          | 1.97±1.08                    | 1.72±1.01                     | 0.028   |
| Lipoprotein-a (g/L)             | 0.23±0.19                    | 0.26±0.29                     | 0.185   |
| Apoprotein A (g/L)              | 1.23±0.25                    | 1.21±0.27                     | 0.341   |
| Apoprotein B (g/L)              | 0.96±0.28                    | 0.91±0.27                     | 0.089   |
| Fasting glucose (mmol/L)        | 6.17±2.08                    | 5.45±1.51                     | <0.001  |
| 2-h postprandial glucose (mmol/L)| 10.13±2.98                | 8.56±2.38                     | <0.001  |
| HbA1c (%)                       | 6.53±1.03                    | 6.24±1.03                     | 0.006   |
| Blood urea nitrogen (mmol/L)    | 5.53±2.01                    | 5.11±1.68                     | 0.032   |
| Creatinine (μmol/L)             | 82±19.7                      | 81.7±29.3                     | 0.901   |
| eGFR (ml·min⁻¹·1.73m⁻²)         | 86.5±24.3                    | 90.1±21.6                     | 0.131   |
| Uric acid (μmol/L)              | 335±79                       | 331±79                        | 0.64    |
| ACEI or ARB (%)                 | 87 (54.0)                    | 138 (57.0)                    | 0.554   |
| β-blocker (%)                   | 84 (52.2)                    | 107 (44.2)                    | 0.117   |
| Calcium-channel blocker (%)     | 44 (27.3)                    | 71 (29.3)                     | 0.662   |
| Statins* (%)                    | 148 (92.0)                   | 230 (95.0)                    | 0.768   |
| Antiplatelet (%)                | 140 (88.2)                   | 225 (93.0)                    | 0.485   |
| LVEF                            | 47.3±11.2                    | 51.2±9.3                      | 0.103   |
| 1-vessel CAD                    | 33 (20.5)                    | 67 (27.7)                     |        |
| 2-vessel CAD                    | 54 (33.5)                    | 90 (37.2)                     | 0.04    |
| 3-vessel CAD                    | 74 (46.0)                    | 85 (35.1)                     |        |
| No. of coronary occlusions      | 2.85±0.9                     | 2.5±0.78                      | 0.107   |
| Total occlusion of the recipient artery (%) | 157 (97.5) | 239 (98.7) | 0.853   |

Data are mean±SD or n (%). *Mainly simvastatin, pravastatin and atorvastatin. *Significant carotid artery stenosis is defined as a peak systolic velocity >125 cm/s on echo-Doppler.

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BP, blood pressure; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin A1c; HDL, high-density lipoprotein; LDL, lower density lipoprotein; LVEF, left ventricular ejection fraction.

Diagnostics, Germany). Serum levels of sVEGFR-1, -2 and -3 were determined using commercially available ELISA kits (Aviscera Bioscience, Santa Clara, CA, USA). Serum VEGF, PLGF and hs-CRP levels were also assayed with ELISA kits (R&D Systems, Minneapolis, MN, USA).

Statistical Analysis
Continuous variables are presented as mean±standard deviation (SD) and categorical data are summarized as frequencies or percentages. For categorical clinical variables, differences between groups were evaluated with the chi-square test. For continuous variables, the existence of a normal distribution was evaluated with the Kolmogorov-Smirnov test, and logarithmic or square-root transformations (for serum sVEGFR-1, sVEGFR-2, sVEGFR-3, VEGF, PLGF and hs-CRP) were performed on the continuous variables of non-normal distribution. Differences among groups were analyzed by Student’s t test. Correlation between variables was determined by the Pearson or Spearman correlation test as appropriate. A multivariable logistic regression model was constructed to assess the independent determinants of coronary collateralization. Receiver-operator characteristic (ROC) analysis of risk factors and biomarkers was performed with and without sVEGFR. The C statistic was used to analyze the discriminatory capacity with and without the addition of sVEGFR.

Results
Baseline Characteristics of the Patients
Overall, low and high coronary collateralization occurred in 161
and 242 patients, respectively. Despite similar age, sex distribution, cigarette smoking, and medical treatment, patients with low collateralization were less hypertensive, and more often had type 2 DM and dyslipidemia than those with high collateralization (for all comparisons, P<0.05), (Table 1). In addition, patients with low collateralization exhibited more severe multivessel disease, longer duration of CAD as compared with those with high collateralization (P<0.05), but the prevalence of total occlusion (no antegrade flow) of the recipient artery and severe carotid artery disease was similar between the 2 groups. When patients were further stratified according to the presence or absence of type 2 DM, duration of DM was longer in the patients with low collateralization (P=0.05), but the difference in the incidence of diabetic retinopathy (20.2% vs. 16.4%) and nephropathy (25.0% vs. 21.3%) between the low and high collateralization subgroups did not reach statistical significance (both P>0.05).

Serum Levels of VEGF, sVEGFR and PLGF

Serum levels of sVEGFR-1, sVEGFR-2 and hs-CRP were significantly higher, whereas VEGF and PLGF levels were remarkably reduced in patients with low collateralization compared with those with high collateralization (for all comparisons, P<0.05) (Table 1). Serum sVEGFR-3 level was similar between the 2 groups. The serum sVEGFR-3 level increased stepwise from 1-vessel disease (2.08±0.68 ng/ml), 2-vessel disease (2.28±0.96 ng/ml) to 3-vessel disease (2.45±1.36 ng/ml), although the difference did not reach statistical significance (P=0.348). In contrast, a stepwise decrease in serum VEGF level was observed across 1-vessel disease (623±172 pg/ml), 2-vessel disease (395±194 pg/ml) and 3-vessel disease (324±177 pg/ml), with significant difference existing between 1- and 3-vessel disease (P<0.01). Moreover, the VEGF level inversely correlated with the sVEGFR-1 values (Pearson’s r=−0.48, P<0.001) and the number of diseased coronary arteries (Spearman’s r=−0.49, P<0.001). PLGF concentrations were also negatively related to the sVEGFR-1 levels (Pearson’s r=−0.23, P<0.01).

