CXCL9: a biomarker for the coronary slow flow phenomenon in patients with coronary artery disease

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Abstract

Background: Atherosclerosis is a chronic inflammatory disease. The pathology underlying the disease consists of accumulation of the extracellular matrix, lipid and inflammatory cells. Coronary Slow Flow Phenomenon (CSFP) is closely related to inflammatory responses, while chemokines play an important role in the progression of atherosclerosis. However, the relationship between chemokines and CSFP is unclear. In this study, our aims were to evaluate the association between CXC Chemokines 9 (CXCL9) levels and CSFP in patients with coronary artery disease. Methods: We studied 46 patients diagnosed with CSFP and classed them as the CSFP group. 50 patients with normal coronary angiography (CAG) were randomly selected as the no-CSFP group in our study. The mean TIMI frame count was used to measure coronary blood flow velocity. The clinical and biochemical index, including serum levels of IL1, IL-6, IL-10, CXCL9, CD40L and interferon-γ (IFN-γ), were analyzed in all subjects. Results: The serum levels of IL-1, IL-6, IL-10, CXCL9, CD40L, IFN-γ and CXCL9 in the CSFP group were significantly higher than those in the no-CSFP group, with the differences being statistically significant ($p<0.001$). Furthermore, Pearson's correlation analysis reflected a significant positive correlation ($r=0.171$, $p=0.01$) in CXCL9 levels. Multivariate logistic regression analysis showed that CXCL9 are important risk factors for CSFP ($\beta=1.795$, $P=0.000$). Subsequent ROC curve analyses indicated that the serum CXCL9 levels demonstrated a high diagnostic value in differentiating patients with CSFP from that of normal controls (Area Under the Curve = 0.758) and the serum CXCL9 level of 131.915 mg/L was a predictor of CSFP, with a sensitivity of 54.3% and a specificity of 96.0%. Conclusions: Our findings are indicative of the potential clinical implications of CXCL9 in the occurrence and development of CSFP.

Background

Coronary Slow Flow Phenomenon (CSFP) is a phenomenon in which the main coronary artery consists of obstructive lesions, resulting delayed filling of distal contrast media in patients with chest tightness or chest pain [1]. Currently, the mechanism behind CSFP has not been fully elucidated. Current literature on CSFP propose abnormal microvascular regulation, [2] endothelial function injury and inflammatory response [3–5], oxidative stress and atherosclerosis of coronary artery [6–8], mean platelet volume or abnormal platelet function and imbalance of vasoactive substances [9–13] underlying CSFP pathology. However, there is general agreement that CSFP is closely related to inflammatory response.

The pathologic T cell-driven inflammatory responses play a pivotal role in the progression of atherosclerosis [14,15], with such T cell-related inflammatory responses involving interactions between cytokines, adhesion molecules and chemokines [16,17]. Research has shown that T-helper (Th) 1 cells and the interferon-γ secreted by pathologic T cells exhibit important functions in the pathogenesis of atherosclerosis [18,19]. For example, Th1-associated chemokines, such as the Monokines Induced by Interferon-*γ* (MIG, also called CXCL9) and Interferon-*γ*-Induced protein 10 (IP–10) are induced by interferon-*γ* and elicit their chemotactic functions by interacting with the CXC chemokine receptor type 3 (CXCR3). Furthermore, expression of interferon-*γ* and interferon-*γ*-inducible CXCR3 chemokines was

found to be increased in patients with coronary artery disease [20,21]. It should be noted that serum CXCL9 levels are independently associated with the carotid IMT and the coronary artery calcium score [22,23]. Our previous studies have shown that CXCL9 is an important risk factor for the occurrence and severity of coronary heart disease [24]. However, there are few reports investigating whether CSFP are related to chemokines. In order to further elucidate the role of chemokines in CSFP, we analyzed the correlation between CXCL9 and CSFP by using methods targeting the study of the pathogenesis of CSFP. Such elucidation might provide new molecular targets for coronary artery disease.

Methods

1. Subjects Between November 2016 and October 2018, subjects were selected based on the premise that they had symptoms of chest tightness, shortness of breath or chest pain, were clinically diagnosed with coronary artery atherosclerotic heart disease and had undergone a coronary angiography (CAG). The CAG provided information concerning the absence of coronary artery stenosis or stenosis less than 40%. 46 patients diagnosed with CSFP via the corrected TIMI blood flow frame method were selected as the CSFP group. During the same period, 50 patients with normal CAG were randomly selected as the normal coronary blood flow group (no-CSFP group).

