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Role of serum CXCL9 and CXCL13 in predicting infection after kidney transplant

A STROBE study

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Abstract

Chemokines are majorly involved in inflammatory and immune responses. The interferon-γ-inducible chemokines C-X-C motif chemokines 9 and 10 (CXCL9 and CXCL10) are considerably associated with Th1 cells and monocytes, and their expression levels rapidly increase during the early episodes of renal allograft rejection and various infectious diseases. CXCL13 is one of the most potent B-cell and T follicular helper-cell chemoattractants. The expression of CXCL13 in the presence of infection indicates an important chemotactic activity in multiple infectious diseases. C-C motif chemokine ligand 2 (CCL2) can attract monocytes and macrophages during inflammatory responses. However, there are no studies on the role of these chemokines in posttransplant infection in kidney transplant recipients.

In this study, CXCL9, CXCL10, CXCL13, and CCL2 were analyzed using the Bio-Plex suspension array system before transplant and 30 days after transplant.

The serum levels of CXCL9 and CXCL13 30 days after kidney transplant were associated with infection within 1 year after transplant ($P = .021$ and $P = .002$, respectively). The serum levels of CXCL9 and CXCL13 before surgery and those of CCL2 and CXCL10 before and after surgery were not associated with infection within 1 year after transplant ($P > .05$). The combination of postoperative day (POD) 30 CXCL9 and postoperative day 30 CXCL13 provided the best results with an area under the curve of 0.721 (95% confidence interval, 0.591–0.852), with a sensitivity of 71.4% and specificity of 68.5% at the optimal cutoff value of 52.72 pg/mL.

As important chemokines, CXCL9 and CXCL13 could be used to predict the occurrence of infection after kidney transplant.

Abbreviations: AUC = area under the receiver operating characteristic curve, CCL2 = C-C motif chemokine ligand 2, CDI = *Clostridium difficile* infection, CXCL9 and CXCL10 = interferon-γ-inducible chemokines C-X-C motif chemokines 9 and 10, CXCR3 = C-X-C chemokine receptor type 3, ESRD = end-stage renal disease, HBV = hepatitis B virus, HR = hazard ratio, KT = kidney transplant, POD 30 = postoperative day 30, ROC = receiver operating characteristic, Tfh = T follicular helper.

Keywords: chemokine, CXCL13, CXCL9, infection, kidney transplant, prediction

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

With the widespread use of new immunosuppressive drugs, kidney transplant (KT) has become the best life-saving procedure for patients with irreversible end-stage renal disease. Although the survival rate and quality of life of KT recipients (KTRs) have greatly improved after successful KT procedures, severe complications have also been frequently reported.[1] One such adverse event after KT is infection.[2] Posttransplant infection is costly and is responsible for nonnegligible morbidity and mortality in KTRs.[3] Therefore, effectively identifying the risk of life-threatening infectious complications is warranted and crucial.

At present, clinicians rely on routine therapeutic drug monitoring of immunosuppressants such as tacrolimus to estimate the posttransplant immunological status of KTRs. As the site of action of tacrolimus is within lymphocytes, measurement of tacrolimus levels within lymphocytes may be more relevant than measurement in whole blood to reflect the immunological status.[4] Nevertheless, this option is technically demanding.[5] These inadequacies of pharmacokinetic measurement limit its application in the prediction and progression evaluation of adverse outcomes, especially infection. A promising technique is pharmacodynamic monitoring of immunosuppressants.[5] Immunocytes and intercellular signal mediators are believed to substantially mirror the immune and inflammatory interactions between the host and the graft.[6] Assays for immunological biomarkers may provide more accurate information with respect to the management of KTRs with posttransplant infection.

Previous studies have demonstrated that chemokines, a subtype of cytokines, serve as either recruiter or activator signals for immune cells by interacting with their receptors.[7] The interferon-γ-inducible chemokines C-X-C motif chemokines 9 and 10 (CXCL9 and CXCL10) are considerably associated with the recruitment and activation of C-X-C chemokine receptor type 3 (CXCR3)-positive immunocytes. CXCL9 and CXCL10 are produced by macrophages, endothelial cells, and fibroblasts. CXCL10 can also be produced by monocytes, dendritic cells, and T cells. Both CXCL9 and CXCL10 exert their biological effects mainly by binding to the CXCR3 receptor.[8] Earlier evidence has revealed that CXCL9 and CXCL10 are associated with the occurrence of cancer, autoimmunity, and renal allograft rejection.[8–13] Recent studies on high expression of CXCL9 and CXCL10 in patients with *Clostridium difficile* infection (CDI), human immunodeficiency virus (HIV) infection, *Porphyromonas gingivalis* infection, and *Mycoplasma* infection suggest that these chemokines play a crucial role in various infectious diseases.[14–17] However, the role of CXCL9 and CXCL10 in the initiation and prediction of posttransplant infection in KTRs is unknown.

