Arthritis Research & Therapy

RESEARCH

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CCL23 is a potential biomarker for antineutrophil cytoplasmic antibody–associated vasculitis

Weiwei Hao[1], Qianqian Liu[2], Xiaoping Li[2], Yiran Xu[3], Wenjuan Guan[1], Lei Zhang[1], Fang Dong[1], Wenjun Cao[1], Shengyun Liu[1*] and Wei Li[1*]

Abstract

Objective The present cohort study aimed to evaluate the value of CCL23 in diagnosis, disease activity, and prognosis in patients with antineutrophil cytoplasmic antibody–associated vasculitis (AAV).

Methods CCL23 levels in serum samples from 317 patients with AAV and 83 healthy controls (HCs) were measured using a customized immune response kit.

Results Patients with AAV had significantly elevated CL23 levels compared with HCs. CCL23 level was closely related to disease activity and was better than birmingham vasculitis activity score (BVAS) in distinguishing disease relapse from remission (area under curve: CCL23 = 0.942, BVAS = 0.84). Elevated CCL23 level was associated with poor prognosis within a 1 year follow-up period in patients with AAV ($p = 0.0001$). The ability of CCL23 to predict the poor prognosis of disease is better than that of five-factor score. Furthermore, elevated CCL23 levels were a risk factor for renal involvement (odds ratio = 1.722, $p = 0.033$), and were significantly related to serum creatinine ($r = 0.381$, $p = 0.009$) and eGFR ($r = -0.382$, $p = 0.01$) at the time of diagnosis. High CCL23 level at diagnosis was associated with increased adverse outcomes during 1 year follow-up in patients with AAV with renal involvement ($p = 0.0242$).

Conclusion Elevated serum CCL23 level was closely related with disease activity and renal involvement in patients with AAV, can be a potential biomarker for diagnosis, and can predict prognosis in patients with AAV, especially adverse renal prognosis.

Keywords ANCA-associated vasculitis, CCL23, Biomarker, Renal involvement, Prognosis

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a group of disorders characterized by the inflammation and destruction of small- and medium-sized blood vessels, involving multiple organs and systems, such as the ears, nose, throat, lungs, and renal system [1, 2].

The 2012 International Chapel Hill Consensus Conference (CHCC) classifies AAV into three clinical diseases: granulomatosis with polyvasculitis (GPA), microscopic polyangiitis (MPA), and eosinophilic GPA [3]. Each of these conditions is commonly associated with a circulating ANCA, and the major target antigens are proteinase

*Correspondence:
Shengyun Liu
fccliusy2@zzu.edu.cn
Wei Li
libuwei2011@163.com
[1] Department of Rheumatology, The First Affiliated Hospital of Zhengzhou University, NO. 1, Jianshe East Road, Zhengzhou 450052, Henan, China
[2] The Third Clinical Medical College of Zhengzhou University, Zhengzhou 450052, Henan, China
[3] Henan Key Laboratory of Child Brain Injury and Henan Pediatric Clinical Research Center, Institute of Neuroscience and The Third Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China

3 (PR3) and myeloperoxidase (MPO). Owing to the overlap between GPA and MPA in clinical features, AAV is categorized into MPO AAV and PR3 AAV according to ANCA type [4, 5].

ANCA, as a decisive serological feature for the identification of AAV, plays an important role in the diagnosis of diseases. However, the absolute ANCA titer has limited value in determining disease activity and target organ involvement and predicting disease prognosis. Elevated ANCA titers have been reported in up to 40% of patients without relapse [6, 7]. The traditional indicators of disease activity, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), have poor specificity. Moreover, the Birmingham vasculitis activity score (BVAS), vasculitis damage index, and five-factor score (FFS) are complex and often result in delayed results when disease is assessed on the basis of clinical symptoms [8, 9]. Therefore, it is of great value to screen biomarkers that can identify disease activity in a timely and effective manner, as well as those related to target organ involvement and different serotypes, and those can predict the prognosis of the disease.

Some serologic biomarkers are related to disease activity in AAV, such as tissue inhibitor of metalloproteinases 1, matrix metalloproteinase 3, CXC motif chemokine 13, interleukin-6, neutrophil extracellular trap remnant levels, and the alternative complement pathway [10–14]. Chemokine may mediate the development and progression of a disease by influencing immune cells and releasing inflammatory mediators. The use of chemokines as markers for diagnosis and prognosis has been reported, such as CXCL9, which is a potential biomarkers for primary Sjögren's syndrome with extra glandular manifestations [15], and CCL2 and CXCL12, which are potential biomarkers for predicting the phenotype of progressive fibrosis in patients with interstitial pneumonia with autoimmune characteristics [16].

A group of proteins differ significantly between samples from healthy controls (HCs) and samples before the onset of AAV symptoms. The comparisons of samples from presymptomatic individuals with AAV before symptom onset and matched controls showed that CCL23 level was positively associated with presymptomatic AAV [17]. Moreover, significant difference in CCL23 level was found between patients with AAV and HCs [18]. CCL23 promotes the chemotaxis of T cells, monocytes, and neutrophils [19]. We speculated that CCL23 plays an important role in the occurrence and development of AAV. However, research into this role is scarce. The present study aimed to evaluate the value of CCL23 in diagnosis, disease activity, organ or system involvement, and disease prognosis in patients with AAV.

Methods

Study populations

Patients with AAV who visited the First Affiliated Hospital of Zhengzhou University between August 2021 and June 2024 and fulfilled the CHCC definitions were enrolled [3]. A total of 317 patients with AAV and 83 HCs were enrolled. Patients who had other autoimmune diseases, were already on regular hemodialysis when enrolled, or had tumors, viral hepatitis, tuberculosis, AIDS, infective endocarditis, allergies, and other diseases that may cause immune disorders at the time of enrollment were excluded. The HCs had no autoimmune diseases, severe allergic disorders, malignancies, or infections. Clinical and laboratory data were collected from inpatient medical records. Disease activity in all the patients was assessed using the BVAS, and prognosie was assessed using the FFS. The demographic data of the subjects are shown in Table 1. All the included patients were followed up for 1 year. Within 1 year, the disease progressed to end-stage renal disease (ESRD), multiple-organ failure, and death due to AAV complications, which were marked as poor prognoses. This study was approved by the institutional review board of the First Affiliated Hospital of Zhengzhou University (2021-KY-0486), and informed consent was obtained from all the study participants.