Multivariable Regression Analysis

Multivariable regression analysis was performed by including the conventional factors and bio-measurements (Tables 1,2) to determine the independent risk factors for impaired coronary collateral development. Duration of CAD, DM and dyslipidemia, and elevated sVEGFR-1 and reduced VEGF and PLGF levels in serum were independently associated with low coronary collateralization (Table 3). The hs-CRP level failed to be a predictor for the risk of low collateralization (P=0.062). Adding sVEGFR-1 significantly improved risk prediction by increas-

![Figure 2. Serum levels of soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) in diabetic and nondiabetic patients with low or high degree of coronary collateralization.](image)

![Table 2. Serum Levels of sVEGFR1, sVEGFR2, sVEGFR3, VEGF and hs-CRP](table)
Coronary collateral formation is a physiological accommodation during severe, gradual vessel narrowing to restore blood flow in ischemic areas. Poor collateral growth has been shown to be associated with adverse outcome in patients with CAD. Our study is the first to demonstrate an elevated serum sVEGFR-1 level in patients with a low degree of coronary collateralization, indicating linkage of this negative regulator of angiogenesis to impaired coronary collateral formation.

Soluble VEGFR-1 is produced in the endothelium by inflammatory induction. The anti-inflammatory enzyme heme oxygenase 1 (HO-1) and simvastatin suppress inflammation-induced sVEGFR-1 release from endothelial cells. Consistent with previous findings, our present results showed that the serum levels of sVEGFR-1 and sVEGFR-2 were significantly elevated, while VEGF and PLGF levels were remarkably reduced, in patients with low collateralization than in those with high collateralization. Increased sVEGFR-1 and decreased VEGF and PLGF levels in serum were independently associated with low coronary collateralization. Furthermore, the serum sVEGFR-1 level increased stepwise from patients with 1-vessel disease to those with 3-vessel disease, and correlated negatively with the VEGF and PLGF levels. Together with previous findings that collateralization is also influenced by VEGF genotype, these observations support the notion that disturbance of pro- and anti-angiogenesis modulation in severe atherosclerosis may be related to poor collateral growth during myocardial ischemia.

In addition, plasma chemokine levels are associated with the presence and extent of coronary collaterals in patients with chronic ischemic heart disease. In our present study, the serum hs-CRP level was higher in patients with low collateralization as compared with those with high collateralization. Such a disparity could partially be explained by the antiangiogenic effect of proinflammatory cytokines in mild to moderate atherosclerosis in the former case, and collateral dysfunction caused by severe endothelial dysfunction in pathophysiological states such as DM and severe atherosclerosis in the latter case, as reflected by an increased hs-CRP level. Our viewpoint is also consistent with other studies.

In our study, the percentages of type 2 DM and dyslipidemia were significantly higher in patients with low coronary collateralization than in those with high collateralization. DM is a crucial factor that negatively affects vascular cell function. Numerous studies have indicated that the development and biological function of coronary collaterals are significantly attenuated in patients with DM, although some have negated this. Previous studies have observed that the myocardial expression of VEGFR-2 is reduced together with downregulation of its signal transduction in diabetic patients, and that advanced glycation endproducts inhibit VEGFR-1-mediated chemotaxis in the monocytes of diabetic subjects. The serum sVEGFR-1 level is increased in diabetic patients and the diabetic condition aggravates vascular inflammation through augmenting reactive oxygen species (ROS) and amplifying a receptor for advanced glycation endproducts (RAGE)-mediated mechanism. Furthermore, in patients with DM and dyslipidemia, glycation of apoprotein A attenuates the atheroprotective function of high-density lipoprotein, and, in contrast, glycation of apoprotein B reinforces the low-density lipoprotein-induced inflammatory response. Thus, diabetic pathophysiology promotes an antiangiogenic process and meanwhile mitigates pro-angiogenic factors in the coronary vasculature during ischemia, jointly leading to impaired collateral growth.

### Study Limitations

We do recognize certain limitations to our study. First, the study is cross-sectional for the point of coronary collateral investigation, thereby allowing us to detect association, not to predict outcome. Second, measurement of sVEGFR-1, -2 and -3 in serum mainly reflects the angiogenic condition of the whole-body vasculature, instead of coronary vessels solely. Third, we observed increased sVEGFR-1 levels in both diabetic and nondiabetic patients with low collateralization, with obvious elevation in diabetic patients with low collateralization vs. nondiabetic counterparts. However, in those with high collateralization, serum level of sVEGFR-1 did not significantly differ between diabetic and nondiabetic patients. Thus, further studies are warranted to investigate both the production of sVEGFR-1 and its effect on angiogenesis and arteriogenesis under diabetic conditions.

### Conclusions

The present study has demonstrated that increased sVEGFR-1 levels in serum are associated with poor coronary collateralization in patients with stable CAD. Type 2 DM is a predominant factor affecting collateral growth in these patients.

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### Disclosures

Conflict of interest: None.

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