CXCL13 is one of the most potent B-cell and T follicular helper (Tfh)-cell chemoattractants when acting through its cognate receptor, CXCR5.[18] In our previous study, the serum CXCL13 levels in KTRs with allograft dysfunction were found to be significantly higher than in recipients with stable function.[19] Recent studies that aimed to detect CXCL13 expression in CDI,[20] systemic candidiasis,[21] and hepatitis B virus (HBV) infection,[22] as well as in acute rejection[23] and chronic antibody-mediated rejection[24] indicated a crucial chemotactic activity in both multiple infectious diseases and KT. Determining the role of CXCL13 in the occurrence of infection after KT is necessary. Additionally, C-C motif chemokine ligand 2 (CCL2), the receptor of which is CCR2, attracts monocytes and macrophages during inflammatory responses or cancer development.[25] CCL2 has also been shown to be crucial in chronic kidney diseases.[26] In our previous study, the serum level of CCL2 was associated with renal injury in liver transplant recipients.[27] CCL2 has been reported to be correlated with pathogenicity and mortality in both avian (H5N1 and H7N9) and human (H1N1 and H3N2) viral infections.[7] However, the role of serum CCL2 in kidney posttransplant infection remains unclear.

In this study, we aimed to investigate whether these four chemokines may be useful predictive biomarkers for impending infection in KTRs. We measured the levels of CXCL9, CXCL10, CXCL13, and CCL2 preoperatively and on postoperative day 30 (POD 30) in KTRs who subsequently did not experience or experienced at least one infection episode within the first 12 months after transplant.

2. Methods

2.1. Patients and assessment

A total of 95 KTRs who underwent KT at West China Hospital of Sichuan University from July 2014 to June 2016 and met the inclusion and exclusion criteria were retrospectively recruited for this study. The inclusion criteria were as follows:

(1) age >18 years at transplant,
(2) living-related KT, and
(3) available 1-year follow-up data at our hospital.

The exclusion criteria were as follows:

(1) diagnosis of other autoimmune diseases, including systemic lupus erythematosus or systemic sclerosis;
(2) diagnosis of chronic infection including viral hepatitis, tuberculosis infection, or human immunodeficiency virus;
(3) history of malignancy;
(4) occurrence of allograft rejection during the follow-up period; and
(5) occurrence of infection within 30 days after transplant.

The study outcome was the occurrence of infection from 1 to 12 months after transplant, which was based on one or two of the following criteria:

(1) pathogenic evidence of infection by bacteria, fungus, or virus, and
(2) clinical diagnosis based on symptoms of infection and results of computed tomography (CT) without culture-confirmed pathogens, or complete resolution of symptoms with antimicrobial treatment.

KTRs who experienced posttransplant infection and required hospitalization at least once were included in the infection group (N=31, among which 15 cases were confirmed by the detection of pathogenic microorganisms and 16 cases were diagnosed on the basis of clinical symptoms and CT findings). Meanwhile, the no-infection group consisted of 64 recipients without any infection episode during the follow-up period. The study was approved by the ethics committee of West China Hospital (2017–397), and all participating recipients provided written informed consent before enrollment. The authors had access to information that could identify individual participants during or after data collection.

Table 1

Demographic and clinical characteristics of kidney transplant recipients and living donors.