Sample acquisition and detection of serum CCL23

Blood samples were collected before glucocorticoids or immunosuppressive treatment for initial patients and were collected before hospital treatment for return patients. Serum was isolated after centrifugation at 1800 rpm for 10 min within 2 h after collection and then stored at − 80 °C for subsequent assays. CCL23 levels were tested with a customized immune response kit (ThermoFisher, LOT 394780–000). Use Luminex™200™ for readings. All assays were performed according to the manufacturer's instructions. Results were analyzed using ProcartaPlex™ Analyst 1.0.

Statistical analysis

Continuous data were described as the mean ± standard deviation or median (interquartile range), and frequency (percentage) was used for categorical data. Mann–Whitney U test was used in comparing the quantitative data of the two groups. Kruskal–Wallis test was used in comparing multiple quantitative data groups. Two sets of rates were compared using chi-square tests ($n \geq 40$ and $T \geq 5$), corrected chi-square tests ($n \geq 40$ and at least one $1 \leq T < 5$), and Fisher's exact probability method ($n < 40$ or at least one $T < 1$; n is the entire study sample size; T is the theoretical value of four table cells). Spearman's rank correlation

Table 1 Characteristics of the study cohort at the time of collecting serum samples

Characteristics	AAV ($n=317$)	HC ($n=83$)	AAV VS HC (p)	Initial patients with AAV ($n=95$)
Age (years), M (IQR)	67 (58–73)	46 (36–56)	**< 0.0001**	67 (61–75)
Gender				
Female subjects, n (%)	161 (50.8%)	47 (56.6%)	0.388	54 (56.8%)
Male subjects, n (%)	156 (49.2%)	36 (43.4%)		41 (43.2%)
Clinical features, n (%)				
MPA, n (%)	253 (79.8%)			79 (83.2%)
GPA, n (%)	64 (20.2%)			16 (16.8%)
Hypertension, n (%)	76 (24.0%)			23 (24.2%)
Diabetes, n (%)	43 (13.6%)			8 (8.4%)
Heart disease, n (%)	44 (13.9%)			10 (10.5%)
Cerebrovascular disease, n (%)	39 (12.3%)			11 (11.6%)
Smoking, n (%)	51 (16.1%)			15 (15.8%)
Alcohol consumption, n (%)	21 (6.6%)			7 (7.4%)
Coinfection, n (%)	146 (46.1%)			50 (52.6%)
Fever, n (%)	98 (30.9%)			46 (48.4%)
Myalgia, n (%)	23 (7.3%)			6 (6.3%)
Arthralgia or arthritis, n (%)	36 (11.4%)			10 (10.5%)
Weight loss, n (%)	43 (13.6%)			21 (22.1%)
Skin involvement, n (%)	11 (3.5%)			1 (1.1%)
Eye involvement, n (%)	20 (6.3%)			3 (3.2%)
ENT involvement, n (%)	65 (20.5%)			23 (24.2%)
Lung involvement, n (%)	247 (77.9%)			84 (88.4%)
CVS involvement, n (%)	43 (13.6%)			12 (12.6%)
GIT involvement, n (%)	7 (2.2%)			3 (3.2%)
Renal involvement, n (%)	138 (43.5%)			46 (48.4%)
NS involvement, n (%)	65 (20.5%)			15 (15.8%)
Laboratory features, n (%)				
MPO ANCA positive, n (%)	191 (60.3%)			79 (83.2%)
PR3 ANCA positive, n (%)	45 (14.2%)			15 (15.8%)
CRP (mg/L), M (IQR)	23.4 (3.6–84.1)			55.2 (14.8–119.8)
ESR (mm/h), M (IQR)	40 (15.8–80.8)			70 (37–107.5)
C3 (g/L), M (IQR)	1.1 (0.9–1.3)			1.2 (0.9–1.4)
C4 (g/L), M (IQR)	0.3 (0.2–0.3)			0.2 (0.2–0.3)
IgG (g/L), M (IQR)	11.1 (8.4–15.1)			14.5 (10.9–16.8)
BVAS (2003), M (IQR)	7 (4–12)			11 (6–16)
FFS (2009), M (IQR)	2 (1–2)			2 (1–2)

Values highlighted in bold represent statistically significant p-values ($p < 0.05$)

Abbreviations: *AAV* Antineutrophil cytoplasmic antibody-associated vasculitis, *HC* Healthy control, *n* number, *M* Median, *IQR* Inter-quartile range, *MPA* Microscopic polyangiitis, *GPA* Granulomatosis with polyvasculitis, *ENT* Ear-nose-throat, *CVS* Cardiovascular system, *GIT* Gastrointestinal tract, *NS* Nervous system, *MPO* Myeloperoxidase, *PR3* Proteinase 3, *ANCA* Antineutrophil cytoplasmic antibody, *CRP* Creactive protein, *ESR* Erythrocyte sedimentation rate, *C3* Complement 3, *C4* Complement 4, *IgG* Immunoglobulin G, *BVAS* Birmingham vasculitis activity score, *FFS* Five-factor Score

coefficient was used in examining the correlations of CCL23 levels and clinical parameters. The optimal cut-off points, sensitivity and specificity of markers distinguishing binary outcomes were determined through receiver operating characteristic (ROC) analysis, and the advantages and disadvantages of a prediction model were evaluated through area under curve (AUC). Kaplan–Meier analysis and log-rank test were used in determining the effect of biomarkers on poor disease prognosis. All statistical analyses were conducted using IBM SPSS Statistics (version 25.0). Figures were drawn using GraphPad Prism (version 9.5.1).

Results

Demographics and clinical features of participants

The clinical features of the participants at the time of serum sample collection are shown in Table 1. A total of 317 patients with AAV (49.2% male subjects) and 83 HCs (43.4% male subjects) were enrolled. The median age was 67 (IQR, 58–73) in the AAV group and 46 (IQR, 36–56) in the HC group. No difference in sex ratio was found between the groups. According to the CHCC clinical classification, 253 cases (79.8%) were MPA, and 64 cases (20.2%) were GPA [3]. Owing to the rarity of EGPA, no patients with this condition were included in our study. Serologic ANCA types were available in all the patients, including 191 (60.3%) MPO ANCA–positive patients and 45 (14.2%) PR3 ANCA–positive patients. Among the 317 patients with AAV, 138 (43.5%) had renal involvement; and 247 (77.9%), lung involvement. The median BVAS for all patients was 7 (IQR, 4–12). The median FFS for all the patients was 2 (IQR, 1–2).

CCL23 levels in patients with AAV and HCs

The levels of CCL23 were significantly elevated in patients with AAV compared with HCs ($p < 0.0001$, Fig. 1A). Binary logistic regression analysis was used in assessing whether elevated CCL23 levels were a risk factor for AAV. After adjusting for age and sex, the probability of developing AAV increased by 51.1% for every 1 ng/mL increase in CCL23 (odds ratio (OR) = 1.511, $p < 0.0001$, Fig. 1B). ROC analysis showed that AAV can be distinguished from HCs on the basis of CCL23 level (AUC, 0.891; cutoff, 6.351; sensitivity, 0.748; specificity, 0.88; Fig. 1C).

