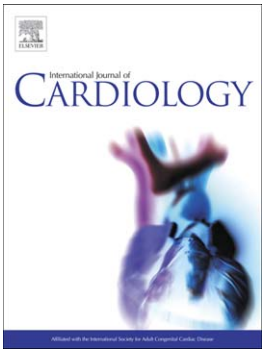

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Title page

Association of CXCR4 Expression with Coronary Collateralization in Patients with Chronic Total Coronary Occlusion: a nested case-control study ☆

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☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

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Conflicts of interest

All authors declared no conflicts of interest.

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Abstract

Objective: CXCR4 signaling contributes to the development and progression of neovascularization. The objective of this study was to investigate whether CXCR4 expression in peripheral CD34+ cells associated with the coronary collateralization (CC) in patients with chronic total coronary occlusion (CTO).

Methods and results: We measured CXCR4 expression in peripheral CD34+ cells and assessed its relation with CC in a nested case-control study including 78 cases and 78 matched controls aged 38-69 years, assessed in January 2011 to December 2012 and with at least 1 year of follow-up before the index date. Cases were defined as good coronary collateralization (GCC) according to the Rentrop scoring system (Rentrop score of 2 or 3); for each case, one age-matched control with poor coronary collateralization (PCC) (Rentrop score 0 or 1) was randomly selected from the study participants. Demographic, biochemical, and angiographic variables were collected. In multivariate analysis, the OR (95% CI) of CXCR4 expression was 0.018 (0.017 to 0.020) in patients with GCC versus PCC. Independent effect of CXCR4 expression on CC was (OR 0.012, 95% CI 0.010-0.014) when adjusted for other variables. A nonlinear relationship between CXCR4 expression and CC was observed. The CC degree increased when CXCR4 expression exceeded the turning point (30%) (OR 0.025, 95% CI 0.022–0.028; $p < 0.001$). When the CXCR4 expression exceeded 75%, increased CXCR4 level could not promoted CC (OR 0.000, 95% CI –0.008–0.007; $p = 0.974$).

Conclusion: increased CXCR4 level in peripheral CD34+ cells was associated with GCC in patients with CTO.

Keywords

Coronary collateralization; CXCR4; Chronic total coronary occlusion; Nested case-control study

1. Induction

Chronic total occlusion (CTO) of coronary arteries is defined by angiographic total occlusion of duration >3 months [1]. CTO is not uncommon in patients with coronary artery disease (CAD); currently, about 20–30% of patients with significant have CTO [2]. Coronary collateral vessels are anastomotic channels between major epicardial coronary arteries and develop as an adaptation to myocardial ischemia [3]. Well-developed coronary collaterals contribute to a reduction of infarct size, preservation of left ventricular function, and an improvement of survival in patients with CAD [4].

The extent of coronary collateralization differs greatly among patients and is affected by multiple clinical, angiographic and biochemical factors and inflammatory cytokines [5]. Studies of the mechanisms underlying the recruitment of coronary collaterals have, in large part, concentrated on the potential contribution of growth factors including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) with inconsistent results [6]. Thus, the mechanisms that contribute to the successful recruitment of coronary collaterals remain obscure.

Transplantation of bone marrow cells as well as circulating endothelial progenitor cells (EPC) may enhance neovascularization after ischemia and contribute to coronary collateralization (CC), while during this progress, the chemokine receptor CXCR4 plays an important role in migration and homing of the cells. Reports show that early up-regulation of cardiomyocyte CXCR4 (CM-CXCR4) at a time of high myocardial SDF-1 expression could be a strategy to engage the SDF-1:CXCR4 axis and improve cardiac repair after myocardial ischemia [7]. The SDF-1/CXCR4 signaling also essential for maturation of the ventricular

coronary endothelial plexus and establishment of functional coronary circulation [8]. Another previous studies indicated that disturbance of CXCR4 signaling may contribute to functional impairment of EPC in patients with CAD, while stimulating CXCR4 signaling might improve functional properties of EPC and may rescue the impaired neovascularization capacity of EPC derived from patients with CAD [9, 10]. However, as far as we know, no preceding work or report has considered the effects of peripheral CD34+ cell CXCR4 expression on coronary collateralization following CTO.

