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ORIGINAL RESEARCH

Cytokine Signatures as Biomarkers of Clinical Remission in Rheumatoid Arthritis

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Objective: Variations in cytokine levels have been observed in patients with rheumatoid arthritis (RA), which contribute to immune dysfunction. This study aimed to investigate the potential of intricate cytokine networks for predicting the clinical remission of RA.

Methods: In total, 164 patients with RA and 69 healthy individuals were included in this study. We investigated the levels of interleukins (ILs, including IL1β, IL2, IL4, IL5, IL6, IL8, IL10, IL12P70, and IL17), interferons (IFNs, including IFNα and IFNγ), tumor necrosis factor-alpha (TNFα), and immunoinflammatory markers, and subsequently analyzed their association and diagnostic potential in RA remission.

Results: In all patients with RA, the prevalence of the release of more than six or seven cytokines was 25.0% or 18.9%, respectively, and presented nearly or the highest consistency with the prevalence of non-remission RA (*Kappa*=0.678 or 0.682, respectively, $P<0.001$). All the 12 cytokines examined were significantly associated with non-remission of RA in both Spearman correlation analysis (ρ=0.28~0.58, $P<0.017$) and univariate logistic regression analysis (*OR*=1.005~1.546, all $P<0.05$). However, multivariate analysis identified only IL-6, IL12P70, and TNFα as independently associated with non-remission RA (*OR*=1.003~1.460, all $P<0.05$). For the diagnosis of clinical remission of RA, the release patterns of these three cytokines yielded areas under the curve of 0.941 and 0.926 in the modeling and validation groups, respectively, with sensitivities of 88.9% and 87.0% and specificities of 87.5% and 87.9%, respectively.

Conclusion: Our study suggests that IL6, TNFα, and IL12P70 may plot a cytokine release pattern for non-remission of RA, and are associated with its initiation, progression, and manifestation.

Keywords: cytokine release, rheumatoid arthritis, clinical remission, prediction, pattern

Introduction

Rheumatoid arthritis (RA) is a lifelong chronic inflammatory and autoimmune rheumatic disease characterized by synovial inflammation and cartilage destruction.[1] Furthermore, RA leads to systemic connective tissue inflammation and autoimmune complications, such as interstitial pneumonia, chronic kidney disease, and RA-associated autoimmune liver diseases.[2–6] The primary goal of RA treatment is to achieve remission and minimize disease activity.[7] The American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) have established classification criteria for RA, including the 1987 ACR and 2011 ACR/EULAR classification criteria.[8,9] In current clinical practice, patients with RA presenting with persistent and/or erosive inflammation can be identified early according to the following four observations: number and location of involved joints, presence of abnormal serological autoantibodies, elevated levels of acute-phase reactants, and duration of synovitis.

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Additionally, the ACR and EULAR have introduced RA remission criteria, such as the 1987 ACR criterion, Disease Activity Score (DAS)/DAS28, Simplified Disease Activity Index (SDAI), Clinical Disease Activity Index (CDAI), and the 2011 ACR/EULAR Boolean criterion.[10–12] However, there are concerns regarding the sensitivity of the 1987 ACR criterion. Furthermore, both the DAS and DAS28 criteria are considered more lenient and may lead to underestimation of disease activity. Additionally, both the SDAI and CDAI simply streamline calculations from the DAS/DAS28 criterion. Therefore, because of its enhanced rigor, the most widely accepted remission criterion is the 2011 ACR/EULAR Boolean criterion.[12] The current core indicators were optimized based on the 2011 ACR/EULAR Boolean criterion, including joint swelling/tenderness count ≤ 1, serum level of C-reactive protein/erythrocyte sedimentation rate returned to normal range, morning stiffness < 15 minutes, no extra-articular manifestations, and VAS pain score ≤ 1 (out of 10). In addition, the EULAR released a consensus on the assessment of imaging progress[13] and suggested a comprehensive judgment by integrating laboratory indicators with imaging examinations in clinical practice.