CCL23 levels in different subgroups of AAV patients

Patients with AAV were divided into different subgroups on the basis of their clinical classification, coinfection, disease activity, and gender. Comparative analysis revealed significantly elevated CCL23 levels in MPA patients versus GPA patients ($p = 0.017$), with substantially higher concentrations observed in active-phase compared to remission-phase patients ($p < 0.001$). Notably, CCL23 expression remained unaffected by either coinfection status or gender differences (Fig. 1D). AAV patients were classified into four distinct subgroups based on ANCA phenotypes: MPO + PR3- group ($n = 188$), MPO + PR3 + group ($n = 3$), MPO- PR3- group ($n = 84$), MPO- PR3 + group ($n = 42$). Among PR3- patients, CCL23 levels showed significant differences between MPO + and MPO- subgroups ($p < 0.001$). However, no significant difference in CCL23 levels was observed between PR3- and PR3 + patients within the MPO- population (Fig. 1E).

Correlation of disease activity and ANCA phenotype with CCL23 level in patients with AAV

According to the median CCL23 levels, patients were divided into low- (CCL23 ≤ 10.39 ng/mL, $n = 159$) and high-CCL23 groups (CCL23 > 10.39 ng/mL, $n = 158$). The ESR and CRP levels, BVAS, FFS, and MPO + ratio in the high-CCL23 group were significantly higher than those in the low-CCL23 group (all $p < 0.001$, Supplementary Table S1). However, there was no significant difference in the proportions of MPA and GPA between the two groups.

Furthermore, we divided the patients with AAV into low-(BVAS < 15, $n = 255$) and high-disease-activity group (BVAS ≥ 15, $n = 62$) according to the standardized BVAS definition. The CCL23 level in the high-disease-activity group was significantly higher than that in the low-disease-activity group, and the CCL23 levels of both groups were significantly higher than the CCL23 level of the HC group (all $p < 0.0001$, Fig. 1F). According to the MPO antibody phenotype, the patients with AAV were divided into MPO + ($n = 191$) and MPO − group ($n = 126$). The CCL23 level of the MPO + group was significantly higher than that of MPO- group, and the CCL23 levels of both groups were significantly higher than the CCL23 level of the HC group (all $p < 0.01$, Fig. 1G). Meanwhile, we observed that the BVAS of patients in the MPO + group was significantly higher than that in the MPO- group ($p < 0.0001$, Fig. 1H). This result confirms that elevated CCL23 in MPO + patients may be associated with higher disease activity. Since it remains unclear whether the elevated levels of CCL23 in MPO + patients are attributable to the MPO ANCA phenotype or heightened disease activity, the patients were further stratified into four subgroups based on MPO phenotype and disease activity (MPO- Low BVAS group, $n = 116$; MPO- High BVAS group, n = 10; MPO + Low BVAS group, $n = 139$; MPO + High BVAS group, $n = 52$). In the MPO + patients, CCL23 levels were significantly higher in the high-disease-activity group than in the low-disease-activity group ($p < 0.0001$). However, no difference in CCL23 levels was observed between the MPO − and MPO + subgroups, neither in the low disease activity group nor in the high disease activity group (Fig. 1I).

To exclude the influence of MPO antibodies and further explore the relationship between CCL23 levels and disease activity, we analyzed the correlation between traditional disease activity indicators and CCL23 levels in all the patients, MPO − patients, and MPO + patients respectively. As shown in Figs. 2A–C, CCL23 level was significantly positively associated with BVAS, CRP, and