In this study, we aimed to evaluate the association between peripheral CD34+ cell CXCR4 expression and the extent of coronary collateralization among patients with CTO in a Chinese population. We hypothesized that increased CXCR4 levels in peripheral CD34+ cells could promote coronary collateralization.

2. Materials and Methods

2.1 Study population

We carried out a nested case-control study in CTO patients who underwent coronary angiography at the First Affiliated Hospital of Xi'an Jiaotong University between January 2011 and December 2012. **This study was approved by the Ethical Committee of the First Affiliated Hospital of Xi'an Jiaotong University, China. All patients provided written informed consent to participate after a full explanation of the study.**

Patients with CTO (2–12 weeks) of at least one major epicardial coronary artery with thrombolysis in myocardial infarction (TIMI) grade 0– I blood flow were recruited. The duration of CTO was estimated from the date of occurrence of myocardial infarction in the area of the myocardium supplied by the occluded vessel, from an abrupt worsening of existing angina pectoris or from information obtained from a previous angiogram. The date of hospitalization served as the index date. Cases were defined as good coronary collateralization (GCC) according to the Rentrop scoring system (Rentrop score of 2 or 3); for each case, one age-matched control with poor coronary collateralization (PCC) (Rentrop score 0 or 1) on the index date of their corresponding case was selected from the study participants. Patients with comorbidities such as cancer, immunological diseases, a serious liver, renal, hematological or neurological disorder or those who had received coronary intervention or coronary artery bypass surgery before were also excluded from the study. The recruited participants were all of the Chinese Han population in an attempt to provide a genetic and environmental background that was similar to the patient population. Baseline demographic, biochemical and angiographic features were recorded for each patient.

All subjects underwent blood collection from the cubital vein and laboratory testing. Aspirin (100 mg) and clopidogrel (600 mg) were administered to patients at the time of the coronary angiography. Aspirin (300 mg/daily) was administered for at least 3 days, followed subsequently by a daily maintenance dose of 100 mg. Clopidogrel (150 mg/daily) was administered for at least 12 months after the intervention. Unfractionated heparin (100 IU/kg) was administered during the procedure.

2.2 **Angiography and Collateral Grading**

Coronary angiography was performed using standard techniques. CTO was defined by the presence of a coronary artery causing complete interruption of antegrade flow in a major epicardial coronary artery or minimal contrast penetration through the lesion without distal vessel opacification (TIMI 0–1 flow). Because patients with acute coronary syndromes within 90 days of enrollment were excluded, the presence of a total occlusion on coronary angiography was assumed to be chronic. Retrograde collateral filling of the vessel distal to a CTO was assessed by experienced interventional cardiologists blinded to other clinical and imaging data, according to the lesion classification scheme of the American College of Cardiology/American Heart Association [11]. The diameter and angiographic flow of collateral vessels was semi-quantitatively assessed by the use of Rentrop scoring (score 0 = no visible filling of collaterals, score 1 = filling of side branches, score 2 = partial filling of the epicardial segment of the occluded vessel, score 3 = total filling of the epicardial segment). The presence of well-developed angiographic collaterals was defined as a Rentrop score of 2 or 3.

2.3 Measurements and outcomes

Standardized physical examination procedures were performed. Subjects were questioned regarding their personal demographic information and medication histories, then measured for height and weight. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m^2). All blood samples were collected and processed within 2 h. Total cholesterol, triglycerides, VEGF, bFGF, high-sensitivity C-reactive protein (hs-CRP), Interleukin (IL)-6 and other biochemical blood measurements were determined by standard laboratory procedures (Beckman coulter chemistry analyzer AU5800 series, Tokyo, Japan). All measurements were taken using blinded quality control specimens in the central laboratory. Each sample was placed in double wells. Flow cytometry analysis was used to assess CXCR4 expression in the peripheral blood cells. After red blood cell lysis, cells were labeled with the following antibodies: phycoerythrin (PE) conjugated CD34 (No. 12-0349; eBioscience), FITC-conjugated CD45 (No. 11-9459; eBioscience), and APC-conjugated CD184 (CXCR4, No. 17-9999; eBioscience) antibodies. Isotype-matched immunoglobulins were used as controls. All antibodies were used at a final concentration of 5 mg/ml. Cells were stained for 20 min at room temperature avoiding lights, then washed with PBS and suspended with binding buffer. CXCR4 expression was determined by a FACScan flow cytometer (Becton Dickinson, NJ, USA).