RA pathogenesis is associated with immune dysfunction, which is characterized by the aberrant activation of T and B lymphocytes. These activated immune cells synthesize and secrete various cytokines to perform diverse functions, including the regulation of immune responses, hematopoiesis, cell proliferation, and tissue repair. Based on their specific roles, cytokines can be classified as interleukins (ILs), interferons (IFNs), tumor necrosis factor superfamily members (TNFs), colony-stimulating factors, chemokines, and growth factors.[14,15] Over the past five decades, the relationship between cytokines and RA has been extensively explored. Multiple cytokines have been associated with early diagnosis, prognosis, treatment management, and synovial pathological manifestations of RA.[16–19] These findings have gradually revealed and refined the characteristics of the cytokine network in patients with RA.[20,21]

Multiple cytokine release (MCR) is common in patients with non-remission RA and has a profound effect on patient health. Recently, the cytokine storm experienced by patients with COVID-19 pneumonia has refreshed our understanding of the detrimental effects associated with MCR.[22,23] Fortunately, although patients with RA also exhibit MCR, they rarely develop serious conditions. Owing to the complex synergistic or antagonistic interactions among cytokines, it remains uncertain which cytokine release pattern holds a more significant diagnostic potential for non-remission RA.

Methods

Ethical Review

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of Mianyang Central Hospital (approval no. S2022092, date Aug. 27, 2022). All the participants provided written informed consent.

Patients

Patients newly diagnosed with RA between January and December 2023 at the Mianyang Central Hospital, School of Medicine, University of Electronic Science and Technology of China were enrolled in this study. The inclusion criteria were as follows: 1) the diagnosis of RA met the 2011 ACR/EULAR criteria[8,9] and 2) all participants underwent concurrent 12 cytokines and complete blood count (CBC) assays. The exclusion criteria were as follows: 1) patients with evident joint injury; 2) patients with gouty arthritis or osteoarthritis caused by wound, infection, or joint overloading; 3) patients complicated with other immune system diseases; co-infections; malignant tumors; cardiovascular, pulmonary, hepatic, or renal diseases; hypertension; or fatty liver disease; 4) patients who had received glucocorticoid therapy within one month prior to the 12 cytokines assay; and 5) pregnant women. Following RA diagnosis, all patients received conventional synthetic disease-modifying antirheumatic drugs (csDMARDs). During this period, patients who transitioned to treatment with biological disease-modifying antirheumatic drugs (bDMARDs) due to an insufficient response to csDMARDs were also excluded. Simultaneously, according to the principle of matching the age range and sex ratio of patients with RA as much as possible, healthy individuals who visited our hospital for general medical examinations with normal liver, kidney, cardiac, and pulmonary function tests were randomly selected as the healthy control (HC) group.

Sample Collection and Preprocessing

After 12 weeks of treatment, two aliquots of 3.0 mL fasting venous blood were collected early in the morning using an SST-II vacuum tube (BD, USA) and an EDTA-2K vacuum tube (BD, USA). The EDTA-2K vacuum tube was reversed five times back and forth for adequate anticoagulation. The SST-II vacuum tube was centrifuged at 4000 rpm (approximately 1500×g) for 10 min to separate the serum. If not immediately analyzed within 4 hours, the serum was stored at −20°C for <7 days.

Before flow cytometry, the serum was preprocessed using a Cytokine Multiplex Detection Kit (CatNo: P010100403, CERGER, CellGene Biotech Co., Ltd., CHN), which designed a 12-color flow cytometry scheme using four types of luciferases and three different sizes of microspheres. Microspheres of identical size conjugated with the same luciferase were coated with the same cytokine-specific antibody that could capture the target cytokines present in the serum. Serum preprocessing was performed according to the manufacturer's instructions.

Multiplex Cytokine Detection by Flow Cytometry

The resuspended microspheres were analyzed using a NAVIOS flow cytometer (Beckman Coulter, USA). Under excitation light at 488 nm and absorption light at 576 nm and 660 nm, 12 cytokines were detected, comprising nine ILs, two IFNs, and one TNF. The kit instruction provided lower and upper detection limits of 2.0 pg/mL and 2500 pg/mL for IL17, and 0.2 pg/mL and 2500 pg/mL for the other cytokines (IL1β, IL2, IL4, IL5, IL6, IL8, IL10, IL12P70, IFNα, IFNγ, and TNFα). The simultaneous release of more than three cytokines is generally considered a manifestation of the release of multiple cytokines. To ensure clarity, we introduced the term "numerical multiple cytokine release (N-MCR)" to describe scenarios involving the release of N cytokines, where N ranges from 3 to 12.