Patients were excluded on the basis of the following criteria: patients with coronary artery dilatation; deformity and spasm; intracoronary thrombosis; heart valvular disease; cardiomyopathy; acute and chronic cardiopulmonary insufficiency; connective tissue disease; severe infectious disease; hypertensive heart disease; hepatorenal insufficiency and metabolic disease; hyperthyroidism heart disease; a patient with a history of disease; patients who took statins, glucocorticoids and other drugs within the last 2 months; and patients who were contraindicated with antiplatelet and/or anticoagulant therapy.

Approval or this study was gained from the ethics committee. Informed consent was obtained and signed by patients, following the principle of voluntariness and harmlessness all throughout the duration of the study. After obtaining the consent of patients and their families, the researchers signed the informed consent for the study, and explained the purpose and significance of the study to the patients and their families in detail.

2. General information and Biomarker Measurements

2.1 General Information Medical data on the patients was obtained, including smoking, hypertension, diabetes and medication history. General information on all patients including gender, age, Body Mass Index (BMI, BMI = body weight/Hight^2 (kg/m^2)), Waist-to-Hip Ratio (WHR), blood pressure was also collected. Blood pressure was measured and categorised according to the Chinese Guidelines for the Prevention and Treatment of Hypertension (Version 2010). The mean values were measured in triplicate and the interval between measurements was approximately 5 minutes.

2.2 Laboratory Biomarkers Blood samples were collected from all eligible patients prior to treatment. Patients fasted for 12 hours before blood samples (5ml) were collected the following morning for

laboratory analysis. Blood lipid analysis (TG, TC, LDL, HDL), fasting blood glucose (Glu) and hematuria were carried out using BECKMAN automatic biochemical analyzer CXCL–9 kits (USA). Acid (UA), highly sensitive C reactive protein (hs-CRP) and homocysteine (Hcy) content were measured.

2.3 Level Detection of Chemokines On the day of admission, venous blood (5 mL) was taken from all subjects using a disposable pyrogen-free and endotoxin-free test tube. EDTA was the recommended anticoagulant. The samples were then centrifuged at a speed of 3000 rpm for 15 minutes, and the serum separated temporally. Samples were frozen at –80℃ for future testing. ELISA KIT was employed by following the standard operation procedure. Finally, the coordinate points of each standard were connected by a smooth line, with the concentration of the standard substance set as abscissa and the OD value as ordinate. Hence, the concentration of a sample was found using the standard curve, via the OD value of the specimen.

3. Assessment of Coronary Blood Flow

3.1 Coronary Angiography With informed consent, all the selected subjects completed a CAG during hospitalization. Nitrate drugs were discontinued for 24 hours prior to the CAG. The CAG was performed through the right radial artery or femoral artery using Judkins method. Two experienced cardiologists performed the CAG.

3.2 Definition of CSFP and TFC CSFP refers to the absence of significant coronary artery lesions (normal or stenosis < 40%) during the CAG and absence of abnormal left ventricular contractile function as well as the exclusion of coronary artery spasm or gas thrombosis, coronary angioplasty or stent implantation, cardiomyopathy, valvular heart disease, connective tissue disease and thyroid function. The CAG demonstrates that following hyperactivity, patients with chest tightness or chest pain do not experience significant obstructive lesions in the main coronary artery, and instead, a phenomenon of delayed filling of distal angiographic agents is observed. The thrombolysis in myocardial infarction(TIMI) frame count method was used to record the number of image frames, observe the blood flow index and quantitatively analyze the results of CAG. The number of TIMI blood flow frames is the number of image frames from the beginning of filling the coronary artery with contrast medium to the distal end of the coronary artery during the CAG.

3.3 Evaluation of CSFP In this study, the mean TIMI frame count was used to measure coronary blood flow velocity. The coronary blood flow of patients was allocated to the CSFP group when the velocity of each coronary artery was greater than the two standard deviations of the published normal coronary blood flow velocity [25]. The no-CSFP group was considered within the two standard deviations of normal blood flow velocity. In order to ensure the accuracy and consistency of blood flow assessment, two researchers evaluated and recorded the number of coronary artery blood frames according to the above criteria on the basis of CAG images.

4. Statistical Analysis The collected data was sorted and analyzed by software SPSS10.0. The resulting data were expressed using the mean±standard deviation, data counts by % and ^{2} test with p<0.05, with

the difference being of statistical significance. A logistic regression method was used for correlation analysis. Receiver Operating Characteristic (ROC) curve analysis were performed to assess the diagnostic value of CXCL9 in the diagnosis of CSFP. The larger the area of the ROC curve, the higher the diagnostic value.