The main outcome of interest was coronary collateralization, which was defined as a Rentrop score of 0 or 1 (low coronary collateralization) or a Rentrop score of 2 or 3 (high coronary collateralization). CXCR4 expression was calculated using the following equation: [$CXCR4^+$ $CD34^+$ cells / $CD34^+$ cells] ×100%.

2.4 **Statistical analysis**

We first compared the data distribution of each covariate between the case and control groups, using a t test (normal distribution) or Kruskal–Wallis rank sum test (non-normal distribution) for continuous variables and χ^2 tests for categorical data (Table 1). Next, Univariate logistic regression (Table 2) and multivariate logistic regression models (Table 3) were used to estimate the ORs and 95% CIs to investigate the risk factors associated with coronary collateralization, then, we examined the independent effect of CXCR4 expression on coronary collateralization when adjusted for other variables (Table 4). Then, χ^2 tests were used to analyze the distribution of CXCR4 expression in patients with different Rentrop scores (Figure 1). We then explored the relationship between CXCR4 levels and coronary collateralization by smoothing plot, with an adjustment for potential confounders (Figure 2). We further applied a three-piecewise linear regression model to examine the threshold effect of CXCR4 expression on coronary collateralization according to the smoothing plot (Table 5). The threshold level of CXCR4 expression at which the relationship between coronary collateralization and CXCR4 expression began to change and became notable was determined using a trial method. The trial inflection point was moved along a pre-defined interval and detected the inflection point that gave the maximum model likelihood.

All data were double entered and then exported to tab-delimited text files. All analyses were performed with R (http://www.R-project.org) and EmpowerStats software (www.empowerstats.com, X&Y solutions, Inc., Boston, MA, USA).

3. Results

Between January 2011 and December 2012, 78 CTO patients with GCC (34 Rentrop score 2 and 44 Rentrop score 3) were enrolled in our study. The average age of these cases was 53.6 ± 10.3 year. Another 78 age-matched CTO patients with PCC (36 Rentrop score 0 and 42 Rentrop score 1) were randomly selected from the study cohort participants. The average age of controls was 53.8 ± 11.7 year. The baseline demographic and clinical characteristics and biochemical measurements for these 156 subjects are summarized in Table 1. There were more patients suffered from diabetes in the PCC group compared with the GCC ones. In addition, plasma levels of VEGF and bFGF were higher in the GCC group, while the hs-CRP, TC, WBC, glucose and creatinine levels were lower compared with the controls. Moreover, the CXCR4 expression level was significantly higher in the case group than the controls. Apart from these two factors, there was no noticeable difference in the basic characteristics between the two groups.

The univariate regression analysis showed that CXCR4 expression in peripheral blood CD34+ cells was significantly correlated with coronary collateralization as well as Rentrop score (Figure 1). In addition, diabetes mellitus, plasma level of WBC, glucose, creatinine, TC, VEGF, bFGF and hs-CRP might also be associated with coronary collateralization (Table 2).

In the multivariate logistic regression model for risk factors associated with coronary collateralization, after adjusted for potential confounding factors, CXCR4 expression, plasma level of VEGF, bFGF were positively associated with coronary collateralization; while plasma level of WBC, glucose, creatinine, TC, and hs-CRP were negatively with coronary collateralization (Table 3).

Further analysis of the independent effect of CXCR4 expression on coronary collateralization, after adjusted for other variables, CXCR4 expression was still positively associated with the coronary collateralization, meanwhile, after trisecting the CXCR4 expression levels, we found that coronary collateralization was obviously positively correlated with high CXCR4 expression levels (OR 0.520, 95% CI 0.389–0.650, $p < 0.001$), but slightly positively correlated with low CXCR4 expression levels (OR 0.167, 95% CI 0.061–0.274, $p = 0.002$) (Table 4), thus, we speculate that there may be threshold or saturation effects between CXCR4 expression and coronary collateralization.

