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RESEARCH ARTICLE

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CXCL8, CXCL9, and CXCL10 serum levels increase in syphilitic patients with seroresistance

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Abstract

Background: Recently, the rise of syphilitic seroresistance brings great confusion to the clinical diagnosis and treatment of syphilis, and no clear diagnostic marker has been found to distinguish syphilitic seroresistance from other progression of syphilis. This study evaluated the serum chemokines levels of CCL2, CXCL8, CXCL9, and CXCL10 and its correlation with blood routine, coagulation, and biochemical indexes in seroresistant syphilitic patients.

Method: Serum levels of chemokines were quantitatively determined by Flow Cytometric Bead Array (CBA). The results expressed in pg/ml. Clinical parameters were detected and analyzed according to the clinical laboratory standards. A correlation analysis was subsequently performed.

Results: The seroresistant syphilitic patients increased significantly serum chemokines levels of CXCL8 (***$p < 0.001$), CXCL9 (***$p < 0.001$), and CXCL10 (**$p < 0.01$) when compared to noninfected individuals, but the CCL2 was not statistically significant, and serum CXCL8 shows a strong association with platelets ($r = 0.51$, **$p = 0.004$) and serum CXCL10 was significantly positively related to INR levels ($r = 0.49$, **$p = 0.007$).

Conclusion: Increasing serum abnormalities in CXCL8, CXCL9, and CXCL10 level combining with platelets of peripheral blood and plasmatic INR in syphilis patients may be helpful for the diagnosis of serofast state.

KEYWORDS
chemokines, CXCL10, CXCL8, CXCL9, syphilitic seroresistance

1 | INTRODUCTION

Syphilis infected by *Treponema pallidum*. As a major global public health problem, as many as 12 million new infections are diagnosed each year worldwide.[1] Parenteral penicillin is crucial to the cure of syphilis at all stages. Because of the difficulty of rapid culture of *T. pallidum* in vitro and the low sensitivity of PCR detection of patients' serum,[2–4] the evaluation of the efficacy of syphilis treatment depends almost entirely on serological testing. Non-treponemal serum antibody titers detected by tolulized red unheated serum test (TRUST) have been linked to disease activity. The efficacy of penicillin in the treatment of syphilis showed the following two results:

Xiaoyan Dong and Junwu Zhang made the same contribution to this article.

After treatment, the titer of non-Treponema decreased four times or the serum level reversed.[5,6] However, about 5%–41% syphilis patients who are not cured thoroughly after adequate anti-syphilis treatment by penicillin show consistently low non-treponemal serum antibody titers (<1:8) post-treatment and syphilis-specific antibody positive for a long time or even for a lifetime,[7] which have been regarded to as the serofast state.[8] About 35% of seroresistant syphilis patients with will redevelop into symptomatic syphilis.[9] It is worrisome that in recent years, syphilis seroresistance is increasing, which makes the treatment of syphilis faces new challenges.

Although antibiotics such as penicillin take a significant role in host resistance to pathogens, the complete cure of an infectious disease and the ability of the body to completely eliminate pathogens ultimately depends on the host's own immune function. Chemokines are a big class of cytokines, which are mainly secreted by monocytes, macrophages, and endothelial cells. They play a leading role in inducing directional migration of cells.[10] So far, approximately 50 kinds of human chemotactic factor and 20 kinds of receptors have been verified. According to the N-terminal cysteine residues of chemokines, they are divided into four subfamilies: C, CC, CXC, and CX3C chemokines.[11] In the past ten years, a growing body of evidence suggests that some inflammatory chemokines, such as monocyte chemoattractant monokine, interleukin-8 (CXCL8), and protein 1 (CCL2), which induced by transferon gama (CXCL9) and interferon gamma inductive protein 10 (CXCL10), take a leading role in the immune monitoring and defense of pathogens by effector cells when natural immunity and adaptive immunity occur.[12] They monitor the invasion of pathogens by inducing immune cells into lymph nodes as well as interacting with antigen-presenting cells and attract monocytes, neutrophils, and other effector cells at the part of infection. What's more, they can lead to tissue damage and inflammatory cells infiltrate, which make patients with chronic syphilis have a pro-inflammatory immune response.[13,14] Therefore, the serum levels of inflammatory chemokines in patients with serum drug resistance to syphilis were analyzed in this study and provide the basis for revealing the possible pathogenesis of serofast state in syphilis from the perspective of immune molecules.