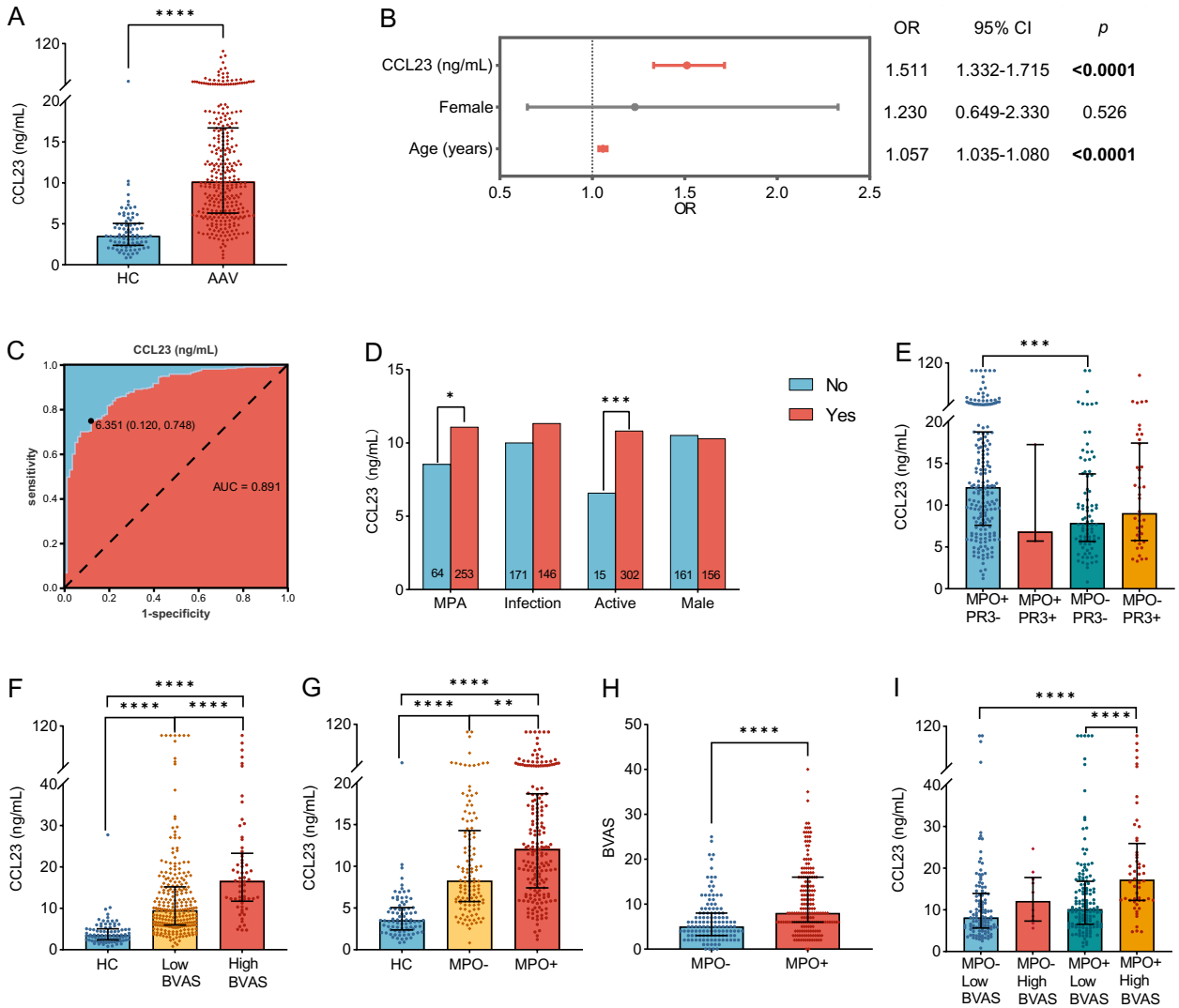

Fig. 1 Serum CCL23 levels were elevated in patients with AAV. **A** Comparison of CCL23 levels in HCs and patients with AAV. **B** Binary logistic regression analysis of variables at diagnosis for patients with AAV. OR adjusted for age, gender, and CCL23 levels. **C** ROC curves that CCL23 differentiate AAV patients from HCs. **D** CCL23 levels in different subgroups of patients with AAV. **E** CCL23 levels across ANCA-based subgroups of patients with AAV. **F** Comparison of CCL23 levels in HCs, low-disease-activity group, and high-disease-activity group. **G** Comparison of CCL23 levels in HCs, MPO−, and MPO + group. **H** Comparison of BVAS in MPO − and MPO + group. **I** CCL23 levels were compared between MPO- Low BVAS, MPO- High BVAS, MPO + Low BVAS, and MPO + High BVAS groups.Symbols represent the subjects, the bars show the median and interquartile range. The numbers on the bar chart represent the number of subjects. * represents significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$), and ns means no difference. Abbreviations: HC, healthy control; AAV, antineutrophil cytoplasmic antibody-associated vasculitis; OR, odds ratio; MPO, myeloperoxidase; PR3, proteinase 3; MPA, microscopic polyangiitis

ESR (all r > 0.314, all $p < 0.001$) in all the patients, MPO- patients, and MPO + patients.

CCL23 levels in different statuses of disease activity

To determine whether the CCL23 levels varied with disease status, we randomly screened 18 patients with active diseases and measured the levels of CCL23 in the same manner as after their disease went into remission. The serum CCL23 levels were compared before and after induce remission. The results showed that the CCL23 levels decreased significantly after induced remission ($p < 0.001$, Fig. 2D). In accordance with the 2022 EULAR guidelines, we screened 16 patients in remission (absence of the typical signs, symptoms, or other features of active AAV with or without immunosuppressive therapy) for the remission group and 15 patients in relapse (recurrence of active AAV after a period of remission) for the relapse group [20]. During sample collection,