CBC and Immunoinflammatory Markers

CBC was performed using a Sysmex XN-9000 blood cell analyzer (Sysmex, JPN) within 2 hours of sampling. Leukocytes and their differential counts were determined using hydrodynamic focusing and flow cytometry, respectively. The platelet count was determined using the sheath flow method. Referring to the hotspots of immunoinflammatory markers in the past decade, we calculated the following immunoinflammatory biomarkers: neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), and systemic inflammation index (SII).[24,25] The SII was calculated as the product of the platelet count and neutrophil count divided by the lymphocyte count.

Evaluations of Clinical Remission

The 2011 ACR/EULAR Boolean criteria were used to evaluate clinical remission in patients with RA. Patients with RA were considered to be in clinical remission if they met either of the following two criteria: 1) tender joint counts, swollen joint counts, C-reactive protein level, and overall patient score are all ≤1; 2) SDAI≤3.3.[12] The C-reactive protein assay was used for the EDTA-anticoagulated blood samples and was conducted simultaneously with CBC measurements on an XN-9000 blood cell analyzer (Sysmex, JPN). The SDAI was calculated as the sum of the tender joint counts, swollen joint counts, C-reactive protein levels, and global scoring from patients and doctors on disease activity (0~10 scores).

Statistical Analysis

MedCalc software v20.1 (MedCalc, Belgium) and SPSS software v22.0 (IBM Corp., USA) were used for statistical analysis. Continuous data are presented as *median* (*Q1, Q3*). The Kruskal–Wallis test and its all-pairwise comparison option were employed to achieve multi-group differences and post-hoc analyses. Adjusted P-values (*Padj*) from post-hoc analyses were used to determine the statistical significance of pairwise comparisons. A Spearman correlation heat-plot was used to illustrate the bivariate correlation among cytokines, immunoinflammatory markers, MCR, and non-remission RA. The McNemar test and Kappa test were used to analyze the differences and consistency in prevalence between MCR and non-remission RA, respectively. The bivariate correlation was analyzed using the Spearman correlation plot with the Bonferroni correction of significance level $\alpha=0.05/3\approx0.017$. After conducting a variance inflation factor > 10 test for multicollinearity, univariate and stepwise multivariate logistic regression analyses were performed to analyze the association of cytokines and immunoinflammatory markers with non-remission RA. After dividing the RA group data

into a 70% modeling cohort and a 30% validation group, logistic regression was used to develop a prediction model for non-remission RA using its independent associated variables. A ROC curve was used to illustrate the area under the curve (AUC) of cytokines to predict non-remission RA. A sensitivity/specificity threshold plot was used to illustrate the changes in sensitivity/specificity with the threshold. Except for the significance level for Spearman correlation coefficient (ρ) that was corrected at $\alpha=0.05/3\approx0.017$ because of three group comparisons, the other significance level was set at $\alpha=0.05$.

Results

Levels of Cytokines and Immunoinflammatory Biomarkers Among HC, Remission RA, and Non-Remission RA Groups

A total of 164 patients with RA participated in this study, including 45 men and 119 women, with ages ranging from 33 to 88 years. Based on clinical remission, patients with RA were divided into a remission RA group (n=119) and a non-remission RA group (n=45). Simultaneously, an HC group consisting of 69 individuals, comprising 31 men and 38 women, with ages ranging from 35 to 84 years, was included.