2 | MATERIALS AND METHODS

2.1 | Patients and ethics statement

A total of 30 seroresistant syphilitic patients from Zhejiang People's Hospital were enrolled in experimental group of this study, 19 males and 11 females, aged between 25 and 86 (mean age, 64.23 ± 17.05 years). There were 3 outpatients (10%), 27 inpatients (90%), 4 single (13.3%), 26 married (86.7%), 16 permanent staff (53.3%), 3 temporary workers (10%), and 11 retirees (36.7%). 30 healthy blood donors from non-syphilitic patients served as normal controls in this study, who had undergone physical examinations in the same hospital, 11 males and 19 females, aged between 21 and 81 (mean age, 36.07 ± 14.70 years). All seroresistant syphilitic patients were serological Treponema Pallidum Particle Assays (TPPA), and serum TRUST titer were positive with 10 of trust (1:1), 12 of trust (1:2), and 8 of trust (1:4) accounted for 33.3%, 40%, and 27.7%, respectively. The remaining serum of patients with syphilis after treponemal and non-treponemal tests was collected and used in this study, in conformity with the ethical standards of the Declaration of Helsinki. This study was supported by the Ethics Committee of Zhejiang People's Hospital (approval No.2019KY311).

2.2 | Diagnostic and excluded criteria

The diagnosis of syphilitic patients with seroresistance was determined according to the Centers for Disease Control in Europe and America's recommendations based on medical history, clinical symptoms, physical examination, and laboratory findings.[5] Serofast state was defined that TPPA and TRUST was positive, and the trust titers did not decline by at least 4 times in the non-treponemal test compared to previous results after one year of continuous treatment by retrospectively analyzing and clinical follow-up, and neurosyphilis and other organic syphilis infections were excluded. Patients with hepatitis C virus (HCV), hepatitis B virus (HBV), human immunodeficiency virus (HIV), and other underlying acute or chronic infectious disease; history of systemic inflammatory, autoimmune disease; receiving anti-inflammatory medications; immunocompromised, or using of antibiotics or immunosuppressive medications during the preceding 6 months were excluded in all participants.

2.3 | Quantitative flow cytometry for Chemokines

All serum of seroresistant syphilitic patients and healthy controls were collected by sterile tubes containing separating glue with centrifuging at 900 *g* for 5 min and stored at −80℃ for subsequent use. The concentrations of serum chemokines were quantitatively measured by Cytometric Bead Array (CBA) Human Chemokine Kit(552990)comprising microbeads coupled with monoclonal antibodies (MoAb) against CXCL8, CCL2, CXCL9, CCL5, and CXCL10. The levels of the corresponding chemokines were acquired using a FACS Verse flow cytometer (Beckman Coulter, Inc.) according to the manufacturer's instructions (Beckman Coulter, Inc.) and analyzed by BD FCAP Array 3.0 software (Becton Dickinson and Company).

2.4 | Clinical parameters

The RBC, HCT, RDW-CV, PLT, HGB, WBC, LYM, NLR, PLR, and NEUT in peripheral blood were determined by an automated blood cell counter (XN-9000, Sysmex). The plasma levels of APTT, PT, INR, TT, fibrinogen, and D-Dimer were measured by a CS-5100 Hemostasis System (Sysmex Corporation). Routine

biochemical analyses including ALT, AST, TB, DB, TP, ALB, ALP, Urea, CR, UA, GLU, TG, TCH, LDLC, HDLC, CK, CKMB, α-HBDH, and LDH were measured by commercial kits using an automated chemistry analyzer (Chemistry Analyzer Au5821, Beckman Coulter, Inc.).

The anti-TP was qualitatively detected in double antigen sandwich method by using Addcare ELISA 600 automatic ELISA workstation (Addcare Biotech Inc.) and Diagnostic Kit for Antibody to Treponema Pallidum (ELISA) (Zhuhai Lizhu Inc.). Serological confirmation for syphilis was performed by the toluidine red unheated serum test (TRUST; Rongsheng Biotech) in combination with treponemal pallidum particle agglutination (SERODIA-TP.PA; FUJIREBIO Inc.).