Results

1. General information and biomarkers between the two groups According to the CAG images and the definition of CSFP a total of 96 patients were enrolled in our study. There were 46 patients in the CSFP group (mean coronary flow velocity > 27 frames) and 50 patients in the no-CSFP group (mean coronary flow velocity < 27 frames). Statistical analysis showed that gender, age, BMI, WHR, DBP and LVEF were statistically similar between the two groups. There was no significant difference in SBP, smoking habits and prevalence of diabetes mellitus ($p<0.05$). Statistical analysis of biomarkers in the CSFP group and no-CSFP group indicated that the levels of LDL-c, hs-CRP and Hcy in the CSFP group were significantly higher compared to the no-CSFP group ($p<0.05$). However, the HDL-c levels were significantly lower in the no-CSFP group ($p<0.05$). The levels of BNP in both groups were within the normal range (<100 pg/ml), with no statistically significant differences ($p>0.05$), as shown in Table–1.
2. Correlation analysis of risk factors for CSFP Pearson's correlation analysis of risk factors affecting CSFP, such as smoking, Hcy, hs-CRP and CXCL9, showed that there was no correlation of CSFP with either blood pressure level or BMI. In contrast, smoking, serum Hcy, hs-CRP and CXCL9 levels had a significant positive correlation ($r = 0.171$, $p = 0.01$), while HDL levels were of a negative correlation with CSFP (mean TIMI frame count), as illustrated in Table–2.
3. Chemokine levels between the two groups Statistical analysis of cytokines showed that the serum levels of IL–1, IL–6, IL–10, CD40L, IFN-γ and CXCL9 in the CSFP group were significantly higher than those in the no-CSFP group respectively. These differences were statistically significant ($p<0.05$), as shown in Figure–1 and Figure–2. The statistically significant positive association was observed between the serum levels of IL–1, IL–6, IL–10 and the mean TIMI frame count and the serum CXCL9 level, as shown in Table–3 and Tanle–4. A statistically significant positive association was observed between the mean TIMI frame count and the serum CXCL9 level ($r = 0.5469$, $p<0.001$, Figure –3).
4. Logistic regression analysis of influencing factors of CSFP Multivariate logistic regression analysis was performed with CSFP as strain (1 = CSFP, 0 = no-CSFP). Age, sex, history of hypertension, smoking history, mean systolic blood pressure, BMI, serological indicators (FPG, DHL, LDL, hs-CRP, Hcy, IL–1, IL–6, IL–10) and chemokine CXCL9 were taken as independent variables. The results showed that smoking, hs-CRP, Hcy and CXCL9 were important risk factors for CSFP ($\beta = 1.795$, $P = 0.000$), as shown in Table–5.
5. Receiver operating characteristic curve analyses in subjects with CSFP and controls To further explore the applicability of serum CXCL9 levels as a potential diagnostic biomarker of CSFP, subsequent ROC curve analyses were performed. The results indicated that the serum CXCL9 levels

demonstrated a high diagnostic value in differentiating patients with CSFP from that of normal controls (Area under the curve = 0.758, Figure–4). The ROC curve revealed that the serum CXCL9 level of 131.915 mg/L was a predictor of CSFP, with a sensitivity of 54.3% and a specificity of 96.0%.

Discussion

According to clinical reports, about 7% of patients suspected of coronary artery disease had slow blood flow after a CAG [26], and about 25% of patients experiencing chest pain also had slow flow [27]. The mechanism of CSFP is complex and the causes numerous. Research suggested that CSFP is the early stage of coronary artery atherosclerosis, which is related to coronary microcirculation dysfunction and endothelial dysfunction [28–29]. When vascular endothelium dependent relaxation function is impaired, various stimulators, such as endothelin, homocysteine, prostaglandin H2 and peroxyanion phase equilibrium, do not produce vasodilation and instead, induce vasoconstriction. This causes abnormal vascular endothelial metabolism, injury of coronary microvascular endothelial function and endothelial dysfunction, eventually leading to CSFP [30–32].

One study showed that the level of serum inflammatory factors in patients with chronic coronary flow was significantly higher, suggesting that an inflammatory mechanism may be involved in the occurrence and development of CSFP [33]. However, the exact mechanisms underlying CSFP caused by early atherosclerosis as well as the role of CXCL9 chemokines are still unclear. Our previous studies have shown that CXCL9 is an important risk factor for the occurrence and severity of coronary heart disease [24]. In our work, we found that there was no significant difference between the CSFP group and the no-CSFP group in gender, age, BMI, WHR, blood pressure and LVEF. On the other hand, there was a significant difference with smoking habits and the prevalence of diabetes between the two groups (p<0.05), consistent with previous clinical studies. This also confirms that such factors may be critical risk factors for atherosclerosis and may also be involved in the occurrence of CSFP. Further analysis indicated that the serum levels of hsCRP, Hcy, IL–1, IL–6, IL–10, CD40Land IFN-γ in the CSFP group were significantly higher than those in the no-CSFP group, suggesting that these factors may play an important role in the occurrence and development of CSFP.