	Total, n=95	No infection, N=64	Infection, N=31	*P* value
Recipient characteristics				
Age (yr)	28.0 (25.0–34.0)	28.0 (24.5–34.0)	29.0 (25.5–34.0)	.395
Male sex, n (%)	71 (74.7%)	46 (71.9%)	25 (80.6%)	.356
Body mass index (kg/m^2)	20.2 (19.3–22.4)	20.2 (19.3–22.8)	20.6 (19.2–22.2)	.880
Pretransplant urine volume (mL/d)	200 (50–575)	200 (50–575)	200 (0–550)	.545
Pretransplant renal replacement therapy, n (%)				.426
Hemodialysis	82 (86.3%)	56 (87.5%)	26 (83.9%)	
Peritoneal dialysis	5 (5.3%)	4 (6.3%)	1 (3.2%)	
No dialysis	8 (8.4%)	4 (6.2%)	4 (12.9%)	
Dialysis duration (mo)	12.0 (7.0–20.0)	10.5 (7.0–18.0)	14.0 (8.0–33.0)	.026
Induction therapy, n (%)				.779
Anti-CD25	74 (77.9%)	50 (78.1%)	24 (77.4%)	
Anti-thymoglobulin antibodies	14 (14.7%)	10 (15.6%)	4 (12.9%)	
No induction	7 (7.4%)	4 (6.3%)	3 (9.7%)	
Pretransplant eGFR (mL/min/1.73m^2)*	5.4 (4.3–7.3)	5.6 (4.1–7.3)	5.4 (4.4–7.4)	.918
eGFR at month 1 (mL/min/1.73m^2)*	71.8±24.4	73.9±24.6	67.6±37.8	.241
Trough level of TAC (ng/mL)	6.7±1.9	6.5±1.8	7.0±2.1	.240
MMF Level (ng/mL)	75.8±20.0	75.0±18.9	77.4±22.4	.588
Event time post KT (d)	–	–	178.0 (79.5–255.0)	
Donor characteristics				
Age (yr)	49 (43–52)	48 (42–52)	51 (44–55)	.156
Male sex, n (%)	30 (31.6%)	21 (32.8%)	9 (29.0%)	.785
HLA mismatches (A, B, DR, DQ)	4.0 (3.0–4.0)	4.0 (3.5–4.0)	4.0 (3.0–4.0)	.464

eGFR=estimated glomerular filtration rate, HLA=human leukocyte antigen, MMF=mycophenolate mofetil, TAC=tacrolimus.
* eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation; *P* value for the no-infection group vs. infection group.

2.2. Measurement of chemokine levels

A total of 173 peripheral blood samples were obtained at baseline (pretransplant, N=91) and 1 month after transplant (N=82). Paired samples of 78 recipients were collected, whereas the remaining recipients only had samples at 1 time point. Freshly isolated serum samples were aliquoted at the date of collection and stored in a -80°C refrigerator until the detection of chemokines (March 2, 2018). The serum levels of CCL2, CXCL9, CXCL10, and CXCL13 were quantified using the Bio-Plex suspension array system (Bio-Rad Laboratories Inc., Hercules, CA) according to the manufacturer instructions. The Human Premixed Multi-analyte Kit was purchased from R&D Systems (Minneapolis, MN).

2.3. Statistical analyses

Statistical analyses were performed using SPSS software (version 23.0; SPSS Inc., Chicago, IL), and graphs were generated using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA). Data are presented as absolute number (relative frequencies), mean ± standard deviation, or median (interquartile range) according to the type of data. The chi-square or Fisher exact tests were used to compare categorical variables between groups. Student *t*-test and Mann-Whitney *U*-test were applied to compare continuous variables with normal and skewed distributions, respectively. Spearman correlation analysis was performed when analysis the correlation between chemokine levels and infection time. Receiver operating characteristic (ROC) curve analysis was conducted to assess the predictive ability of chemokines and to determine the optimal cutoff values according to the maximum value of the Youden-J indices (sensitivity+specificity-1). Logistic regression analysis was conducted to calculate the combined predictor values for the combination of potential markers. The regression equation was presented as logit $(P)=a*marker1+b*marker2+c$. The combined predictor value was obtained from the following formula: marker1+b/a*marker2. These values were used to estimate the area under the ROC curve (AUC) for marker combinations and to determine the cutoff values. All variables of the recipients and donors were entered into univariate Cox proportional hazard regression model and the potential factors of the recipients and donors were entered into multivariate Cox proportional hazard regression model to show their influence on posttransplant infection.[2] A 2-tailed P value of $<.05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

Table 1 shows the baseline characteristics and outcomes of KTRs with and without infection. All recipients received tacrolimus-based triple immunosuppression therapy (tacrolimus plus mycophenolate mofetil plus steroids) after KT. No significant difference was found in recipient and donor demographics except for pretransplant dialysis duration, which was significantly longer in KTRs with subsequent infection than in those without any episode of infection. Table 2 shows the infection site and pathogenic classification in all 31 patients with infection.

3.2. Pretransplant and POD 30 chemokine levels in the infection and no-infection groups

Pretransplant chemokine levels were assessed in 91 KTRs. No significant differences in pretransplant CCL2, CXCL9, CXCL10, and CXCL13 levels were observed between recipients with

Table 2

Infection site and pathogen classification.