2.5 | Bioinformatic analysis

Differentially expressed chemokine analysis of enriched pathways, gene ontology (GO), and diseases were performed by KOBAS 3.0 database (http://kobas.cbi.pku.edu.cn/kobas3/genelist/), [15] and the corrected p-value was used to evaluate the significance of those terms in the pathogenesis of serofast.

2.6 | Statistical analysis

Statistical analysis was performed by SPSS version 24.0 and GraphPad Prism 5.01 software. For analysis between groups, the Dunn's multiple comparison test was used. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ were considered statistically significant. The numeric variables were displayed as mean ± standard deviations (SD), and the categorical variables were analyzed by chi square test and displayed by frequency and percentage, while the data of the middle and four digit numbers were shown in the abnormal distribution. Paired t test was used for normal distribution data. Kruskal-Wallis test and Mann-Whitney U test were used for non-normal distribution variables.

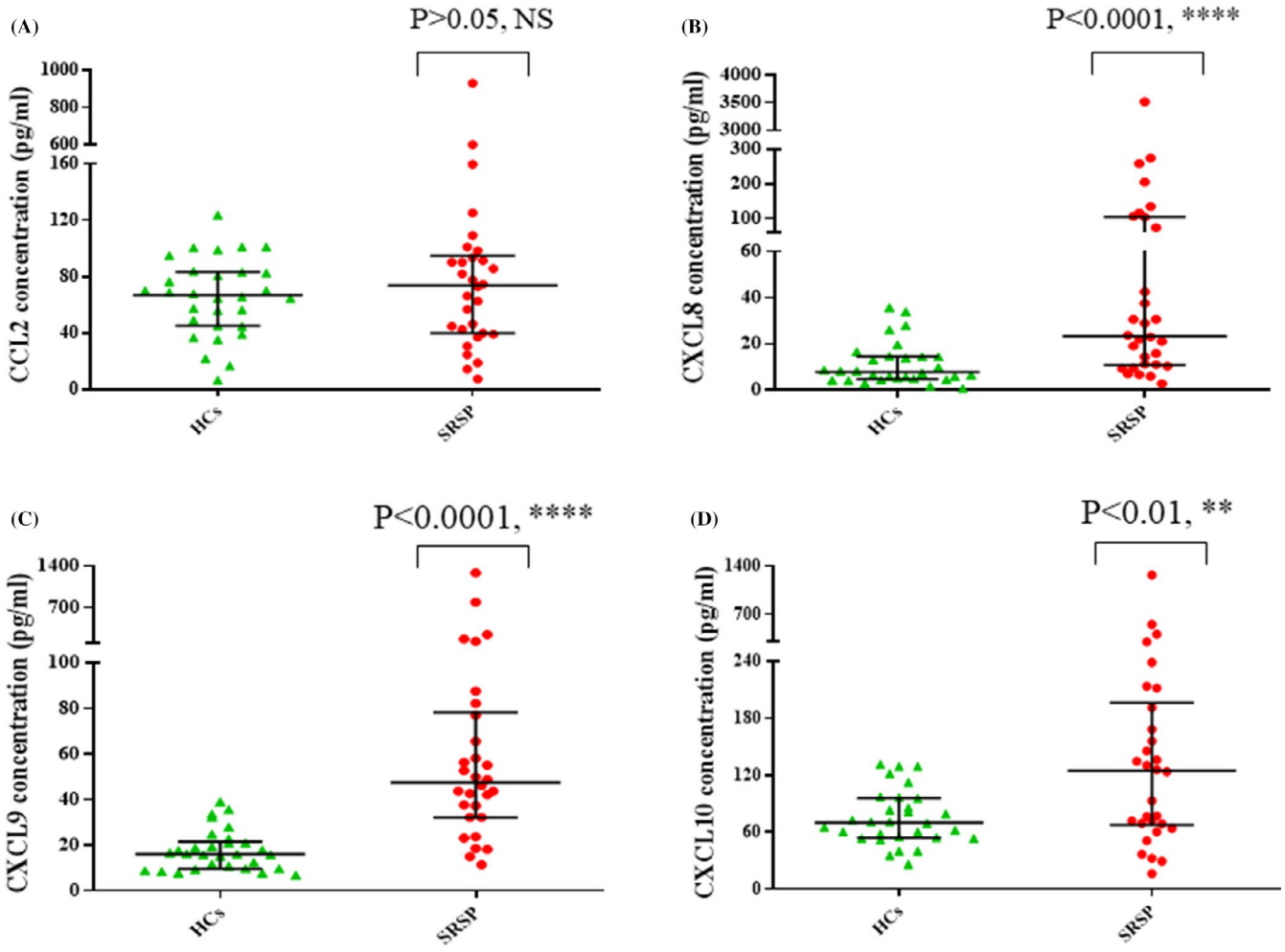

FIGURE 1 The serum concentrations of inflammatory chemokines CCL2 (A), CXCL8 (B), CXCL9 (C), and CXCL10 (D) from seroresistant syphilitic patients and healthy controls were quantitatively determined by CBA. Abbreviation: HCs, healthy controls; SRSP, seroresistant syphilitic patients; NS, not significant. Each data point represented an individual subject, error bars and median with interquartile range were displayed. $^{*}p < 0.05$ versus HCs

3 | RESULT

3.1 | CXCL8, CXCL9, and CXCL10 serum levels significant increase in syphilitic patients with seroresistant than non-syphilitic healthy individuals

The statistical significance of the multivariate analysis of the association between syphilitic seroresistance and chemokines is represented in Figure 1. The serum chemokine concentration (pg/ml) measured in seroresistant syphilitic patients group was 73.84 (39.76, 94.75) for CCL2, 23.34 (10.87, 105.12) for CXCL8, 47.51 (32.17, 78.39) for CXCL9, and 124.86 (67.66, 196.55) for