After adjusting for possible factors related to coronary collateralization including age, gender, diabetes, WBC, glucose, creatinine, VEGF, bFGF, and hs-CRP, a nonlinear relationship between CXCR4 expression and coronary collateralization was observed (Figure 2). Coronary collateralization increased with CXCR4 level up to the turning point (30%) (OR 0.025, 95% CI 0.022–0.028; $p < 0.001$). When the CXCR4 level was less than 30% (OR 0.000, 95% CI -0.006–0.005; $p = 0.908$) or more than 75% (OR 0.000, 95% CI -0.008–0.007; $p = 0.974$), the level of CXCR4 was not associated with coronary collateralization (Table 5).

4. Discussion

Coronary collateral circulation is an important prognostic marker of ischemic heart disease [12]. To the best of our knowledge, the present study is the first clinical study to demonstrate that an increase of CXCR4 levels in peripheral blood $CD34^+$ cells is positively associated with well-developed coronary collaterals in patients with CTO.

Human coronary arteries are not functionally end arteries, but are instead, interconnected by a rich network of collateral vessels [13]. It is estimated that approximately one fourth of individuals have functional collateral vessels able to reduce or prevent myocardial ischemia induced by brief abrupt reduction of antegrade flow [14]. One previous study showed that the presence of well-developed collateral vessels was inversely correlated with the severity of transmural injury and was also associated with a lower frequency of abnormal ECG with Q waves [1]. Other studies also documented a reduction in major adverse cardiac events and improved survival in patients with well-developed coronary collaterals [15, 16]. Thus, the existence of a collateral circulation supplying the myocardium distal to severe stenosis or total occlusion may be essential for CTO treatment.

It is well known that collateral growth in patients with CAD is highly heterogeneous and influenced by a number of factors. Diabetes mellitus has been confirmed to affect coronary collateralization in several studies. As expected, in the present study, data also show a negative correlation between diabetes mellitus, as well as plasma level of glucose and coronary collateralization, which is consistent with the previous study [17].

Substantial preclinical data have implicated growth factors as critical mediators of collateral formation in animal models of hind limb ischemia [18, 19]. Some previous studies showed a

positive association between plasma growth factors and the presence of collaterals, while another showed that there was no significant difference of plasma VEGF, PDGF, or bFGF levels between patients with and without coronary collaterals [20]. In this study, we found that VEGF and bFGF levels were positively correlated with the presence of collaterals. Immune factors might also play an important role in coronary collateralization. Serum levels of cytokines such as hs-CRP, TNF-α, and IL-6 were reported to inhibit the key components of angiogenesis, particularly EPC differentiation, survival and function [21, 22]. According to our findings, the hs-CRP level was negatively correlated with Rentrop scores in CTO patients, and may be a risk factor for collateral development, while no difference was found in IL-6 concentration between cases and controls. We also found the plasma level of WBC may also be a risk factor for collateral development. Renal function may also influence the collateral formation. In this study, the plasma level of creatinine was significantly higher in the patients with PCC than the ones with GCC.

The chemokine receptor CXCR4 is essential for the migration and homing of hematopoietic stem cells and circulating EPC, which help to enhance neovascularization after ischemia [23, 24]. Previous studies have shown that dual stem cell therapy after myocardial infarction acts specifically by enhanced homing via the SDF-1/CXCR4 axis [25], and Tanshinone IIA increases the recruitment of bone marrow mesenchymal stem cells to the infarct region by up-regulating the SDF-1/CXCR4 axis in a myocardial ischemia model [26]. Thus, these observations support the notion that CXCR4 may be either a protective factor for collateral growth in patients with CTO. In the current study, a significant difference was found in CXCR4 expression in peripheral blood CD34+ cells between patients with good and poor collateralization. Both unadjusted and adjusted data showed a significantly positive correlation between CXCR4 levels and coronary

collateralization. Interestingly, when adjusting for these possible factors related to coronary collateralization, a nonlinear relationship was observed between CXCR4 expression and coronary collateralization. Threshold and saturation effects were then found at the turning point when CXCR4 levels reached 30% and 75%, respectively. Coronary collateralization increased when the CXCR4 level was between 30% and 75%, while when the CXCR4 level was less than 30% or more than 75%, the level of CXCR4 was not associated with coronary collateralization. We speculated this phenomenon may be attributed to the minimal receptor concentration needed for SDF-1 / CXCR4 signaling pathway activation, and the maximum binding capacity of SDF-1 and CXCR4. This will be further studied in the future.