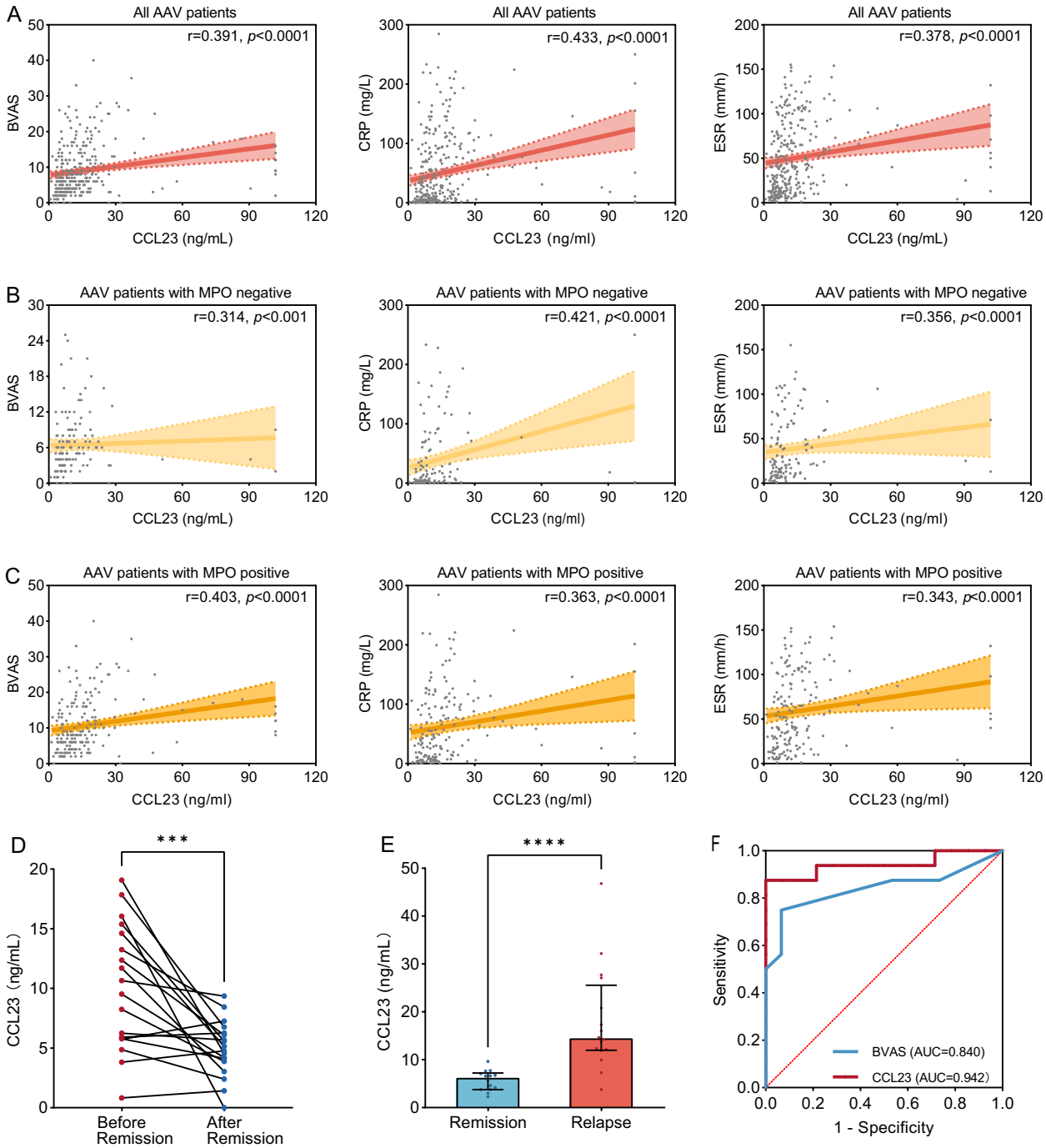

Fig. 2 Increased CCL23 levels are associated with disease activity in patients with AAV. **A** Correlation of CCL23 level with BVAS, CRP, and ESR in all the patients with AAV. **B** Correlation of CCL23 levels with BVAS, CRP, and ESR in MPO – patients with AAV. **C** Correlation of CCL23 level with BVAS, CRP, and ESR in MPO + patients with AAV. **D** CCL23 levels in paired samples from patients with AAV before and after treatment remission. **E** Comparison of CCL23 levels in the remission and relapse groups of patients with AAV. **F** ROC curves showing that CCL23 facilitated differentiation between patients with AAV in relapse and those in remission. Symbols represent the subjects, the bars show the median and interquartile range. * represents significance (*** $p < 0.001$, **** $p < 0.0001$). Abbreviations: AAV, antineutrophil cytoplasmic antibody-associated vasculitis; MPO, myeloperoxidase; BVAS, Birmingham vasculitis activity score; CRP, creactive protein; ESR, erythrocyte sedimentation rate

no significant difference in glucocorticoid dosage was found between the two groups ($p=0.484$, Supplementary Table S2). Disease activity (BVAS) was significantly higher in the relapse group ($p<0.001$, Supplementary Table S2), which had significantly higher CCL23 levels than the remission group ($p<0.0001$, Fig. 2E). We performed ROC analysis on BVAS and CCL23 levels to distinguish patients in relapse from those in remission. The results showed that BVAS and CCL23 levels were effective in differentiating between patients in relapse and those in remission and CCL23 outperformed BVAS (AUC of CCL23 = 0.942, AUC of BVAS = 0.840, Fig. 2F).

CCL23 levels predicted the poor outcome of patients with AAV

To further investigate the association between CCL23 levels and disease prognosis in patients with AAV, we included 95 initial patients with AAV and tested CCL23 levels before treatment to prevent the influence of drug therapy (the clinical characteristics of 95 initial patients with AAV are shown in Table 1). All the included patients were followed up for 1 year. During the 1 year follow-up period, 17 patients (17.9% of the cohort; $n=95$) experienced severe clinical outcomes: 10 patients (58.8% of adverse events) progressed to ESRD, 3 (17.6%) developed acute respiratory failure necessitating mechanical ventilation, and 4 (23.5%) died from AAV complications. The effectiveness of baseline CCL23 levels in the prediction of poor prognosis was assessed on the basis of clinical outcomes at 1 year of follow up. The AUC of CCL23 was 0.775 (cutoff, 24.643 ng/mL; sensitivity, 0.529; specificity, 0.885), which was larger than the FFS (0.727; cutoff, 2.5; sensitivity, 0.5; specificity, 0.948; Fig. 3A). Subsequently, the Kaplan–Meier curves were constructed based on the determined cutoff values. High CCL23 levels (> 24.634 ng/mL) was linked to an increased risk of poor prognosis within 1 year of follow-up period (log-rank test, $p=0.0001$; Fig. 3B).

Association of serum CCL23 levels and organ or system involvement

The 95 initial patients were divided into different subgroups according to the involvement of organs or systems, and the CCL23 levels of the two subgroups were compared. CCL23 levels were significantly higher in patients with lung or renal involvement than in patients without lung or renal involvement (all $p<0.05$; Fig. 4A). No difference was found in other subgroups (Supplementary Figure S1A). Next, we compared the potential clinical parameters of the two subgroups with and without lung or renal involvement. No differences in these potential risk factors were found between the two subgroups (Table 2). To further determine whether elevated CCL23 level is a risk factor for renal and lung involvement in patients with AAV, we conducted a binary logistic regression analysis. As illustrated in Fig. 4B, for every 10 ng/mL increase in CCL23, the risk of renal involvement increased by 72.2% (OR = 1.722, $p=0.033$). However, CCL23 levels were not significantly associated with lung involvement ($p=0.056$).

Spearman correlation analysis was used in evaluating the relationship of CCL23 with the indicators of renal damage in patients with renal involvement of initial AAV. As demonstrated in Fig. 4C, CCL23 level was significantly positively correlated with serum creatinine level (r = 0.381, $p=0.009$) and negatively correlated with eGFR ($r=-0.382$, $p=0.01$) and serum albumin levels (r = −0.397, $p=0.006$). According to the median CCL23 level (13.75 ng/mL), the initial patients were divided into low- ($n=48$) and high-CCL23 group ($n=47$). The prevalence rate of renal involvement in the high-CCL23 group (59.6%) was significantly higher than that in the low-CCL23 group (37.5%; $p=0.031$; Fig. 4D).

CCL23 level and prognoses of patients with renal involvement

To further explore the relationship between CCL23 levels and disease prognosis in patients with renal involvement, we included 46 patients who were first diagnosed with AAV and complicated with renal damage and conducted a 1 year follow-up. Diseases that progressed to ESRD, multiple-organ failure, and death due to AAV complications within 1 year were marked as poor prognoses, whereas decreases in creatinine level and gradual stability were marked as good prognoses. The effectiveness of baseline CCL23 levels in predicting poor outcomes was assessed on the basis of outcomes at 1 year of follow up. As shown in Fig. 4E, the AUC of CCL23 was 0.761 (cutoff value, 28.407 ng/mL; sensitivity, 0.5; specificity, 0.938). Then, Kaplan–Meier curves were constructed according to the determined cutoff values. High CCL23 levels (> 28.407 ng/mL) were associated with increased adverse outcomes during 1 year follow-up (log-rank test, $p=0.0242$, Fig. 4F).