CXCL10. The levels of CXCL8, CXCL9, and CXCL10 differed significantly (****$p = 0.0001$, ****$p = 0.0001$, and **$p = 0.008$, respectively), while the CCL2 was not statistical significance ($p = 0.600$, ns) in comparison with the non-syphilitic healthy controls with chemokine levels of 65.49 ± 27.55 for CCL2, 7.82 (4.85, 14.50) for CXCL8, 16.17 (9.66, 21.52) for CXCL9 and 70.03 (54.05, 95.88) for CXCL10. All the results are shown in Figure 1.

3.2 | Enriched pathways, GO, and diseases of CXCL8, CXCL9, and CXCL10 were visualized in bubble plot

A total of 109 terms including 73 GO, 3 diseases, and 33 KEGG pathway of the inflammatory chemokines was retrieved in the KOBAS 3.0 online database. Top 5 with the highest enrich ratio of each cluster are displayed in Figure 2. Terms of chemokine-mediated signaling pathway, CXCR chemokine receptor binding, and antimicrobial humoral immune response, which mediated by antimicrobial peptide, leukocyte chemotaxis, and chemokine activity, had the lowest corrected p-value and the highest enrich ratio in all retrieved data. All the results are shown in Figure 2.

3.3 | Differences in clinical characteristics of seroresistant syphilitic patients and non-syphilitic healthy individuals

Bivariate analysis between seroresistant syphilitic patients and non-syphilitic healthy controls above mentioned was demonstrated at Table 1. The neutrophil-to-lymphocyte ratio of peripheral blood and the serum concentrations of D-Dimer urea in seroresistant syphilitic patients were obvious rise in comparison with that in healthy controls (***$p < 0.001$), while the INR, TP, and ALB were significantly decreased (***$p < 0.001$). All the results are shown in Table 1.

3.4 | PLT and INR might be most sensitive for the increasing of CXCL8, CXCL9, and CXCL10 level in the rapid serological response of syphilis, and can be used as the predictive indicators of rapid serological response of syphilis.

Serum concentration of CCL2 was not correlated with all the clinical parameters we detected. Serum concentration of CXCL8 was