In order to find new molecular targets for CSFP, we compared the serum levels of CXCL9 between the two groups. Remarkably, we found that serum CXCL9 levels in CSFP patients were significantly higher than those in the no-CSFP group (p<0.01). Furthermore, the statistically significant positive association was observed between the serum levels of IL–1, IL–6, IL–10 and the mean TIMI frame count and the serum CXCL9 level. So we speculate that CXCL9 may play an important role in the occurrence and development of CSFP through IL–1, IL–6 and IL–10. The multivariate logistic regression analysis revealed that serum CXCL9 levels may be an important risk factor for CSFP. To further explore the applicability of serum CXCL9 levels as a potential diagnostic biomarker of CSFP, ROC curve analyses were performed. The results indicated that the serum CXCL9 levels has high diagnostic value to CSFP (AUC = 0.758), and the

ROC curve revealed that the serum CXCL9 level of 131.915 mg/L was a predictor of CSFP, with a sensitivity of 54.3% and a specificity of 96.0%.

Although the role of CXCL9 in CSFP has not been reported previously, some scholars have pointed out that CXCL8 may be an important factor in CSFP. The expression of CXCL8 in CSFP patients is significant [23,30]. This study offers perspective on the role of chemokines in CSFP. We found that CXCL9 is positively correlated with CSFP, hence providing a new molecular target for the pathogenesis of CSFP. Based on our results, we speculate that the up-regulation of inflammatory cytokines (such as IL−1, IL−6 and IL−10) in CSFP patients may be regulated by CXCL9. Our analysis suggests that the role of CXCL9 in CSFP, which supports the existing literature that inflammation promotes slow coronary flow, is a complex interaction between CXCL9 and the interleukin family. For example, previous studies have shown that IL−18 can promote the expression of CXCL9, while the latter affects vascular endothelial function through vascular factors such as vascular endothelial growth factor [34−35]. In addition, CXCL9 can activate more interleukins and exacerbate the prognosis of cardiovascular diseases. CXCL9 may also be involved in more complex inflammation signaling pathways in the progress of coronary CSFP, such as inflammation corpuscles. In particular, CXCL9 may further induce interleukin production and obstruct coronary blood flow via recruitment or activation of NLRP1 and NLRP3 inflammatory corpuscles. However, such hypotheses must be explored in animal and basic histology studies.

In brief, in our study we explored the association between the CXCL9 and CSFP in patients with CAD. It speculate that CXCL9 may play an important role in the occurrence and development of CSFP. Although our research has unveiled a potential correlation between CXCL9 and the occurrence of CSFP, it should be further lager sample numbers study to clarify the role and mechanism of CXCL9 in CSFP in depth.

Conclusions

The role of CXCL9 in CSFP has not been reported previously, so in our study we explored the association between the CXCL9 and CSFP in patients with CAD. According to research and analysis, we found that the serum CXCL9 levels in CSFP patients was significantly higher than in the no-CSFP group. It speculate that CXCL9 may play an important role in the occurrence and development of CSFP. Statistical analysis also showed that CSFP was found to be positively correlated with serum CXCL9 levels and the serum CXCL9 levels may be an important risk factor for CSFP. It should be further lager sample numbers study to clarify the role and mechanism of CXCL9 in CSFP in depth.

Abbreviations

CAD: Coronary artery disease; AS: Athevosclerosis; CSFP: Coronary slow flow Phenomenon; CXCL−9: CXC Chemokines−9; MIG: Monokine Induced by Gamma interferon;TFC: TIMI Frame Count; ELISA: Enzyme Linked Immunosorbent Assay; LVEF: Left Ventricular Ejection Fraction; BMI: Body Mass Index; WHR: Waist-hip Ratio; SBP: Systolic Blood Pressure; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; FPG: Fasting Plasma Glucose; BNP: Brain Natriuretic Peptide B; IFN-*γ:* Interferon-γ.

Declarations

- Ethics approval and consent to participate: All experimental procedures were conducted in strict accordance with the guidelines for the care of Anhui Medical University and were approved by the Ethics Committee. All participants were informed and agreed to participate in the study, and the written informed consent was obtained from all participants.

- Consent to publish: Not Applicable

- Availability of data and materials: The data that support the findings of this study are available from Hefei Health Committee of Anhui Province but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Hefei Health Committee of Anhui Province.

- Competing interests: There are no any financial or non-financial competing interest and no any potential conflicts of interest in our manuscript.

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- Authors' Contributions:Liang yf designed this study, provided the diagnosis of the CSFP /no-CSFP patients and analyzed the data, wrote the manuscript. Lin xh gave the guidance throughout the research process and participated in the design of the study. Xu yy performed level detection of chemokines and participated in the analysis of the data, wrote the manuscript. Wang cm and Zhou q preparation of the blood samples for detecting, and collected the general information and biomarker measurements. All the experiments were performed in performed the laboratory preparations, analyzed the data and wrote the manuscript.