Discussion

The potential value of CCL23 as a biomarker for AAV was evaluated. We examined the CCL23 levels of patients diagnosed with AAV in comparison with those of HCs. CCL23 level was significantly elevated in patients with AAV and was more significantly elevated in patients had showed high disease activity, were MPO ANCA positive, and had renal damage. In addition, in all the patients with AAV and those with renal impairment, elevated CCL23 level was associated with poor disease prognosis. CCL23 as a serum biomarker has remarkable value in the

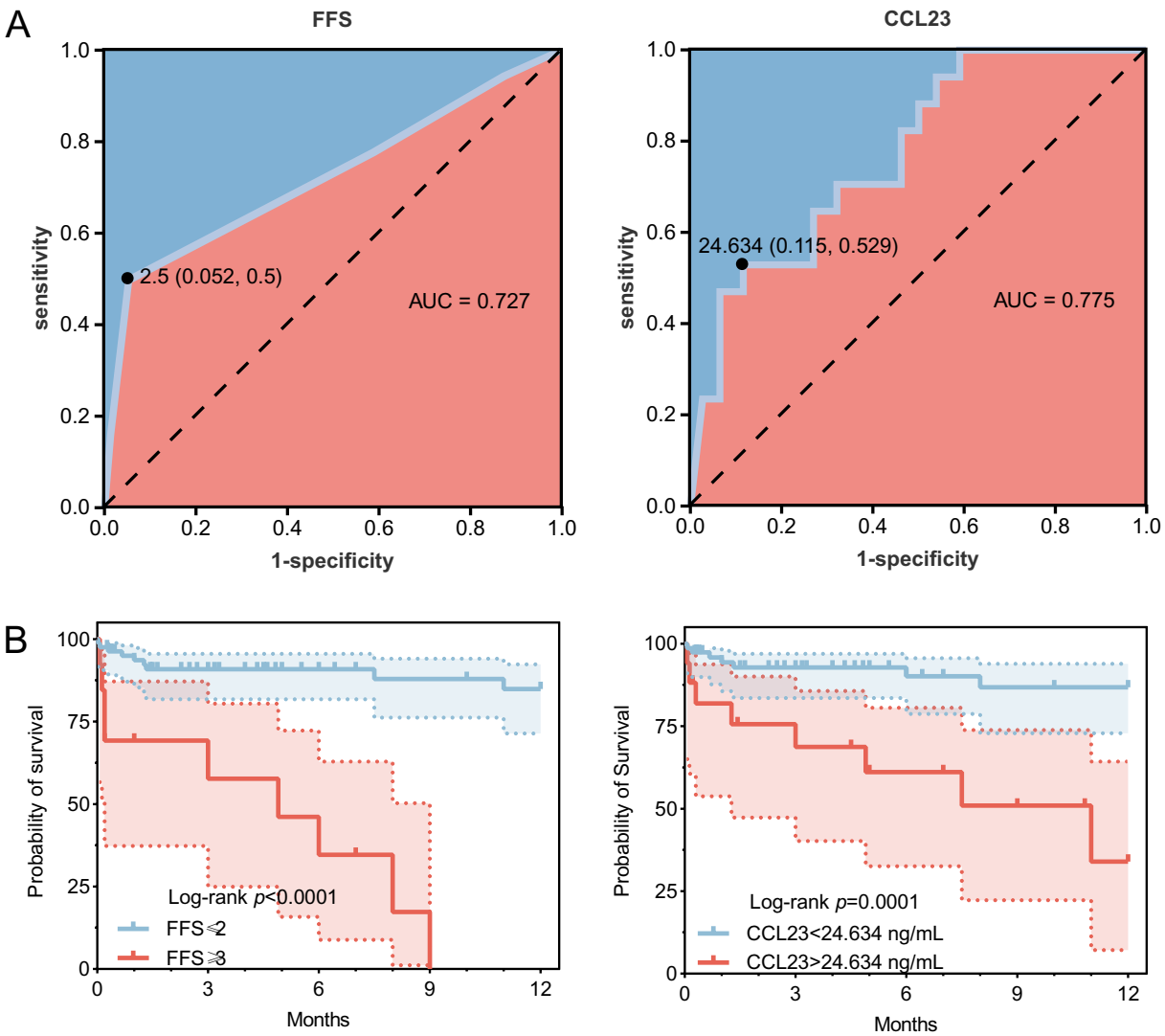

Fig. 3 Increased CCL23 levels are associated with poor prognosis in patients with AAV. **A** ROC curves showing that FFS and CCL23 levels predicted poor prognosis of AAV within 1 year of follow-up. **B** Kaplan–Meier curves constructed on the basis of the determined cutoff values for FFS and CCL23. Abbreviations: FFS, five-factor Score

diagnosis of AAV, disease activity, renal involvement, and prognosis evaluation.

CCL23, also known as myeloid progenitor inhibitory factor-1 (MPIF-1) or macrophage inflammatory protein (MIP)-3, was originally isolated from aortic endothelium and discovered by cDNA sequencing effort [19]. It is a specific ligand and potent agonist for CCR1 [19, 21], displaying chemotactic activity on resting T lymphocytes, monocytes, endothelial cells, dendritic cells, and neutrophils [19, 22–24]. CCL23 inhibited colony formation in the human bone marrow, granulocyte–macrophage, and erythroid [21]. We found that CCL23 was significantly positively correlated with peripheral blood neutrophil count and proportion in the patients with AAV and significantly negatively correlated with erythrocytes (Supplementary Table S3). These correlations seems to reflect the chemotaxis of CCL23 on neutrophils and its inhibitory effect on bone marrow erythrocytes.

CCL23 levels were significantly positively correlated with disease activity regardless of MPO antibody phenotype in patients with AAV. However, after patients the patients were stratified according to disease activity, no significant difference in CCL23 level was found between MPO − and MPO + patients (Fig. 1H). CCL23 levels increased in the synovial fluid of rheumatoid arthritis [25]. In addition, elevated serum CCL23 level is associated with disease activity [26]. Serum CCL23 levels are elevated and associated with disease activity in

