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

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Research article

Keywords: Chemokine; CXCL9; Coronary slow flow; Coronary artery disease

Posted Date: December 16th, 2019

DOI: https://doi.org/10.21203/rs.2.13298/v2

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 s low f low p henomenon (CSFP) is closely related to inflammatory responses, while chemokines plays an important role in the progression of atherosclerosis. However, the relationship between chemokines and CSFP is still 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 classifyed 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 (TFC) 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 is an important risk factor 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%, respectively. 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 absence 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] underly CSFP pathology. However, there is a 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 using methods targeted at 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 principle 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. At the same time, 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; coronary deformity and spasm; intracoronary thrombosis; heart valvular disease; cardiomyopathy; acute or chronic heart failure or respiratory failure after hospitalization; connective tissue disease; severe infectious disease; hypertensive heart disease; hepatorenal insufficiency and metabolic disease; hyperthyroidism heart disease; a patients with a history of previous cardiac surgery or percutaneous coronary intervention; patients who took statins, glucocorticoids and other drugs, for example, antiplatelet and/or anticoagulant, nicorandil within the last 2 months.

Approval of 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 of 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 categorized 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 two 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 on the following morning for laboratory analysis. Blood lipid analysis (TG, TC, LDL and 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 (5mL) 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 test. 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 The coronary angiographies were performed using the standard Judkins technique. Nitrate drugs were discontinued for 24 hours prior to the coronary angiography either in the form of sublingual spray or intracoronary injection. Other agents, such as verapamil, and nicorandil, were not administrated. Obstructive coronary lesion refers to the existence of significant coronary artery lesions (compromising the luminal diameter by 50% or more) on CAG. A normal coronary angiogram was defined as one with the absence of any visible angiographic signs of atherosclerosis, thrombosis or spontaneous spasm. 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. All recordings were subsequently examined by two independent cardiologists in order to estimate the coronary blood flow according to the thrombolysis in myocardial infarction frame count (TFC) proposed by Gibson et al.[25] Images were acquired at 15 frames/s and thus all values were multiplied by 2. Left anterior descending artery was divided by a correction factor of 1.7 for its longer length. Any frame count exceeding 27 was considered to be abnormal and indicative of coronary slow flow based on the recommendations of Gibson et al. [25]

3.2 Evaluation of CSFP The TIMI frame counts (TFC) were calculated for each coronary vessel as described by the method of Gibson et al.[25] Threshold values obtained after correction of the cut-off values were 36.2±2.6 frames for the left anterior descending coronary artery, 22.2±4.1 frames for the left circumflex artery, and 20.4±3 frames for the right coronary artery. Patients in the present study who had cTFC values exceeding these thresholds by greater than 2 standard deviations (SD) for the particular vessel were recognized as having CSFP. Patients who had cTFC values not exceeding these thresholds were recognized as having normal coronary flow.

4. **Statistical Analysis**

The collected data was sorted and analyzed with software SPSS10.0. The resulting data were expressed using the mean±standard deviation, data counts by % and c^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 was 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 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 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 (β=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

Although there has been a great interest in identifying the underlying mechanisms of CSFP, the etiology and pathogenesis remain uncertain. Coronary microcirculation dysfunction and impaired endothelial functions seem to play an important role in the etiopathogenesis of CSFP [26-27]. Zengin et al.[28] found a significant positive correlation between Urotensin-II and CSFP, and demonstrated that Urotensin-II may be one of the underlying factors in the pathogenesis of CSFP. 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 [29-31]. One study found that the abnormalities in nail fold capillaries suggesting the presence of inflammation and anatomical changes were significantly higher in patients with CSFP [32].

Moreover, Soylu et al.[33] encountered significant elevations in the hematocrit level, and erythrocyte, eosinophil and basophil counts in the CSFP patients compared to those with normal coronary blood flow, despite the causative mechanisms are unclear, the results presented to the increases in hematocrit levels and in the eosinophil and basophil counts may have direct or indirect effects on the rate of coronary blood flow. Aksan et al. [34] found that the elevated neutrophil gelatinase-associated lipocalin levels might be a useful tool in predicting slow coronary flow phenomenon in patients. CSFP can leads to significant alterations in the myocardial deformation parameters of the left ventricle, specifically, circumferential deformation parameters are affected in CSFP patients [35].

One study showed that the level of serum inflammatory factors in patients with chronic coronary flow was significantly high, suggesting that an inflammatory mechanism may be involved in the occurrence and development of CSFP [36]. 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 this 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. However, there was a significant difference with smoking habits and the prevalence of diabetes between the two groups (p<0.05), which is 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, and CD40L and 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, 29]. 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 [37-38]. 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, we explored the association between the CXCL9 and CSFP in patients with CAD in the study. It indicates 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, there should be a larger sample coherents to clarify the role and mechanism of CXCL9 in CSFP.