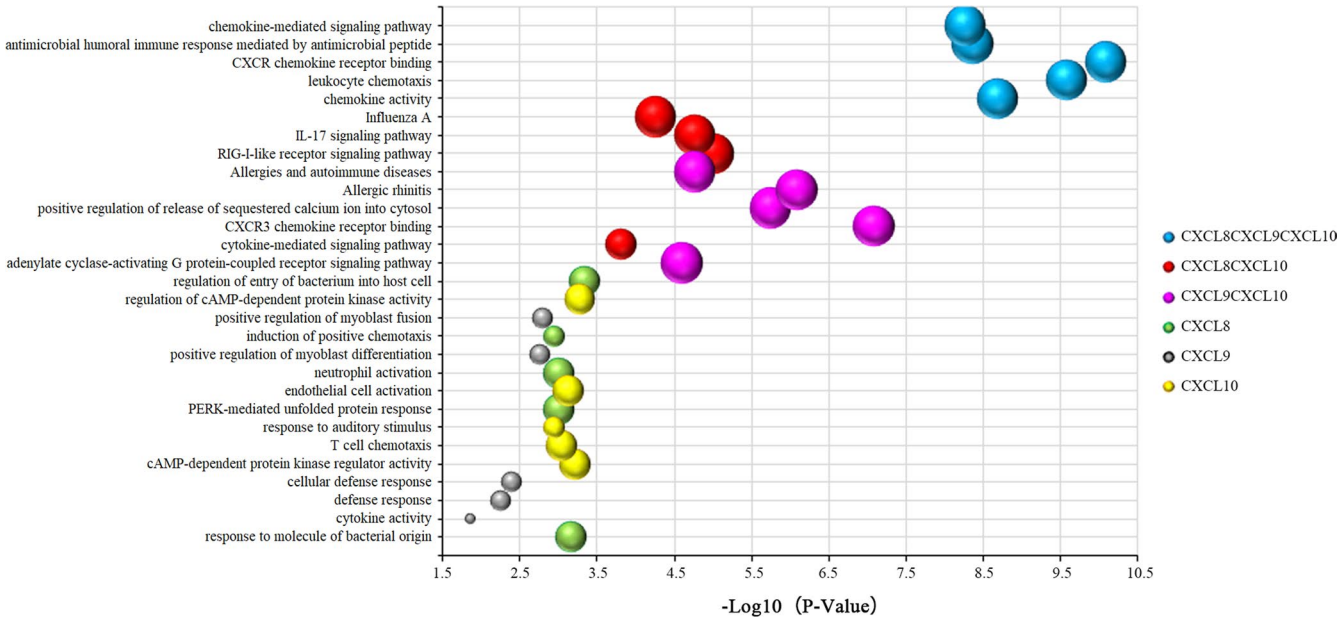

FIGURE 2 Enrichment result of KEGG pathways, GO, and diseases in the pathogenesis of serofast by bioinformatics analysis. Each bubble represents an enriched function, and the size of the bubble is set with six gradients according to the p-value, from small to large, representing the different significance levels: ns (p-value >= 0.05), * (0.01 <= p-value < 0.05), ** (0.001 <= p-value < 0.01), *** (0.0001 <= p-value < 0.001), **** (1e-10 <= p-value < 0.0001), ***** (p-value <1e-10). The color in the circular network represents different clusters. For each cluster, if there are more than 5 terms, top 5 with the highest enrich ratio will be displayed

significantly positively correlated with PLT levels ($r = 0.51$, $^{**}p = 0.004$) and was correlated with NEUT ($r = 0.38$, $^{*}p = 0.04$) in the peripheral blood of seroresistant syphilitic patients, and was negatively correlated with ALB ($r = -0.42$, $^{*}p = 0.024$) and HDLC in serum ($r = -0.39$, $^{*}p = 0.036$). Serum concentration of CXCL9 was positively correlated with urea ($r = 0.39$, $^{*}p = 0.033$), Cr ($r = 0.42$, $^{*}p = 0.022$), and LDLC ($r = 0.39$, $^{*}p = 0.049$) levels and was negatively correlated with WBC ($r = -0.42$, $^{*}p = 0.019$) and LYM in serum ($r = -0.44$, $^{*}p = 0.014$). Serum

TABLE 1 Comparison of hematological characteristics of the seroresistant syphilitic patients and healthy controls