Pathogenic diagnosis (N=15)		Clinical diagnosis (N=16)	
Infection site			
Lung	12	Lung	16
Skin	2		
Urethra	1		
Pathogen			
Bacteria	6		
Fungus	3		
Virus	3		
Co-infection (≥2 types)	3		

infection and those without infection. POD 30 chemokine levels were evaluated in 82 KTRs. Infected recipients had significantly higher serum levels of CXCL9 (247.7 [193.7–294.3] vs. 193.7 [193.7–247.7] pg/mL, $P=.021$; Fig. 1B) and CXCL13 (30.1 [19.7–42.8] vs 17.3 [12.6–26.3] pg/mL, $P=.002$; Fig. 1D) than noninfected recipients. No significant differences in the POD 30 levels of CCL2 and CXCL10 were observed between the two groups (Fig. 1A,C). The chemokine levels in the infection group were further compared between cases diagnosed on the basis of pathogenic evidence and clinically diagnosed cases. No difference was observed between these two subgroups (see **S1 Table**, Supplemental Content, which illustrates the differences of chemokine levels between pathogenic diagnosed infection group and clinical diagnosed infection group, http://links.lww.com/MD/F675).

Correlations between each pretransplant or POD 30 chemokine level and the time that an infection occurred has been performed. No correlation between the pretransplant or POD 30 chemokine levels (CCL2, CXCL9, CXCL10, and CXCL13) and infection time was observed. These results were provided in (see **S2 Table**, Supplemental Content, which illustrates the correlations between pretransplant or POD 30 chemokine levels and infection time, http://links.lww.com/MD/F676).

3.3. Predictive performances of POD 30 CXCL9 and CXCL13 and survival analysis for posttransplant infection

ROC curve analysis was further performed to assess the predictive abilities of POD 30 CXCL9, POD 30 CXCL13, and their combination (POD 30 CXCL9+CXCL13) in the early identification of the risk of posttransplant infection, and to define optimal cutoff values that distinguish between KTRs with and without subsequent infection. The AUC of POD 30 CXCL9 was 0.651 (95% confidence interval [95%CI] 0.520–0.782), with a sensitivity of 60.7% and a specificity of 66.7% when the cutoff value was set at 234.8 pg/mL (Fig. 2). The AUC of POD 30 CXCL13 was 0.709 (95% CI 0.584–0.833), with a sensitivity of 57.1% and a specificity of 83.3% when the cutoff value was set at 27.9 pg/mL (Fig. 2). On the basis of POD 30 CXCL9 cutoff value, the patients were divided into two groups (POD 30 CXCL9 <234.8 pg/mL, N=47; POD 30 CXCL9 ≥234.8 pg/mL, N=35). Survival analysis was performed. Patients with POD 30 CXCL9 ≥234.8 pg/mL had a significantly lower infection-free survival rate than patients with POD 30 CXCL9 <234.8 pg/mL (hazard ratio [HR] 2.614, 95% CI 1.214–5.626, $P=0.0097$; Fig. 3A). On the basis of POD 30 CXCL13 cutoff value, the patients were divided into two groups (POD 30 CXCL9 <27.9 pg/mL, N=46; POD 30 CXCL13 ≥27.9 pg/mL, N=36). Patients with POD 30 CXCL9 ≥27.9 pg/mL had a significantly lower infection-free survival rate than patients with POD 30 CXCL9 <27.9 pg/mL (hazard ratio [HR] 2.952, 95% CI 1.405–6.205, $P=.0043$; Fig. 3B).

In addition, the logistic regression analysis of POD 30 CXCL9 +CXCL13 revealed that the regression equation was logit $(P)=0.039$*(POD 30 CXCL13)+0.005*(POD 30 CXCL9)-2.97, with the combined predictor value=POD 30 CXCL13