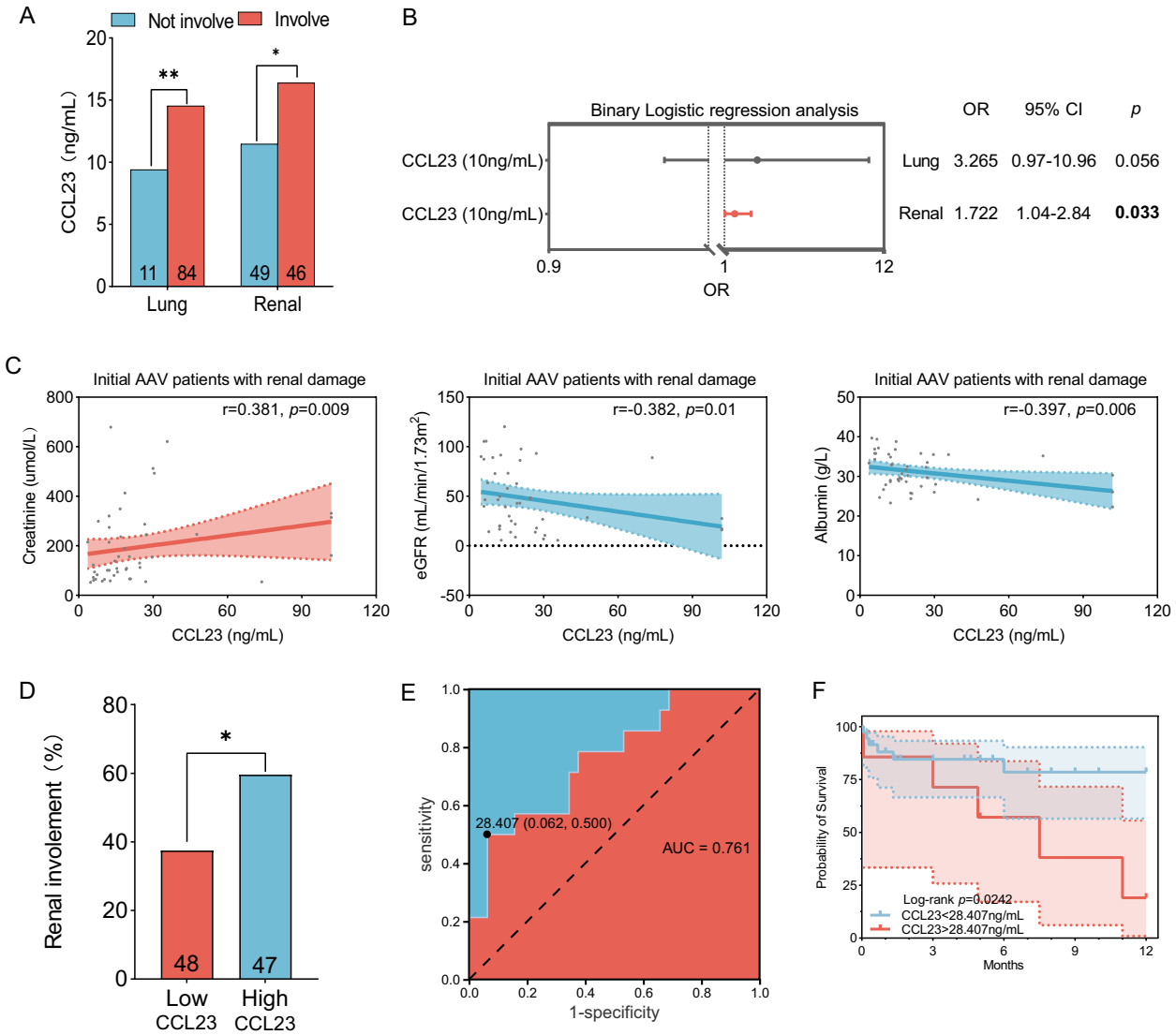

Fig. 4 Increased CCL23 levels are associated with renal involvement in patients with AAV. **A** CCL23 levels in different subgroups of patients with AAV. **B** Binary logistic regression analysis of CCL23 at diagnosis for lung and renal involvement. **C** Correlation of CCL23 level with serum creatinine, eGFR, and blood albumin levels in patients with renal damage in initial AAV. **D** Comparison of renal damage ratio between low- and high-CCL23 groups. **E** ROC curves showing that CCL23 levels predicted the poor prognosis of patients with AAV and renal damage within 1 year of follow-up. **F** Kaplan–Meier curves constructed on the basis of the determined cutoff values for CCL23. Symbols represent the subjects. The bars show the median. The numbers on the bar chart represent the number of subjects in this group. * represents significance (* $p < 0.05$, ** $p < 0.01$). Abbreviations: OR, odds ratio; eGFR, estimated glomerular filtration rate

patients with systemic sclerosis (SSc),and the expression of CCL23 in the dermal fibroblasts of patients with SSc is significantly higher than that in HCs [27]. In addition, CCL23 expression is significantly elevated in systemic lupus erythematosus with cardiovascular involvement [28] possibly because the mechanisms of injury in these disease are associated with the inflammation and regeneration of blood vessels [29–31]. CCL23 possibly plays an important role in angiogenesis. On the one hand, CCL23 up-regulates the expression of the KDR/Flk-1 receptor in endothelial cells, promotes VEGF-induced endothelial proliferation and migration, and helps to enhance the role of VEGF in angiogenesis [32]. On the other hand, CCL23 enhances the up-regulation of matrix metalloproteinase-2, thereby inducing endothelial cell migration and proliferation via CCR1, thus promoting vascular repair and regeneration [24]. The present study revealed that CCL23 is significantly associated with the disease activity of AAV. The possible reason is that blood vessel damage and repair are more active during disease activity.

Table 2 Characteristics of the participants according to renal and lung involvement

	Renal			Lung		
	Not involve ($n=49$)	Involve ($n=46$)	p	Not involve ($n=11$)	Involve ($n=84$)	p
Age, years	69 (62.5–75)	66.5 (57–75)	0.362	64 (45–74)	78 (62–75)	0.143
Male subjects, n (%)	22 (44.9%)	19 (41.3%)	0.836	7 (63.6%)	34 (40.5%)	0.257
Hypertension, n (%)	11 (22.4%)	12 (26.1%)	0.811	1 (9.1%)	22 (26.2%)	0.384
Diabetes, n (%)	1 (2.0%)	7 (15.2%)	0.052	0 (0%)	8 (9.5%)	0.590
Heart disease, n (%)	6 (12.2%)	4 (8.7%)	0.819	2 (18.2%)	8 (9.5%)	0.721
Cerebrovascular disease, n (%)	6 (12.2%)	5 (10.9%)	1.000	0 (0%)	11 (13.1%)	0.352
Smoking, n (%)	8 (16.3%)	7 (15.2%)	1.000	0 (0%)	15 (17.9%)	0.203
Alcohol consumption, n (%)	5 (10.2%)	2 (4.3%)	0.437	0 (0%)	7 (8.3)	1.000
Infection, n (%)	28 (57.1%)	22 (47.8%)	0.414	6 (54.5%)	44 (52.4%)	1.000
MPA, n (%)	38 (77.6%)	41 (89.1%)	0.173	10 (90.9%)	69 (82.1%)	0.684
GPA, n (%)	11 (22.4%)	5 (10.9%)		1 (9.1%)	15 (17.9%)	
MPO (+), n (%)	39 (79.6%)	40 (87.0%)	0.416	10 (90.9%)	69 (82.1%)	0.763
PR3 (+), n (%)	10 (20.4%)	5 (10.9%)	0.264	1 (9.1%)	14 (16.7%)	0.835
CRP(mg/L), M (IQR)	48.9 (8.9–117.5)	70.5 (19.2–123.3)	0.399	42.6 (17.2–86.8)	59.7 (14.8–125.3)	0.479
C3 (g/L), M (IQR)	1.2 (0.9–1.4)	1.1 (0.9–1.2)	0.212	0.9 (0.9–1.2)	1.2 (0.9–1.4)	0.213
C4 (g/L), M (IQR)	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.452	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.447
IgG (g/L), M (IQR)	14.5 (10.9–16.9)	14.1 (10.8–16.4)	0.771	12.7 (10.1–15.3)	14.5 (10.9–16.9)	0.424
CCL23 (ng/mL)	11.5 (8.5–18.0)	16.4 (11.3–26.8)	**0.02**	9.4 (5.7–12.2)	14.6 (9.8–20.8)	**0.007**