Study limitations

One of the main limitations of this study is the small number of cases, which made it unfeasible to perform subgroup or individual analyses. Secondly, not all inflammatory and angiogenic factors have yet been described, thus many other inflammatory and angiogenic factors may have a role in collateral development but it is not feasible to study all of these factors. Thirdly, we evaluated the presence and extent of coronary collateralization by Rentrop scoring system instead of collateral flow index since collateral flow index is an invasive procedure. However, angiographic assessment of coronary collaterals is easy to incorporate into routine clinical practice. Finally, our study had a case-control design and not a cohort design, which is supposed to be a stronger method. However, our cases and controls were selected from a well-defined cohort, reducing the possibility of selection bias and information on treatment use and comorbidities, making differential misclassification of the exposure less likely.

Conclusions

In conclusion, the present study is the first to demonstrate an association between increased CXCR4 levels in peripheral blood CD34+ cells and induced coronary collaterals in patients with CTO. Peripheral blood CD34+ cell CXCR4 expression was increased in patients with good coronary collaterals. These findings may pave the way for other studies investigating the potential therapeutic benefit of CXCR4 in patients with CTO who are not candidates for percutaneous or surgical revascularization.

Conflicts of interest

All authors declared no conflicts of interest.

Acknowledgement

This work was supported by National Natural Science Foundation of China (NO. 81300117) and China Postdoctoral Science Foundation (2013M532060).

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Figure legends

Figure 1 Correlation between CXCR4 expression in peripheral blood CD34+ cells and Rentrop scores of the study population. CXCR4 levels in patients with Rentrop scores of 0 and 1 were not significantly different, but CXCR4 levels in patients with Rentrop scores of 2 and 3 were markedly different from those with Rentrop scores of 0 and 1.

Figure 2 Relationship between CXCR4 expression level and coronary collateralization following CTO. A nonlinear relationship between the CXCR4 expression level and coronary collateralization was observed after adjusting for age, gender, diabetes, WBC, glucose, creatinine, VEGF, bFGF, and hs-CRP.