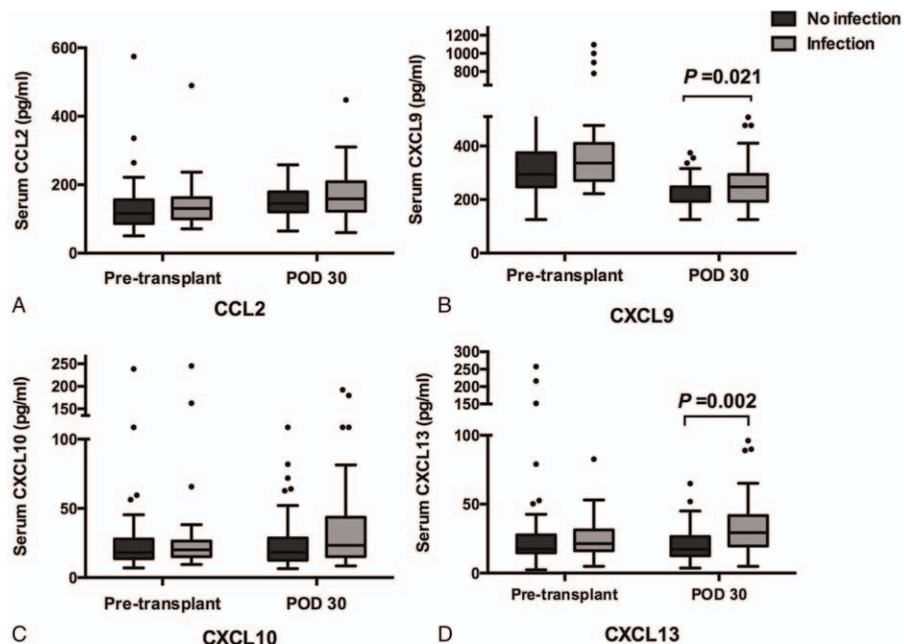

Figure 1. Comparison of baseline and POD 30 serum chemokine levels between KTRs without and with an infection episode during the first 12 months after transplant: (A) serum CCL2, (B) serum CXCL9, (C) serum CXCL10, and (D) serum CXCL13.

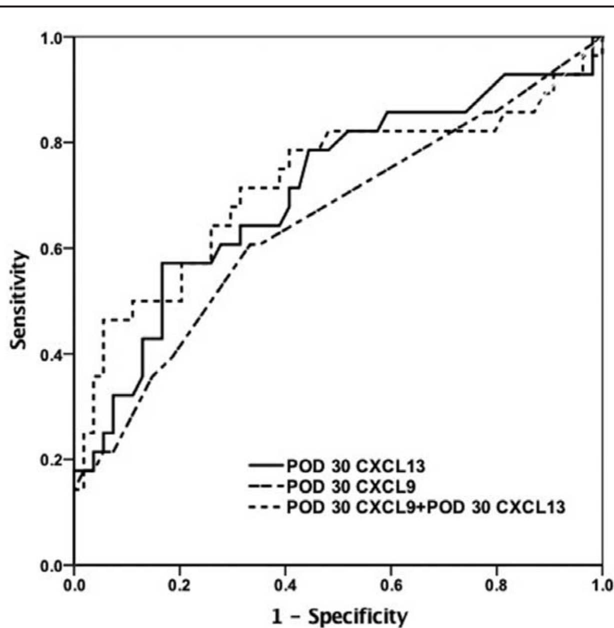

Figure 2. ROC curves of POD 30 CXCL9, POD 30 CXCL13, and POD 30 CXCL9+CXCL13 as predictors of posttransplant infection. The AUC for POD 30 CXCL9, POD 30 CXCL13, and the combined predictor was 0.651 (95% CI 0.520–0.782), 0.709 (95% CI 0.584–0.833), and 0.721 (95% CI 0.591–0.852), respectively. The optimal cutoff value for predicting posttransplant infection for POD 30 CXCL9 was 234.8 pg/mL, with a sensitivity of 60.7% and a specificity of 66.7%. For POD 30 CXCL13, the cutoff was 27.9 pg/mL, with a sensitivity of 57.1% and a specificity of 83.3%. For the combined predictor, a sensitivity and specificity of 71.4% and 68.5%, respectively, were obtained for a cutoff value set at 52.72 pg/mL.

+0.005*(POD 30 CXCL9)/0.039. We found that the combination of POD 30 CXCL9 and POD 30 CXCL13 provided the best results with an AUC of 0.721 (95% CI 0.591–0.852), with a sensitivity of 71.4% and a specificity of 68.5% at the optimal cutoff value of 52.72 pg/mL (Fig. 2). On the basis of this cutoff value, the patients were divided into a high-risk group (predictor value ≥52.72 pg/mL, N=37) and a low-risk group (predictor value <52.72 pg/mL, N=45). Survival analysis was performed. The high-risk group had a significantly lower infection-free survival rate than the low-risk group (hazard ratio [HR] 3.926, 95% CI 1.837–8.394, P=.0004; Fig. 3C).