Variables	Healthy controls (HCs)	Syphilitic patients with seroresistance	χ^2	*p* value
Blood routine				
RBC (10^{12}/L)	4.67 (4.42, 5.03)	4.35 (3.95, 4.79)	2.876	0.004**
HGB (g/L)	141 (134.75, 153.50)	132 (121.75, 142.50)	2.330	0.020*
HCT (L/L)	0.42 (0.40, 0.45)	0.39 (0.37, 0.44)	2.684	0.007**
RDW-CV (%)	12.65 (12.30, 13.10)	12.85 (12.40, 13.53)	1.215	0.224, NS
PLT (10^9 /L)	224 (211.00, 273.75)	219 (165.75, 246.25)	2.151	0.031*
WBC (10^9 /L)	6.50 (5.05, 7.380	6.10 (4.66, 7.18)	0.266	0.790, NS
NEUT (10^9 /L)	3.40 (3.05, 4.33)	4.05 (2.87, 4.78)	0.984	0.325, NS
LYM (10^9 /L)	2.15 (1.68, 2.53)	1.63 (1.18, 2.03)	3.101	0.002**
NLR	1.70 (1.40, 1.94)	2.43 (1.79, 3.63)	3.615	0.000***
PLR	118.37 (97.37, 133.51)	127.53 (88.82, 201.00)	1.161	0.246, NS
Coagulation function				
APTT (s)	27.80 (26.00, 29.73)	26.69 (25.50, 27.80)	1.967	0.049*
PT (s)	11.85 (11.28, 12.30)	11.15 (10.50, 11.600	2.998	0.003**
INR	1.10 (1.04, 1.13)	0.99 (0.95, 1.06)	3.938	0.000***
TT (s)	17.40 (16.70, 17.73)	17.95 (17.36, 18.73)	3.159	0.002**
FIB (g/L)	2.44 (2.23, 2.69)	2.83 (2.38, 3.77)	2.484	0.013*
D-Dimer	100 (80, 475)	480 (232.50, 1042.50)	3.841	0.000***
Blood chemistry				
ALT (U/L)	16.00 (10.75, 23.00)	17.00 (11.50, 34.00)	1.200	0.230, NS
AST (U/L)	20.00 (14.5, 32.25)	22.00 (17.75, 30.50)	0.814	0.416, NS
TP (g /L)	76.10 (72.73, 76.30)	68.20 (64.30, 74.05)	3.657	0.000***
ALB (g /L)	46.32 ± 2.50	38.86 ± 5.60	5.300	0.000***
ALP (U/L)	63.00 (55.00, 76.00)	86.50 (68.50, 116.25)	3.100	0.002**
TB (μmol /L)	14.58 ± 5.35	15.10 (8.60, 19.90)	0.026	0.979, NS
DB (μmol /L)	2.66 ± 1.07	2.60 (1.63, 4.55)	0.451	0.652, NS
Urea (mmol /L)	4.22 ± 1.12	5.69 (4.93, 6.75)	3.979	0.000***
Cr (μmol /L)	66.30 (62.10, 84.30)	77.90 (69.25, 89.10)	1.936	0.053, NS
UA (μmol /L)	306.00 (238.00, 359.00)	311.26 ± 71.035	0.383	0.702, NS
TG (mmol/L)	1.00 (0.75, 1.49)	1.13 (0.98, 1.530)	1.447	0.148, NS
TCH (mmol/L)	5.02 ± 0.71	4.56 (3.52, 5.47)	1.741	0.082, NS
LDLC (mmol/L)	2.86 (2.55, 3.16)	2.32 (1.82, 3.22)	1.643	0.100, NS
HDLC (mmol/L)	1.37 ± 0.30	1.14 ± 0.42	2.119	0.034*
GLU (mmol/L)	4.74 (4.62, 6.47)	5.01 (4.42, 6.47)	0.959	0.337, NS

Note: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ were considered statistically significant. NS was not statistically significant versus HCs. The data of normal distribution are represented by mean ± SD, and the data of skewness are represented by median and quartile.

Abbreviations: ALB, albumin; ALP, alkalinephosphatase; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; Cr, creatinine; DB, direct bilirubin; FIB, fibrinogen; Glu, blood glucose; HCT, hematocrit; HDLC, high-density lipoprotein cholesterol; HGB, hemoglobin concentration; INR, international normalized ratio; LDLC, low-density lipoprotein cholesterol; LYM, lymphocyte; NEUT, neutrophil; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PLT, platelet; PT, prothrombin time; RBC, red blood cell; RDW-CT, red cell distribution width coefficient of variation; TB, total bilirubin; TCH, total cholesterol; TG, triglyceride; TP, total protein; TT, thrombin time; UA, uric acid; WBC, white blood cell.