Values highlighted in bold represent statistically significant p-values ($p<0.05$)

Abbreviations: *n* number, *M* Median, *IQR* Inter-quartile range, *MPA* Microscopic polyangiitis, *GPA* Granulomatosis with polyvasculitis, *MPO* Myeloperoxidase, *PR3* Proteinase 3, *CRP* Creactive protein, *C3* Complement 3, *C4* Complement 4, *IgG* Immunoglobulin G

To date, how CCL23 plays a role in the disease development of AAV is unclear. The interaction of CCL23 with its chemokine receptor, CCR1, can stimulate pro-inflammatory cytokine production, including IL-1β, TNF-α, and MIP-1α [33]. Pro-inflammatory cytokines are important inflammatory mediators in AAV. Increased TNF-α and IL-1β levels were measured in patients with active AAV [34]. IL-1β produces an inflammatory effect that promotes the development of most autoimmune diseases [35, 36]. In an anti-MPO antibody–induced experimental model of ANCA necrotizing crescentic glomerulonephritis (NCGN), neutrophil serine proteases promote IL-1β generation and NCGN, and the absence of IL-1β or the interruption of the IL-1β signaling cascade prevents anti-MPO antibody–induced NCGN [37]. In vitro studies have shown that TNF-α can induce the membranes of neutrophils and monocytes to express the autoantigens PR3 and MPO, which are involved in vasculitis [38]. In the rodent models of nephritis, TNF-α enhances glomerular injury and TNF-α blockade using soluble TNF-α receptor or anti-TNF-α antibody ameliorates diseases [39–41]. Thus, CCL23 is involved in the pathogenesis of AAV and interacts with pro-inflammatory cytokines.

We found that CCL23 is closely related to renal damage. Serum CCL23 levels were significantly positively correlated with serum creatinine levels ($r=0.5314$, $p<0.0001$) and negatively correlated with eGFR ($r=-0.585$, $p<0.0001$) and serum albumin levels ($r=-0.345$, $p=0.001$) in patients with initial AAV (Supplementary Figure S1B). These associations remained significant when only patients with initial AAV and renal damage were analyzed, suggesting that elevated CCL23 levels were associated not only with renal involvement but also with the severity of renal damage. Moreover, high CCL23 levels at diagnosis were associated with poor prognosis during the 1 year follow-up in patients with initial AAV and renal involvement. In addition, MPO ANCA was closely related to renal damage, and the proportion of renal damage in MPO + patients was higher than that in the MPO − patients ($p=0.008$; Supplementary Table S4). CCL23 promotes the production of MPO ANCA and the development of nephropathy by promoting the production of IL-1β and TNF-α, and this function may be an important reason for the association between CCL23 with renal damage.

The strength of the present study is that it is the first to demonstrate the clinical implications of serum CCL23 levels at diagnosis and predicting poor prognosis during the follow-up of patients with AAV. High levels of CCL23 were significantly associated with high disease activity and the risk of relapse in AAV. Furthermore, elevated serum CCL23 levels were significantly associated with

renal involvement. When all the patients were included, we found that differences in serum CCL23 levels were evident in patients with or without renal involvement ($p < 0.0001$, Supplementary Table S5). Given the potential effect of drug therapy on CCL23 level, when only the initial patients were included, CCL23 differences remained significant in patients with or without renal involvement ($p < 0.05$, Fig. 4A). Increase in CCL23 level indicate increases in disease activity, risk of disease relapse, and severity of organ or system involvement, all of which may be important factors in poor disease prognosis.

The limitation of this study is that it is a single-center study despite the use of clinical data from a prospective observational cohort of AAV. In addition, our serum samples were mainly collected from inpatients in the department of rheumatology. Some patients with mild symptoms may not need hospitalization, and some patients were treated in other departments. These factors lead to certain inclusion sample selection bias. Finally, the number of patients with remission and relapse was small, and thus the value of CCL23 in assessing disease relapse was limited. CCL23 level begins to increase 5 years before the onset of AAV symptoms [17]. We look forward to prospective studies with more patients and series of clinical data and to studies exploring the pathogenesis of AAV to address the relationship between CCL23 level and AAV. In conclusion, the detection of CCL23 as a biomarker for AAV patients may be an important method to help early diagnosis of AAV and is of great value for evaluating disease activity and renal involvement after the onset of AAV and predicting disease prognosis.

Conclusion

Serum CCL23 levels significantly increased in patients with AAV and were closely related to the disease activity of AAV. CCL23 level can be used as an effective biomarker for identifying AAV relapse. In addition, elevated CCL23 is associated with renal damage and has a significant predictive effect on poor prognosis in patients with AAV. Thus, our findings supported further investigation of CCL23 as a biomarker of AAV and potential therapeutic target for AAV.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-025-03552-5.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.

Acknowledgements
We gratefully acknowledge the financial supports. We acknowledge assistance with the access of analytic instruments from Institute of Neuroscience and The Third Affiliated Hospital of Zhengzhou University, Translational Medicine Center the First Affiliated Hospital of Zhengzhou University, and Ophthalmology and the Laboratory for Ophthalmology and Vision Science at The First Affiliated Hospital of Zhengzhou University. Finally, we sincerely thank all the patients who participated in this study and the healthy volunteers.

Authors' contributions
Study conception and design: WL, WWH, and SYL. Subject recruitment and sample collection: WL, WWH, LZ, WJG, WJC, and FD. Experimental operation and record： WWH, QQL, XPL, and YRX. Data analysis and draft writing: WWH. Reviewing and revising of the manuscript: WL and SYL. All authors read and approved the final manuscript.

Funding
The work was supported by the Chinese National Key Technology R&D Program, Ministry of Science and Technology (2021YFC2501303).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the institutional review board of the First Affiliated Hospital of Zhengzhou University (2021-KY-0486). Informed consent was obtained from all study participants.

Consent for publication
All authors have approved the manuscript and agree with submission to *Arthritis Research & Therapy*.

Competing interests
The authors declare no competing interests.

Received: 26 January 2025 Accepted: 31 March 2025
Published online: 10 April 2025

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