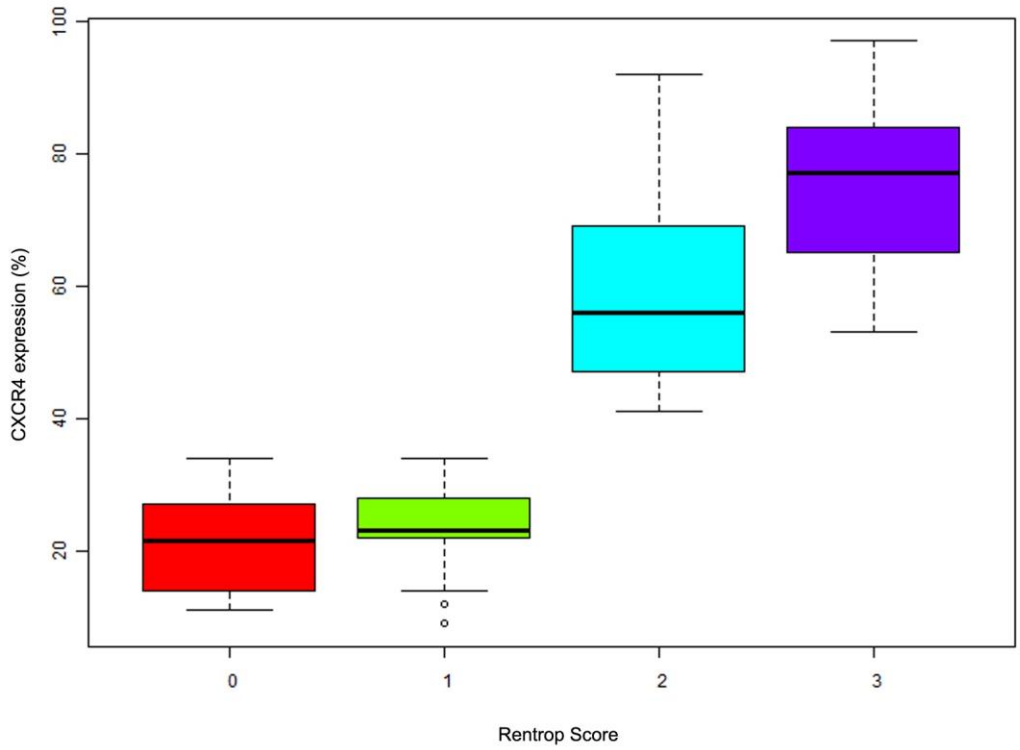

Fig. 1

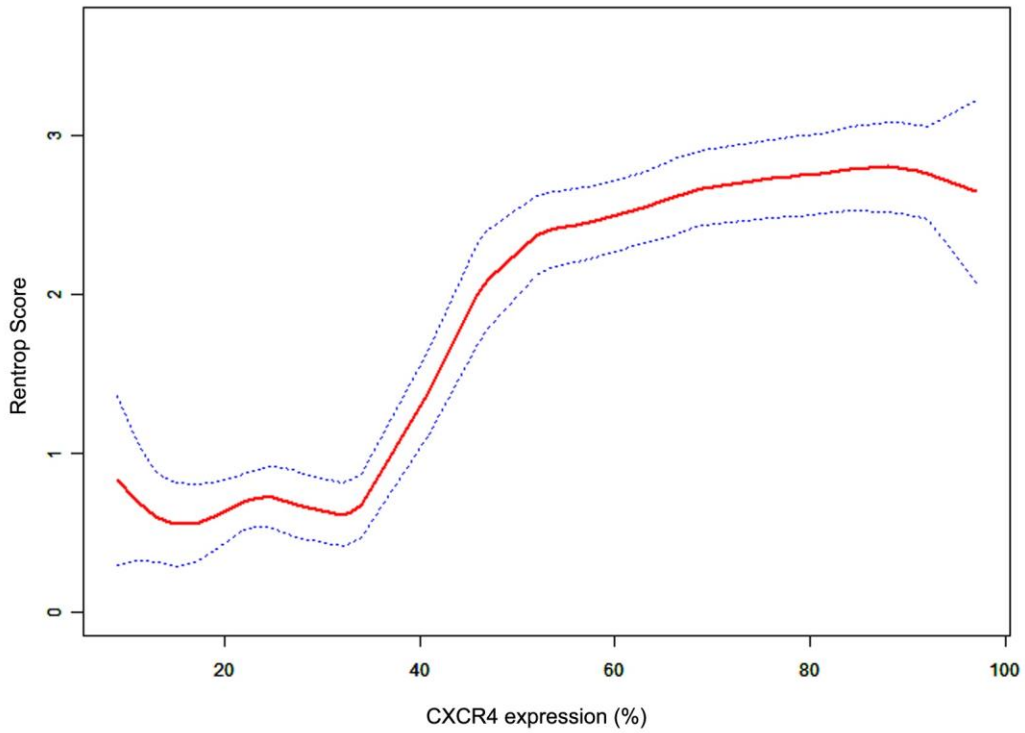

Fig. 2

Table 1 Demographic and clinical characteristics of the cases included in the study (N=156)