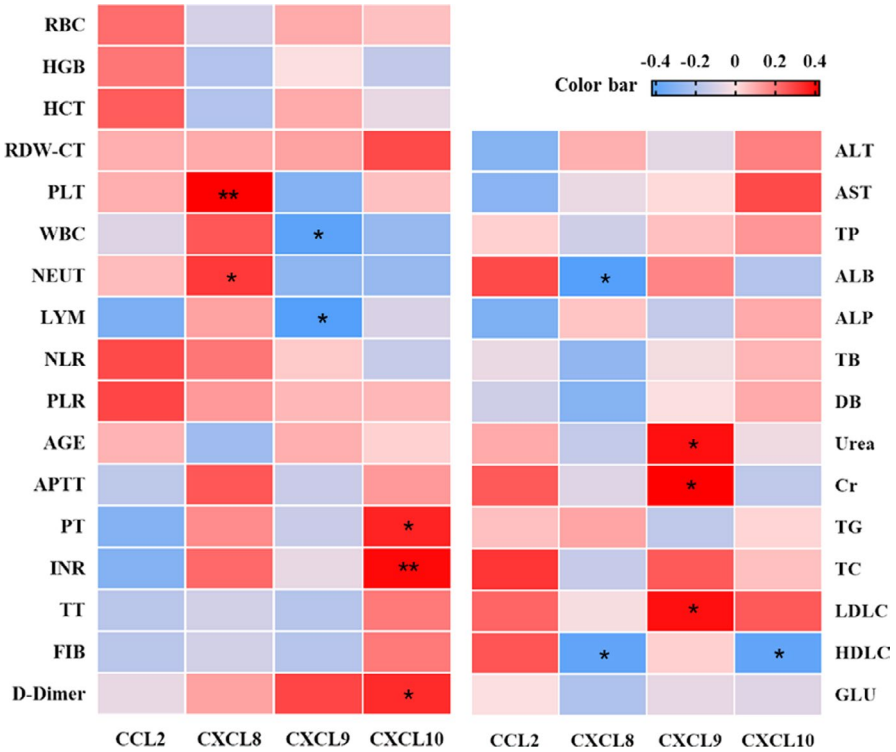

FIGURE 3 The associations between the concentration of CCL2, CXCL8, CXCL9, CXCL10, and clinical hematological indexes in seroresistant syphilitic patients. Heatmap representation of the correlations between the serum concentration of CCL2, CXCL8, CXCL9, CXCL10 and RBC, HCT, RDW-CV, PLT, HGB, WBC, LYM, NLR, PLR and NEUT, APTT, PT, INR, TT, FN, D-Dimer, ALT, AST, TB, DB, TP, ALB, ALP, Urea, CR, UA, GLU, TG, TCH, LDLC, and HDLC levels in seroresistant syphilitic patients ($n = 30$, Pearson coefficient)

concentration of CXCL10 was significantly positively correlated with INR levels ($r = 0.49$, $^{**}p = 0.007$) and was correlated with PT ($r = 0.43$, $^{*}p = 0.018$) and D-Dimer ($r = 0.41$, $^{*}p = 0.029$) in seroresistant syphilitic patients, and was negatively correlated with HDLC in serum ($r = -0.39$, $^{*}p = 0.036$). All the results are shown in Figure 3.

4 | DISCUSSION

The inflammatory chemokines are groups of small (8–12 kDa) chemotactic cytokines that specifically induce migration of immune effector cells to the sites of injury or infection.[16] It is shown in past research that some chemokines take a significant role in the occurrence, development, and clinical presentation of symptomatic syphilis in which the presence of Treponema pallidum or its antigens leads to disturbance humoral and / or cellular immune response. Although there are many studies on chemokines in neurosyphilis, it has been confirmed that the increased CSF concentrations of CXCL8, CXCL10, and CXCL13 could be potential biological markers for neurosyphilis, especially asymptomatic neurosyphilis,[17] while rare studies on chemokines in seroresistant syphilitic patients were reported due to deficiency of significant clinical symptoms of syphilis. Our data suggested that seroresistant syphilitic patients observably elevation levels of the serum chemokines CXCL8 ($^{***}p < 0.001$), CXCL9 ($^{***}p < 0.001$), and CXCL10 ($^{**}p < 0.01$) when compared to noninfected individuals (Figure 1), but the CCL2 was not statistically significant.