Conclusions

The association between CXCL9 and CSFP in patients with CAD was first analyzed in this study. According to the results, we found that the serum CXCL9 levels in CSFP patients were significantly higher

than that in the no-CSFP group. It suggests that CXCL9 may play an important role in the occurrence and development of CSFP. Statistical analysis also showed that CSFP was positively correlated with serum CXCL9 levels and the serum CXCL9 levels may be an important risk factor for CSFP. However, more samples should be further studied to clarify the role and mechanism of CXCL9 in CSFP.

Limitations of study

This was an observational single centre study with a small sample size and need the multi-central study to further clarify the role and mechanism of CXCL9 in CSFP in depth. At the same time, our study didn't analyzed about the single or multiple coronary artery disease in study population, presence of multi-vessel CSFP may be affected of CXCL9 level.

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.

- Funding: This work was supported by The Key Medical Research Project of Hefei Health Committee of Anhui Province, No. Hwk2016zd009. The Health Committee of Anhui Province to grant financial support and to participate in the design of the study.

- Authors' Contributions:

YL designed this study, provided the diagnosis of the CSFP /no-CSFP patients and analyzed the data, wrote the manuscript; XL, gave the guidance throughout the research process and participated in the design of the study; YX, performed level detection of chemokines and participated in the analysis of the data, wrote the manuscript; CM and QZ, 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.

- Acknowledgements: Not applicable

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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