3.4. Univariate/multivariate Cox regression analyses and risk of posttransplant infection

To estimate the putative risk factors for the development of posttransplant infection in KTRs with increased POD 30 CXCL9 and CXCL13 and the combined predictor, a univariate analysis and three separate multivariate analyses were performed. In particular, multivariate Cox regression analysis was conducted with infection episodes as a dependent variable. All variables shown in Table 1 were entered into univariate Cox regression analyses as covariables.[28]

Univariate analysis results demonstrated that pretransplant dialysis duration, POD 30 CXCL9, POD 30 CXCL13, and POD 30 CXCL9+CXCL13 were significantly associated with the endpoint, whereas recipient age, trough level of TAC, level of MMF, and donor age failed to reach statistical significance. On the basis of univariate analysis results, dialysis duration (P=.002) were chosen in multivariate analysis. On the basis of the potential clinical significance on posttransplant infection, recipient age, level of Tac, level of MMF and donor age were also chosen in multivariate analysis.

Multivariate Cox regression analyses confirmed that levels of POD 30 CXCL13 (HR 0.38 [0.14–1.00], P=.049) and POD 30 CXCL9+CXCL13 (HR 0.26 [0.10–0.72], P=.009), whereas the predictive ability of POD 30 CXCL9 was lost in multivariate analysis (HR 0.67 [0.25–1.79], P=.420). In addition, longer pretransplant dialysis duration was significantly correlated with the risk of posttransplant infection in all three multivariate regression models. The detailed univariate and multivariate Cox analysis results are summarized in Table 3.

4. Discussion

In the present retrospective cohort study, the serum levels of CXCL9 and CXCL13 at 30 days after KT were found to be associated with infection within 1 year after transplant. The serum levels of CXCL9 and CXCL13 before surgery and the serum levels of CCL2 and CXCL10 before and after surgery were not associated with infection within 1 year after transplant.

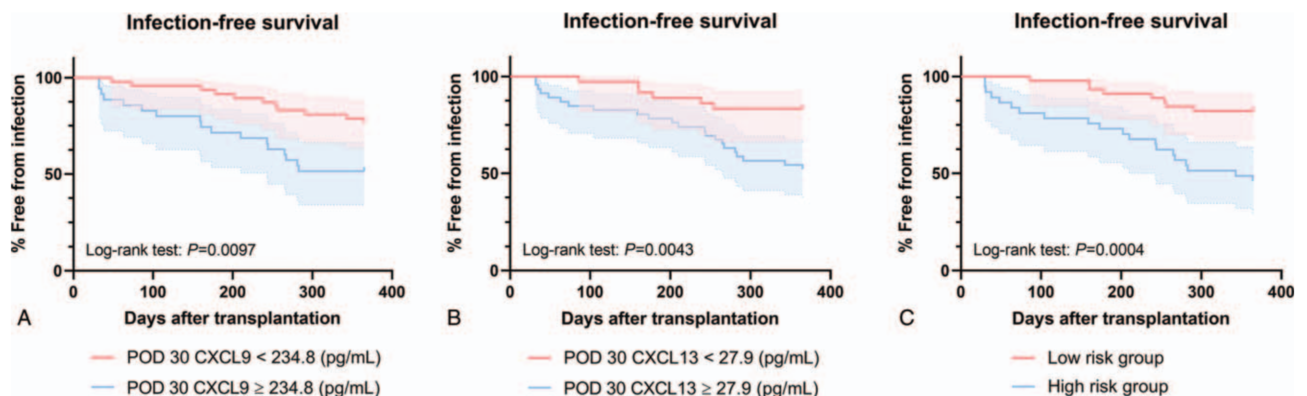

Figure 3. Infection-free survival analysis based on POD 30 CXCL9, CXCL13, and the combined predictor value. (A) POD 30 CXCL9 <234.8 pg/mL (N=47, red line). POD 30 CXCL9 ≥234.8 pg/mL (N=35, blue line). (B) POD 30 CXCL13 <27.9 pg/mL (N=46, red line). POD 30 CXCL9 ≥27.9 pg/mL (N=36, blue line). (C) Low-risk group (N=45, red line): predictor value <52.72 pg/mL. High-risk group (N=37, blue line): predictor value ≥52.72 pg/mL. The regression equation was logit (P)=0.039*(POD 30 CXCL13)+0.005*(POD 30 CXCL9)-2.97; combined predictor value=POD 30 CXCL13+0.005*(POD 30 CXCL9)/0.039.

Table 3

Univariate and multivariate Cox hazard regression models for posttransplant infection between 1 and 12 months after transplant, with serum chemokine entered after dichotomization using the optimal cutoff value.