Variable	Poor CC (n=78)	Good CC (n=78)	P-value
Age (year)	53.8 ± 11.7	53.6 ± 10.3	0.926
Men	62 (79.5)	58 (74.4)	0.447
Family history of CTO	26 (33.3)	34 (43.6)	0.188
Smoke	48 (61.5)	50 (64.1)	0.740
Diabetes mellitus	52 (66.7)	30 (38.5)	<0.001
Hyperlipidemia	40 (51.3)	34 (43.6)	0.336
Hypertension	36 (46.2)	40 (51.3)	0.522
BMI (kg/m^2)	25.6 ± 4.4	25.7 ± 4.3	0.820
EF (%)	47.4 ± 11.7	49.9 ± 8.6	0.132
Platelet count ($\times10^3/mm^3$)	233.9 ± 57.2	228.3 ± 86.0	0.630
WBC ($\times10^3/mm^3$)	9.6 ± 1.4	8.5 ± 1.2	<0.001
Glucose (mg/dl)	138.8 ± 50.2	102.4 ± 30.7	<0.001
Creatinine (mg/dl)	1.0 ± 0.2	0.8 ± 0.2	<0.001
TC (mmol/l)	4.3 ± 1.7	3.7 ± 1.5	0.021
TG (mmol/l)	2.4 ± 0.8	2.4 ± 1.2	0.967
LDL (mmol/l)	2.6 ± 0.9	2.7 ± 1.0	0.477
HDL (mmol/l)	0.9 ± 0.3	0.9 ± 0.3	0.723
UA (μmol/l)	334.5 ± 93.7	329.2 ± 111.5	0.748
ApoA (mg/dl)	114.5 ± 26.6	113.3 ± 26.6	0.787
ApoB (mg/dl)	94.0 ± 23.9	102.0 ± 26.6	0.050
VEGF (pg/ml)	248.4 ± 40.2	288.8 ± 67.4	<0.001
bFGF (pg/ml)	19.2 ± 3.9	35.9 ± 13.4	<0.001
hs-CRP (pg/ml)	7.3 ± 3.1	2.9 ± 1.3	<0.001
IL-6 (pg/ml)	168.9 ± 29.4	176.3 ± 30.7	0.128
CXCR4 (%)	23.2 ± 7.2	68.4 ± 15.3	<0.001
Drugs			
Aspirin	43 (55.1)	38 (48.7)	0.423
ACEI	27 (34.6)	33 (42.3)	0.323
Beta blockers	48 (61.5)	51 (65.4)	0.230
Statin	44 (56.4)	41 (52.6)	0.630

CC, coronary collateralization; CTO, chronic total coronary occlusion; BMI, body mass index; EF, ejection fractions; WBC, white blood cell; TC, total cholesterol; TG, triglycerides; LDL, low density lipoprotein; HDL, high density lipoprotein; UA, uric acid; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; hs-CRP, high-sensitivity C-reactive protein; IL, Interleukin; CXCR4, C-X-C chemokine receptor type 4; ACEI, angiotensin converting enzyme inhibitors. Variables are expressed as n (%) or mean±SD.

Table 2 Effects of risk factors on coronary collateralization by univariate analysis (N=156)

Variable	Total	Odd ratio (95% CI)	P-value
Age	53.7 (10.3)	0.000 (-0.008, 0.007)	0.926
Men	120 (76.9)	-0.072 (-0.259, 0.115)	0.450
Family history of CTO	60 (38.5)	0.108 (-0.053, 0.270)	0.190
Smoke	98 (62.8)	0.027 (-0.136, 0.191)	0.742
Diabetes mellitus	82 (52.6)	-0.283 (-0.435, -0.131)	<0.001
Hyperlipidemia	74 (47.4)	-0.077 (-0.235, 0.081)	0.339
Hypertension	76 (48.7)	0.051 (-0.106, 0.209)	0.525
BMI	25.6 (4.3)	0.002 (-0.016, 0.020)	0.820
EF	48.7 (10.3)	-0.006 (-0.014, -0.002)	0.132
Platelet count	231.1 (72.8)	0.000 (-0.001, 0.001)	0.630
WBC	9.0 (1.4)	-0.132 (-0.184, -0.081)	<0.001
Glucose	120.6 (45.3)	-0.004 (-0.006, -0.003)	<0.001
Creatinine	0.9 (0.2)	-1.070 (-1.403, -0.736)	<0.001
TC	4.0 (1.6)	-0.058 (-0.106, -0.009)	0.021
TG	2.4 (1.0)	-0.002 (-0.080, 0.077)	0.967
LDL	2.6 (1.0)	0.030 (-0.053, 0.113)	0.477
HDL	0.9 (0.3)	0.056 (-0.253, 0.365)	0.723
UA	331.8 (102.7)	0.000 (-0.001, 0.001)	0.748
ApoA	113.9 (26.5)	0.000 (-0.003, 0.003)	0.787
ApoB	98.0 (25.5)	0.003 (0.000, 0.006)	0.050
VEGF	268.6 (58.9)	0.003 (0.002, 0.004)	<0.001
bFGF	27.5 (12.9)	0.025 (0.020, 0.030)	<0.001
hs-CRP	5.1 (3.2)	-0.106 (-0.124, -0.088)	<0.001
IL-6	172.6 (30.2)	0.002 (-0.001, 0.005)	0.128
CXCR4	45.8 (25.6)	0.017 (0.016, 0.019)	<0.001

CTO, chronic total coronary occlusion; BMI, body mass index; EF, ejection fractions; WBC, white blood cell; TC, total cholesterol; TG, triglycerides; LDL, low density lipoprotein; HDL, high density lipoprotein; UA, uric acid; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; hs-CRP, high-sensitivity C-reactive protein; IL, Interleukin; CXCR4, C-X-C chemokine receptor type 4. Variables are expressed as n (%) or mean (SD).