References

1. Tambe AA, Demany MA, Zimmerman HA, Mascarenhas E. Angina pectoris and slow flow velocity of dye in coronary arteries—a new angiographic finding. [J]. Am Heart J, 1972, 84:66–71.
2. Tarantino G, Caputi A. JNKs, insulin resistance and inflammation: A possible link between NAFLD and coronary artery disease[J]. World J Gastroenterol.2011,17: 3785–3794.
3. Barutcu I, Sezgin AT, Sezgin N, Gullu H, Esen AM, Topal E, et al. Increased high sensitive CRP level and its significance in pathogenesis of slow coronary Flow[J]. Angiology, 2007, 58:401–407.
4. Liu R, Deng Y, Bi X, Liu Y, Xiong L, Chen L. Assessment of myocardial perfusion and systolic function in patients with coronary artery disease after coronary artery bypass surgery by myocardial contrast echocardiography and two-dimensional strain echocardiography[J]. J Huazhong Univ Sci Technolog Med Sci,2009,05:664–668.

5. Li JJ, Xu B, Li ZC, Qian J, Wei BQ. Is slow coronary flow associated with inflammation? [J]. Med Hypotheses, 2006, 66:504–508.
6. Pekdemir H, Polat G, Cin VG, Camsari A, Cicek D, Akkus MN, et al. Elevated plasma endothelin–1 levels in coronary sinus during rapid right atrial pacing in patients with slow coronary flow[J]. Int J Cardiol, 2004, 97:35–41.
7. Ruparelia N, Kharbanda RK. Role of coronary physiology in the contemporary management of coronary artery disease[J]. World J Clin Cases, 2015, 3:148–155.
8. Lubrano V Balzan S. Consolidated and emerging inflammatory markers in coronary artery disease[J]. World J Exp Med, 2015, 5:21–32.
9. Sen N, Basar N, Maden O, Ozcan F, Ozlu MF, Gungor O, et al. Increased mean platelet volume in patients with slow coronary flow[J]. Platelets, 2009, 20:23–28.
10. Gökçe M, Kaplan S, Tekelioğlu Y, Erdoğan T, Küçükosmanoğlu M. Platelet function disorder in patients with coronary slow flow[J]. Clin cardiol, 2005, 28:145–148.
11. Camsarl A, Pekdemir H, Cicek D, Polat G, Akkus MN, Döven O, et al. Endothelin–1 and Nitric Oxide Concentrations and Their Response to Exercise in Patients with Slow Coronary Flow[J]. Circ J, 2003, 67:1022–1028.
12. Sezgin N, Barutcu I, Sezgin AT, Gullu H, Turkmen M, Esen AM, et al. Plasma nitric oxide level and its role in slow coronary flow phenomenon. [J]. Int Heart J, 2005, 46:373–382.
13. Rim SJ, Leong-Poi H, Lindner JR, Wei K, Fisher NG, Kaul S. Decrease in coronary blood flow reserve during hyperlipidemia is secondary to an increase in blood viscosity[J]. Circulation, 2001, 104:2704–2709.
14. Zhou X, Nicoletti A, Elhage R, Hansson GK. Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice[J]. Atherosclerosis, 2000, 151:196–196.
15. Robertson AK, Hansson GK. T Cells in Atherogenesis For Better or For Worse? [J]. Arterioscler Thromb Vasc Biol, 2006, 26:2421–2432.
16. Libby P, Paul M, Ridker, Maseri A. Inflammation and atherosclerosis[J]. Circulation, 2002,105:1135–1143.
17. Zernecke A Weber C. Chemokines in Atherosclerosis Proceedings Resumed[J]. Arterioscler Thromb Vasc Biol, 2014; 34: 742–750.
18. Frostegård J, Ulfgren AK, Nyberg P, Hedin U, Swedenborg J, Andersson U, et al. Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage stimulating cytokines[J]. Atherosclerosis, 1999, 145:33–43.
19. Libby P, Lichtman AH, Hansson GK. Immune Effector Mechanisms Implicated in Atherosclerosis: From Mice to Humans[J]. Immunity, 2013, 38:1092–1104.
20. Fernandes JL, Mamoni RL, Orford JL, Garcia C, Selwyn AP, Coelho OR, et al. Increased Th1 activity in patients with coronary artery disease[J]. Cytokine, 2004, 26:131–137.