	Univariate analysis		Multivariate analysis[1]		Multivariate analysis[2]		Multivariate analysis[3]	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Multivariate Cox hazard regression models				0.098		0.030		.010
Recipient age (yr)	1.03 (0.99–1.07)	.178	1.00 (0.94–1.06)	0.975	1.01 (0.96–1.07)	0.700	0.98 (0.92–1.04)	.517
Recipient sex	1.32 (0.54–3.23)	.545						
Recipient BMI	1.04 (0.93–1.16)	.518						
Pretransplant urine volume (mL)	1.00 (1.00–1.00)	.582						
Pretransplant renal replacement therapy	1.24 (0.72–2.13)	.434						
Dialysis duration (months)	1.03 (1.01–1.05)	.002	1.02 (1.00–1.05)	0.050	1.03 (1.00–1.05)	0.038	1.03 (1.01–1.06)	.018
Induction therapy	0.99 (0.34–2.87)	.987						
Pretransplant eGFR (mL/min/1.73m^2)	0.98 (0.86–1.12)	.798						
eGFR at month 1 (mL/min/1.73m^2)	0.99 (0.98–1.00)	.152						
Trough level of TAC	1.12 (0.93–1.35)	.222	1.09 (0.87–1.36)	0.450	1.07 (0.85–1.33)	0.573	1.13 (0.89–1.44)	.309
MMF Level	1.00 (0.98–1.02)	.890	1.00 (0.98–1.02)	0.946	1.00 (0.98–1.03)	0.869	1.01 (0.98–1.03)	.688
Donor age (years)	1.02 (0.98–1.07)	.277	1.02 (0.97–1.07)	0.459	1.03 (0.98–1.09)	0.239	1.04 (0.98–1.09)	.169
Donor sex	0.99 (0.45–2.17)	.979						
HLA mismatches	0.95 (0.74–1.21)	.661						
POD 30 CXCL9 ≥234.8 (pg/mL)	0.35 (0.16–0.76)	.008	0.67 (0.25–1.79)	0.420	–	–	–	–
POD 30 CXCL13 ≥27.9 (pg/mL)	0.31 (0.12–0.76)	.011	–	–	0.38 (0.14–1.00)	0.049	–	–
POD 30 CXCL9+13 ≥52.7 (pg/mL)	0.27 (0.12–0.62)	.002	–	–	–	–	0.26 (0.10–0.72)	.009

BMI = body mass index, CI = confidence interval, CXCL9 and CXCL13 = interferon-γ-inducible chemokines C-X-C motif chemokines 9 and 13, eGFR = estimated glomerular filtration rate, HLA = human leukocyte antigen, HR = hazard ratio, MMF = mycophenolate mofetil, POD 30 = postoperative day 30, TAC = tacrolimus.
Multivariate analysis[1]: multivariate Cox regression model for POD 30 CXCL9.
Multivariate analysis[2]: multivariate Cox regression model for POD 30 CXCL13.
Multivariate analysis[3]: multivariate Cox regression model for POD 30 CXCL9+CXCL13.

CXCL9 is also called interferon-induced mononuclear protein, the main function of which is to promote chemotaxis of immunocytes.[8] CXCL9 can be specifically expressed after inflammatory stimulation and attracts CXCR3-positive immune cells to the site of high CXCL9 gradient.[18] Inflammatory cytokines, bacterial toxins, or other pathological conditions can induce CXCL9 expression and eventually disrupt homeostasis.[8,29] Increased expression of CXCL9 could promote immune stimulation, antiangiogenesis, and increased Th1 and natural killer cell activity.[29] CXCL9 has been shown to be correlated with multiple infections, and even with the severity and mortality of infection.[7,15,30] However, previous studies had a cross-sectional design and did not demonstrate a causal relationship between CXCL9 and infection. In an early study, CXCL9 was suggested to be a predictor of allograft injury, although mostly as a urine biomarker.[9,12] In the present study, the expression of serum CXCL9 in the infection group was higher than that in the no-infection group before transplant; however, the difference was not statistically significant. The level of CXCL9 in the infection group was significantly higher than that in the no-infection group at 1 month after transplant ($P = 0.021$). This suggests that CXCL9 may be involved in inflammation and immune cell migration in both posttransplant rejection and infection. The high levels of inflammation not only implied the occurrence of allograft rejection but also suggested the risk of posttransplant infection. Although it was traditionally believed that rejection and infection result from opposite immunological states, this view may be disputed by the increasing knowledge on the correlation between infection and rejection after transplant. A study on risk stratification from Switzerland, which included 630 KTRs, demonstrated that 26.1% of the KTRs were at a high risk for both infection and rejection.[28] Therefore, a high level of CXCL9 was assumed to indicate a highly inflammatory status, which is detrimental to the maintenance of immune homeostasis.