CCL2 is a key chemotactic factor which plays important roles in wound healing, the regulating endothelial cell migration and angiogenesis as well as migration and infiltration of immune effector cells. These cells include memory T lymphocytes, monocytes, and natural killer cells but not neutrophils or eosinophils.[18,19] CCL2 has been related to the pathogenesis of diseases characterized by monocytic infiltrates and was demonstrated to be significantly elevated for promoting cell migration after treating with Tp0136, an outer membrane protein of *T. pallidum*.[20] The increased expression of CCL2 (MCP-1) incubated with *T. pallidum* has been confirmed as a momentous role in the *T. pallidum*-induced inflammation by recruiting immune cells to inflammatory sites.[21] Surprisingly, in our study, the level of CCL2 in syphilitic patients with seroresistance was not observably different from that in normal controls, showing that CCL2 may play an important role in acute syphilis infection but not in seroresistant stage.

In this study, the features of seroresistant syphilitic patients are high plasmatic levels of CXCL8, which are chemoattractants for neutrophils, basophils, and T cells, but not monocytes, and show a strong association with platelets and neutrophils. There are some characters of high plasmatic levels of CXCL9 and CXCL10, which shown that Th1 type immune response is common.[18,22,23] CXCL9 and CXCL10 affect the growth, movement, and activation state of T lymphocytes and monocytes that involve in immune and inflammatory response by binding to CXCR3. Cunha-Neto et al suggested that CXCL9 and CXCL10, which was known as IFN-γ- dependent chemokines, might increase the chemotactic signal and lead to migration of more CCR5+ CXCR3+ T lymphocytes to the inflammatory sites. As the past studies, there were some factors were associated with serofast like age, gender, stage of infection, initial nontreponemal antibody titer, treatment drug, treponema pallidum occult infection, treponema pallidum repeat genes, and the number of sex partners. However, if patients have rapid serum reaction, serological cure cannot be achieved even though the drug dose is increased or the treatment time is prolonged.

Thus, it is generally believed that the appearance of syphilis serofast may be directly related to the imbalance of immune cell subsets and other factors, which lead to incomplete elimination of Treponema pallidum in hosts. There are many chemokines, and their functions are different. Our results suggested that abnormal increasing levels of CXCL8, CXCL9, and CXCL10 might contribute to inflammation and immunosuppression of syphilitic seroresistance instead of CCL2.

We speculate that immune allergy of host itself caused by infection of Treponema pallidum is the main pathogenic factor in patients with syphilis serofast, rather than bacteremia caused by the reproduction of Treponema pallidum in the body. The abnormally increasing levels in chemokines of CXCL8, CXCL9, and CXCL10 may be master regulators of antisyphilitic inflammatory cell migration. They may affect the clinical progress and outcome of syphilis antibody.

4.1 | Limitation

There were some potential limitations in our study. First, most of the patients in this study came from Zhejiang Province, which might lead to regional limitations in the results of this study. The research results need to be further supplemented by more regional data and results in future. Second, we analyzed the abnormal levels of CCL2, CXCL8, CXCL9, and CXCL10 in syphilitic patients with seroresistance compared with normal healthy subjects through experiments. However, syphilis is a unique infectious disease with different stages. We should further investigate whether there are abnormal levels of chemokines in syphilis in other stages, such as primary syphilis, latent syphilis, neurosyphilis and other tertiary syphilis. This will be the focus of our later research. In conclusion, our results reflect the abnormal level of serum chemokines in patients with syphilis serofast in Zhejiang Province, and increasing serum abnormalities in CXCL8, CXCL9, and CXCL10 level combining with platelets of peripheral blood and plasmatic INR in syphilis patients may be helpful for the diagnosis of serofast state.

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CONFLICTS OF INTEREST

The author(s) declare(s) that there are no conflicts of interest regarding the publication of this article.

AUTHOR CONTRIBUTIONS

XYD, FFY, and JWZ tested the concentration of cytokines in the samples. YMP and JLL interpreted the patient data. XYD and YMG analyzed the data. XYD and YMG draw the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study was approved through the local ethics committee of Zhejiang Provincial People's Hospital. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during this study are available from the corresponding author Yumei Ge on reasonable request.

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