21. Niki T, Soeki T, Yamaguchi K, Taketani Y, Yagi S, Iwase T, et al. Elevated concentration of interferon-Inducible protein of 10 kD (IP–10) is associated with coronary atherosclerosis[J]. Int Heart J 2015; 56: 269–272.
22. Yu HT, Lee J, Shin EC, Park S. Significant Association between Serum Monokine Induced by Gamma Interferon and Carotid Intima Media Thickness[J]. J Atheroscler Thromb, 2015, 22:816–822.
23. Yu HT, Oh J, Chang HJ, Lee SH, Shin EC, Park S. Serum monokine induced by gamma interferon as a novel biomarker for coronary artery calcification in humans[J]. Coron Artery Dis, 2015, 26:317–321.
24. Liang YF, Yang C, Zhou Q, Pan WB, Zhong WS, Ding RY, et al. Serum Monokine Induced by Gamma Interferon Is Associated with Severity of Coronary Artery Disease[J]. Int Heart J, 2017, 58:24–29.
25. Gibson CM, Cannon CP, Daley WL, Dodge JT, Alexander B, Marble SJ, et al. TIMI frame count: a quantitative method of assessing coronary artery flow[J]. Circulation, 1996,93:879–888.
26. Mangieri E, Macchiarelli G, Ciavolella M, Barillà F, Avella A, Martinotti A, et al. Slow coronary flow: Clinical and histopathological features in patients with otherwise normal epicardial coronary arteries[J]. Cathet Cardiovasc Diagn, 1996, 37:375–381.
27. Goel PK, Gupta SK, Agarwal A, Kapoor A. Slow coronary flow: a distinct angiographic subgroup in syndrome X[J]. Angiology, 2001, 52:507–514.
28. Erdogan D, Caliskan M, Gullu H, Sezgin AT, Yildirir A, Muderrisoglu H. Coronary flow reserve is impaired in patients with slow coronary flow[J]. Atheroselerosis, 2007,191:168–174.
29. Beltrame JF, Limaye SB, Wuttke RD, Horowitz JD. Coronary hemodynamic and metabolic studies of the coronary slow flow phenomenon[J]. Am Heart J, 2003, 146:84–90.
30. Tanriverdi H, Evrengul H, Enli Y, Kuru O, Seleci D, Tanriverdi S, et al. Effect of homocysteine induced oxidative stress on endothelial function in coronary slow flow[J]. Cardiology, 2007, 107:313–320.
31. Riza Erbay A, Turhan H, Yasar AS, Ayaz S, Sahin O, Senen K, et al. Elevated level of plasma homocysteine in patients with slow coronary flow[J]. Int J Cardiol, 2005, 102:419–423.
32. Barutcu I, Sezgin AT, Sezgin N, Gullu H, Esen AM, Topal E, et al. Elevated plasma homocysteine level in slow coronary flow[J]. Int J Cardiol, 2005, 101:143–145.
33. Li JJ, Qin XW, Li ZC, Zeng HS, Gao Z, Xu B, et al. Increased plasma C-reactive protein and interleukin–6 concentrations in patients with slow coronary flow[J]. Clinica Chim Acta,2007, 385:43–47.
34. Kanda N, Shimizu T, Tada Y, Watanabe S. IL–18 enhances IFN-γ-induced production of CXCL9, CXCL10, and CXCL11 in human keratinocytes[J]. Eur J Immunol, 2007, 37:338–350.
35. Huang B, Wang W, Li Q, Wang Z, Yan B, Zhang Z, et al. Osteoblasts secrete CXCL9 to regulate angiogenesis in bone[J]. Nat Commun, 2016, 14:13885.

Tables

Table-1. Clinical and biochemical parameters including biochemical parameters and echocardiographic estimation of LVEF and TFC in the two groups.

	CSFP Group n=46	no-CSFP group N=50	P-value
male, n (%)	31 (67.40)	34(68.00)	0.472
Age (y)	63± 10	66 ± 15	0.281
BMI (kg/m2)	22.76±5.56	25.75±5.32	0.092
WHR	0.90± 0.15	0.93±0.11	0.157
SBP (mmHg)	156.2± 21.3	142.5±22.6	0.030
DBP (mmHg)	86± 15	85±11	0.104
Hypertension,n (%)	14 (30.43)	16 (32.00)	0.083
Diabetes, n (%)	12 (26.09)	14 (28.00)	0.041
smoking, n (%)	17 (36.96)	13 (26.00)	0.019
LVEF (%)	52 ±8 %	53 ±9 %	0.270
TFC□frame□	31.2 ± 2.6	22.7±2.5	0.000
TC (mmol/L)	4.51 ± 1.04	4.21 ± 0.93	0.139
HDL-c (mmol/L)	0.95 ± 0.11	1.09 ± 0.10	0.000
LDL-c (mmol/L)	2.44 ± 0.62	2.17 ± 0.58	0.028
FPG (mmol/L)	5.57 ± 0.97	5.46 ± 1.13	0.608
Hcy(umol /L)	21.57 ± 7.76	15.14 ± 6.33	0.000
hsCRP (umol /L)	6.74 ± 1.93	3.05 ± 2.21	0.000
BNP(pg/ml)	89.17 ± 6.53	87.82 ± 6.15	0.298

Table-2. Correlation between CSFP and clinical variables including serum inflammatory cytokines levels.