CXCL13 belongs to the B-cell chemokine 1 of CXC chemokines. It specifically acts to recruit and position CXCR5+ cells within lymphoid follicles, primarily B cells and Tfh cells.[31,32] The Tfh cells of humans, in turn, produce large amounts of CXCL13.[33] Wang et al demonstrated that serum CXCL13 level was associated with CDI and positively correlated with blood markers of inflammation.[20] Liu et al found that the level of CXCL13 expression in Kupffer cells isolated from HBV-positive livers was much higher than that in non-HBV-infected controls. The expression of CXCL13 could promote the accumulation of CD4+ T and B cells in follicular-like structures in the liver. In this study, CXCL13 also showed a protective effect in patients with HBV infection who showed a complete response.[22] In this study, no significant difference was observed in the level of CXCL13 between the infection and no-infection groups before transplant. The CXCL13 level in the infection group was significantly higher than that in the no-infection group at 1 month after transplant ($P = .002$). This suggests that CXCL13 expression increased before the occurrence of posttransplant infection. Therefore, CXCL13 could not only be a promising biomarker for diagnosis and progression valuation of infection but also a potential immunological predictor for a high risk of infection, especially in KTRs. Whether CXCL13 plays an important role in promoting B cells to participate in anti-infective immunity needs to be further identified.

Combination analysis has been increasingly used in prediction and diagnosis research. The combination of CXCL9, CXCL10, and CXCL11 levels during primary HIV infection could predict HIV disease progression.[15] In the present data, the combination of POD 30 CXCL9 and POD 30 CXCL13 increased the sensitivity to 71.4%, which was higher than that of POD 30

CXCL9 (60.7%) and POD 30 CXCL13 (57.1%), at the optimal cutoff value of 52.72 pg/mL. In the survival analysis, the high-risk group with a predictive value of not lower than 52.72 pg/mL showed a significantly lower infection-free survival rate than the low-risk group with a hazard ratio of 0.26 (95% CI 0.10–0.72) ($P=0.009$). This suggests that CXCL9 and CXCL13 could be detected in further multiple-factor combined prediction studies with large numbers of candidates. The specificity of the combination (68.5%) was lower than that of POD 30 CXCL13 (83.3%). With respect to prediction markers, sensitivity is more important than specificity in identifying high-risk patients. A marker with high sensitivity could contribute to clinical monitoring and early intervention to decrease the occurrence of infection.

Intervention suggestions could be given when patients with high levels of CXCL9 and CXCL13 after transplantation:

(1) Reduce the concentration or dose of immunosuppressants, such as mycophenolic acid;
(2) Avoid getting a cold and avoid going to crowded areas;
(3) Detect CXCL9 and CXCL13 more frequently after transplantation. Whether these intervention suggestions would benefit the patients relies on prospective studies focusing on the adjustment of immunosuppressant dose based on CXCL9 and CXCL13 levels.

CXCL10 has been observed to be correlated with various infectious diseases.[7,14,16,17] However, in this study, no difference in CXCL10 expression was found between the infection and no-infection groups. The same results were obtained for CCL2. These results may be related to the use of immunosuppressants, leading to controlled levels of CXCL10 and CCL2 after transplant. These data suggest that CXCL9 and CXCL13 had more potential than CXCL10 or CCL2 in predicting infection or an inflammatory status under the condition of immunosuppressant use. Further, chemokines may be a double-edged sword in the regulation of the occurrence of inflammation and the treatment of the resulting damage.[34] The inflammatory status of infection and the ability to treat the allograft damage would eventually determine the transplant outcome.

This study had some limitations, including the low numbers of analyzed chemokines and the limit of detection time points. Further studies with accurate etiological or pathogenic classification would provide more persuasive results.

In conclusion, CXCL9 could promote anti-infection immune activation and CXCL13 could contribute to B-cell activation and antibody production. The high levels of CXCL9 and CXCL13 indicated the enhancement of chemotaxis of Th1 and B cells in the infection group in this study. The results of this study showed that CXCL9 and CXCL13, as important chemokines, could be used to predict the occurrence of posttransplant infection in KTRs. Monitoring the trend of these chemokines after transplant is useful to reflect the humoral and cellular immunological status of recipients. More studies on inflammatory biomarkers for routine monitoring are needed.

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