Table 3 Multivariate logistic regression model for risk factors associated with coronary collateralization (N=156)

Exposure	Model I		Model II		Model III	
	Odd ratio (95% CI)	p-value	Odd ratio (95% CI)	p-value	Odd ratio (95% CI)	p-value
CXCR4	0.017 (0.016, 0.019)	<0.001	0.018 (0.016, 0.019)	<0.001	0.018 (0.017, 0.020)	<0.001
TC	-0.058 (-0.106, -0.009)	0.021	-0.069 (-0.120, -0.018)	0.009	-0.062 (-0.111, -0.013)	0.015
VEGF	0.003 (0.002, 0.004)	<0.001	0.003 (0.002, 0.005)	<0.001	0.003 (0.002, 0.005)	<0.001
bFGF	0.025 (0.020, 0.030)	<0.001	0.025 (0.020, 0.030)	<0.001	0.024 (0.019, 0.028)	<0.001
hs-CRP	-0.106 (-0.124, -0.088)	<0.001	-0.107 (-0.125, -0.089)	<0.001	-0.103 (-0.122, -0.083)	<0.001
WBC	-0.132 (-0.184, -0.081)	<0.001	-0.137 (-0.190, -0.085)	<0.001	-0.125 (-0.176, -0.075)	<0.001
Glucose	-0.004 (-0.006, -0.003)	<0.001	-0.005 (-0.006, -0.003)	<0.001	-0.004 (-0.006, -0.002)	<0.001
Creatinine	-1.070 (-1.403, -0.736)	<0.001	-1.068 (-1.405, -0.731)	<0.001	-1.044 (-1.365, -0.722)	<0.001

CXCR4, C-X-C chemokine receptor type 4; TC, total cholesterol; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell.

Model I adjust for: None

Model II adjust for: gender; age

Model III adjust for: gender; age; diabetes.

Table 4 Independent effect of CXCR4 expression on coronary collateralization (N=156)

Exposure	Model I		Model II		Model III	
	Odd ratio (95% CI)	p-value	Odd ratio (95% CI)	p-value	Odd ratio (95% CI)	p-value
CXCR4	0.018 (0.017, 0.020)	<0.001	0.018 (0.016, 0.020)	<0.001	0.012 (0.010, 0.014)	<0.001
Tertile						
Tertile 1	Reference		Reference		Reference	
Tertile 2	0.498 (0.384, 0.613)	<0.001	0.444 (0.333, 0.554)	<0.001	0.167 (0.061, 0.274)	0.002
Tertile 3	1.011 (0.888, 1.134)	<0.001	0.867 (0.733, 1.001)	<0.001	0.520 (0.389, 0.650)	<0.001

CXCR4, C-X-C chemokine receptor type 4.

Model I adjust for: gender; age; diabetes

Model II adjust for: gender; age; diabetes; TC; WBC; glucose; creatinine

Model III adjust for: gender; age; diabetes; TC; WBC; glucose; creatinine; hs-CRP; bFGF; VEGF.

Table 5 Threshold effect analysis of CXCR4 expression on coronary collateralization (N=156)

Inflection point of CXCR4 expression (%)	Odd ratio (95% CI)	p-value
＜30	0.000 (-0.006, 0.005)	0.908
30-75	0.025 (0.022, 0.028)	<0.001
≥75	0.000 (-0.008, 0.007)	0.974

CXCR4, C-X-C chemokine receptor type 4.

The 30-75% threshold for the CXCR4 expression existed for protective effects of coronary collateralization.

Adjusted: age, gender, diabetes, VEGF, bFGF, hs-CRP. WBC, glucose and creatinine.