	Correlation coefficient	P
BMI	0.110	0.07
SBP	0.072	0.34
Hypertension	0.083	0.25
Smorking	0.153	0.05
HDL	- 0.146	0.05
hsCRP	0.132	0.05
Hcy	0.183	0.01
CXCL9	0.171	0.01

Table-3. Correlation between CSFP and the serum IL-1/IL-6/IL-10 levels

	Correlation coefficient	P
IL-1	0.106	0.021
IL-6	0.141	0.020
IL-10	0.119	0.001

Table-4. Correlation between the serum CXCL9 and the serum IL-1/IL-6/IL-10 levels

	Correlation coefficient	P
IL-1	0.122	0.037
IL-6	0.176	0.028
IL-10	0.134	0.015

Table 5. Logistic regression analysis of the factors regarding CSFP.

Factor	regression coefficient	Standard error	Wald	P	OR	95%CI
Smorking	1.107	0.503	7.098	0.004	1.010	1.000-10.010
hsCRP	1.190	0.593	4.083	0.038	3.264	1.030-10.437
Hcy	1.315	0.584	5.059	0.024	3.721	1.179-11.582
CXCL9	1.795	0.447	16.612	0.000	6.228	2.597-14.896
IL-1	0.182	0.479	6.778	0.003	4.134	0.710-1.207
IL-6	0.165	0.365	5.237	0.011	4.026	1.016-1.983
IL-10	0.154	0.418	9.651	0.002	5.978	0.793-2.056

Figures

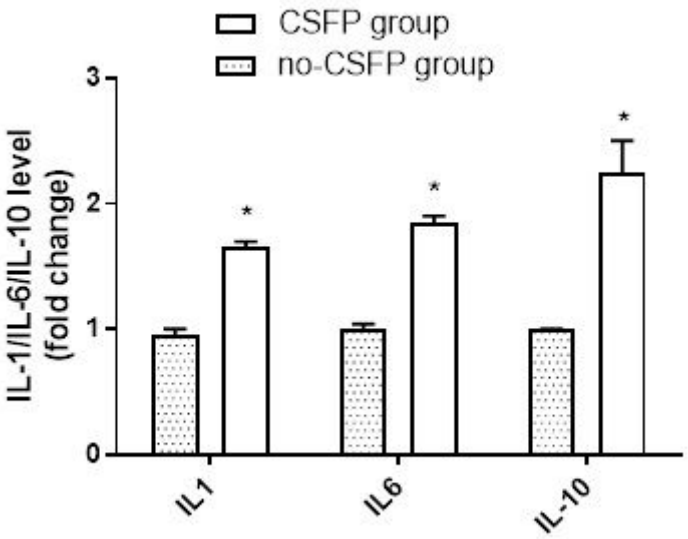

Figure 1

Serum level of IL-1, IL-6 and IL-10 in the CSFP group and no-CSFP group (*, p<0.001).