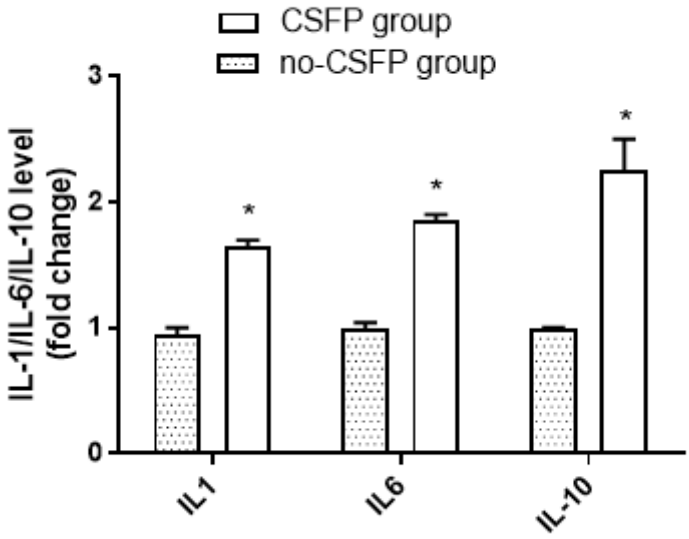

Figure 1

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

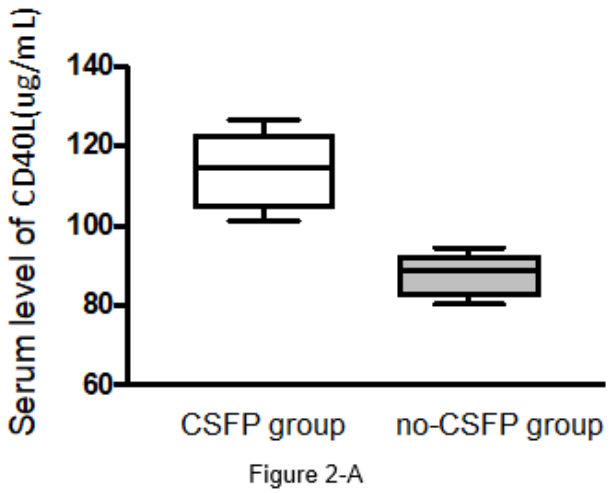

Figure 2-A

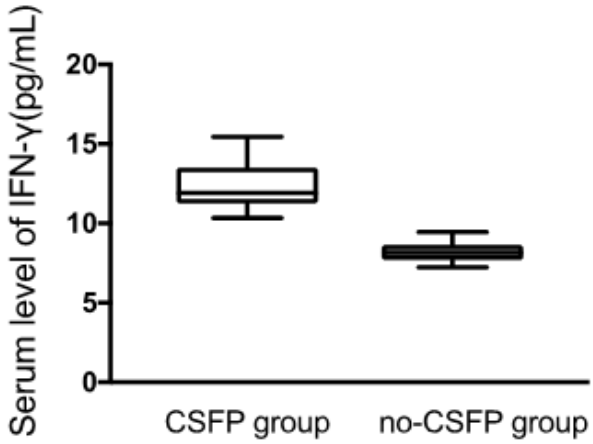

Figure 2-B

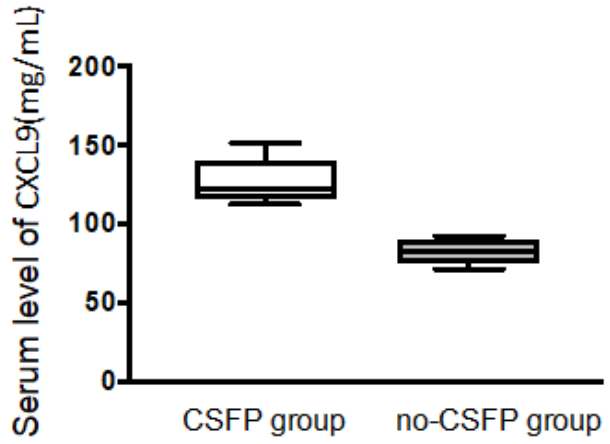

Figure 2-C

Figure 2

Serum level of CD40L (Figure 2-A), IFN-γ (Figure 2-B) and CXCL9 (Figure 2-C) in the CSFP group and no-CSFP group ($p<0.001$).

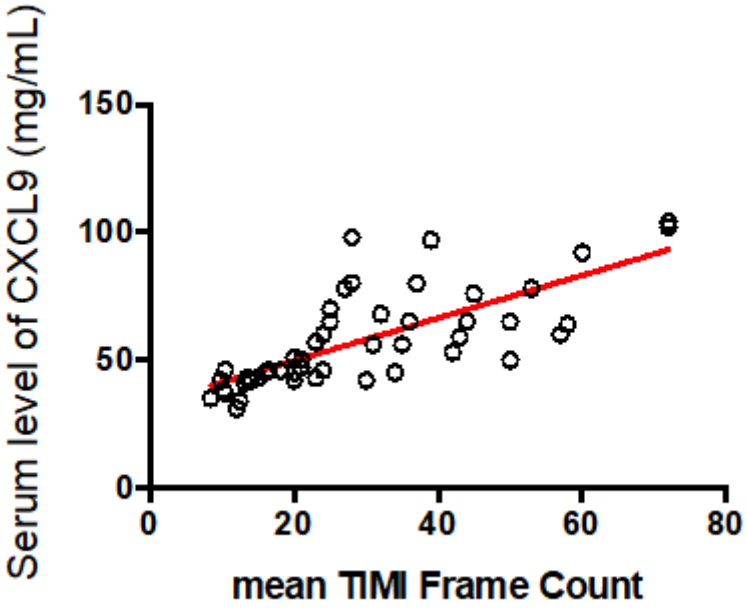

Figure 3

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

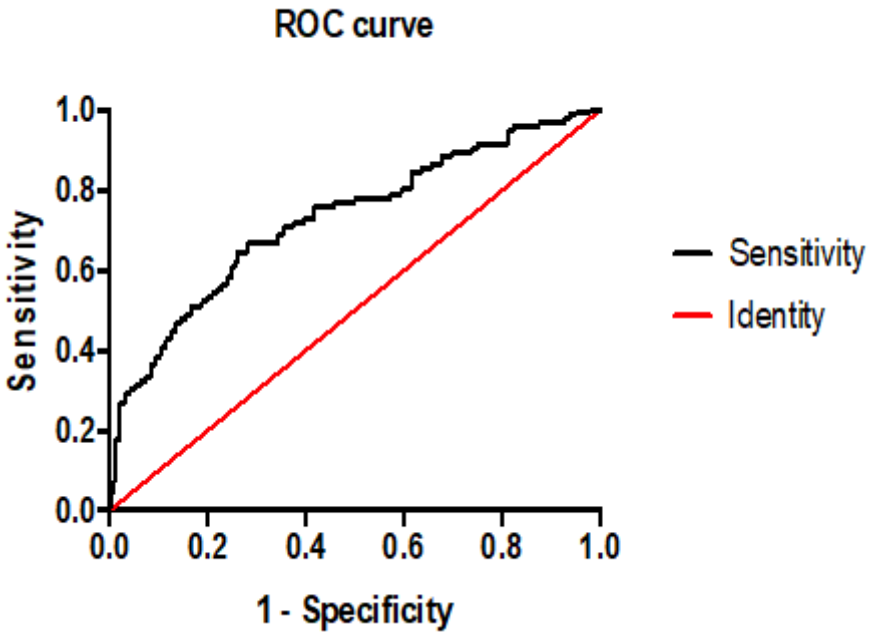

Figure 4

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).