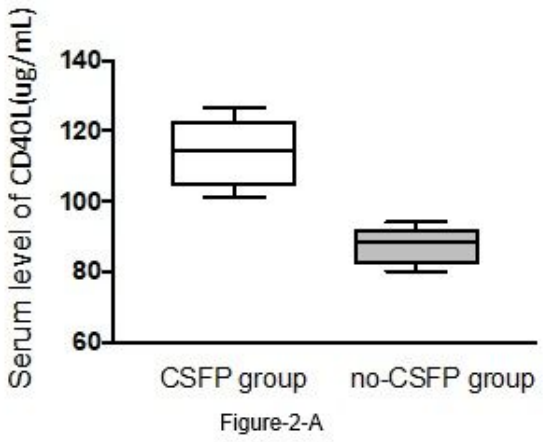

Figure-2-A

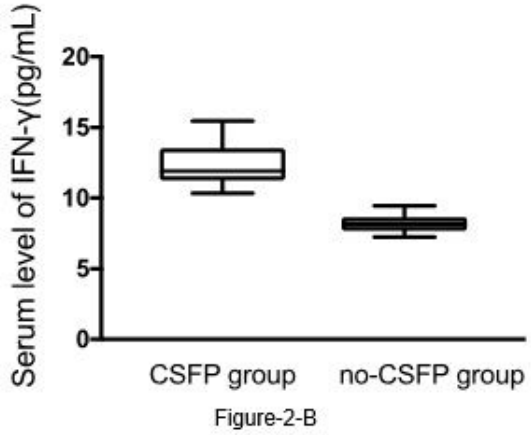

Figure-2-B

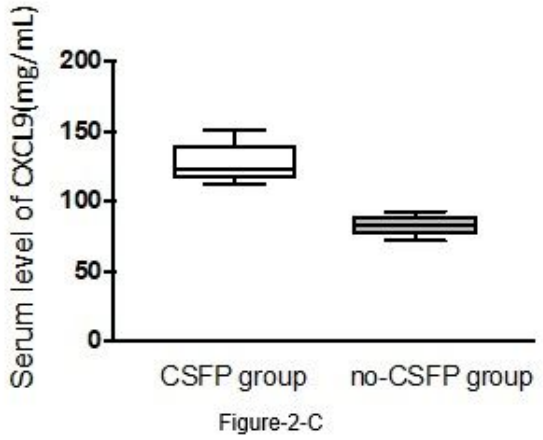

Figure-2-C

Figure 2

Serum level of CD40L (Figure 1-A), IFN-γ (Figure 1-B) and CXCL9 (Figure 1-C) in the CSFP group and no-CSFP group ($p<0.001$).

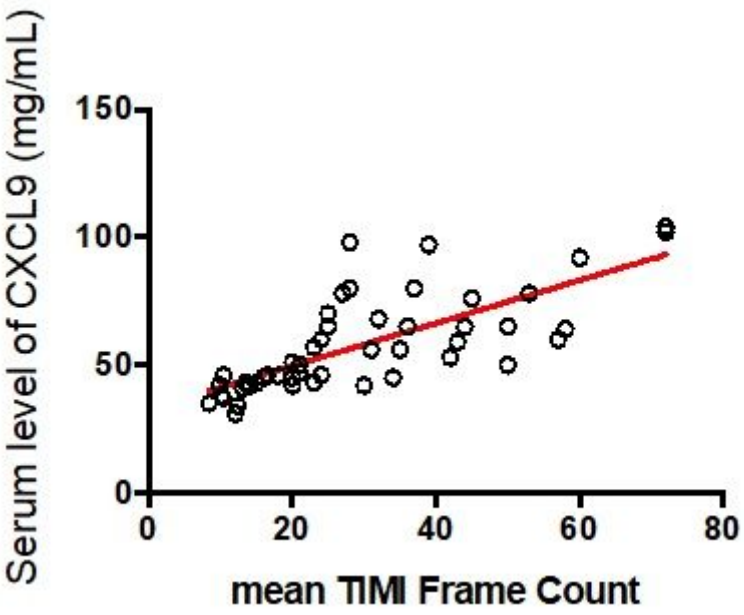

Figure 3

Correlation between mean TFC and serum levels of CXCL9 (r = 0.5469, p<0.001).

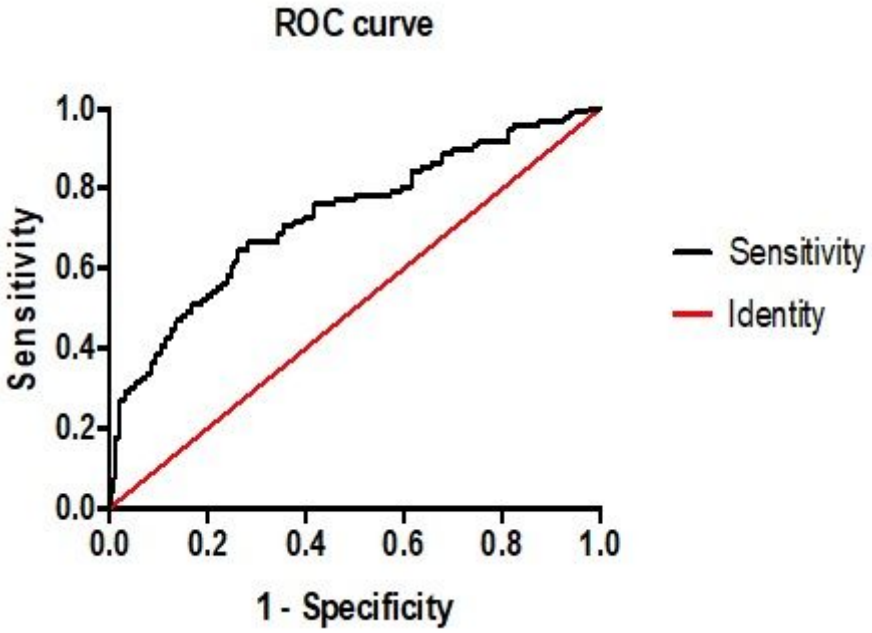

Figure 4

The ROC curve of CXCL9 used to dierentiate the CSFP cases from the control individuals. AUC (95% CI) was 0.758 (0.661 - 0.856).