As shown in Table 1, there were no differences in the ratio of men ($\chi2=5.464$, $P=0.065$) and age ($\chi2=1.062$, $P=0.479$) among the HC, remission RA, and non-remission RA groups. However, the levels of 12 cytokines and four

Table 1 Cytokine Levels and Other Laboratory Marker Levels in RA Patients

Item	HC (n=69)	Non-Active RA (n=119)	Active RA (n=45)	χ^2	P
Male [n (%)]	31 (44.9)	39 (32.8)	11 (24.4)	5.464	0.065
Age (years old)	58 (51, 68)	58 (53, 69)	57 (53, 65)	1.062	0.479
IL1β (pg/mL)	0.91 (0.36, 1.47)	2.04 (0.99, 4.38)$^{\Delta}$	18.58 (5.88, 41.37)$^{\Delta\blacktriangle}$	1.398	<0.001
IL2 (pg/mL)	0.76 (0.29, 1.33)	1.81 (0.88, 3.31)$^{\Delta}$	20.73 (8.85, 44.49)$^{\Delta\blacktriangle}$	86.808	<0.001
IL4 (pg/mL)	0.82 (0.47, 1.56)	1.58 (0.76, 2.70)$^{\Delta}$	12.36 (4.39, 23.84)$^{\Delta\blacktriangle}$	87.240	<0.001
IL5 (pg/mL)	0.3 (0.14, 0.62)	0.56 (0.27, 1.21)$^{\Delta}$	2.68 (1.34, 5.97)$^{\Delta\blacktriangle}$	67.325	<0.001
IL6 (pg/mL)	2.9 (2.2, 4.8)	11.1 (3.7, 43.3)$^{\Delta}$	56.9 (29.4, 220.2)$^{\Delta\blacktriangle}$	65.806	<0.001
IL8 (pg/mL)	13.6 (7.1, 21.2)	14.6 (10.1, 22.0)$^{\Delta}$	34.1 (18.0, 95.7)$^{\Delta\blacktriangle}$	75.578	<0.001
IL10 (pg/mL)	1.85 (1.19, 2.79)	2.54 (1.68, 4.12)$^{\Delta}$	8.62 (4.95, 25.01)$^{\Delta\blacktriangle}$	29.288	<0.001
IL12P70 (pg/mL)	1.27 (0.53, 2.33)	2.47 (1.19, 3.97)$^{\Delta}$	9.66 (4.76, 14.88)$^{\Delta\blacktriangle}$	70.229	<0.001
IL17 (pg/mL)	5.60 (2.10, 10.70)	6.91 (3.03, 13.06)$^{\Delta}$	19.58 (7.48, 32.61)$^{\Delta\blacktriangle}$	62.851	<0.001
IFNα (pg/mL)	0.59 (0.29, 1.05)	1.40 (0.61, 3.08)$^{\Delta}$	12.97 (5.44, 23.17)$^{\Delta\blacktriangle}$	24.169	<0.001
IFNγ (pg/mL)	0.99 (0.60, 1.88)	1.72 (0.95, 2.86)$^{\Delta}$	3.04 (1.45, 6.88)$^{\Delta\blacktriangle}$	85.678	<0.001
TNFα (pg/mL)	1.21 (0.63, 2.16)	2.34 (1.28, 4.30)$^{\Delta}$	25.9 (11.8, 41.8)$^{\Delta\blacktriangle}$	26.480	<0.001
MLP	0.220 (0.151, 0.315)	0.312 (0.205, 0.418)$^{\Delta}$	0.312 (0.206, 0.365)$^{\Delta}$	81.280	0.004
NLP	2.74 (1.72, 4.60)	3.79 (2.77, 5.39)$^{\Delta}$	3.79 (3.15, 6.85)$^{\Delta}$	10.936	0.001
PLR	129 (89, 166)	179 (119, 246)$^{\Delta}$	179 (147, 246)$^{\Delta}$	14.474	<0.001
SII	488 (308, 854)	838 (470, 1397)$^{\Delta}$	853 (725, 1501)$^{\Delta}$	21.384	<0.001
SCDI (score)	NA	3 (2, 4)	7 (6, 8)$^{\blacktriangle}$	2.410	0.010

Notes: Using the Chi-square test for male ratio, the Mann–Whitney test for EULAR Score, and the Kruskal–Wallis test for the else. $^{\Delta}$vs HC, *Padj*<0.05; $^{\blacktriangle}$vs Non-active RA, *Padj*<0.05.

immunoinflammatory biomarkers exhibited significant differences among the three groups ($\chi 2$=5.464, all P<0.01). Compared to the HC group, the levels of all cytokines and immunoinflammatory biomarkers were significantly increased in the non-remission RA group (z=2.397~9.339, all $Padj$<0.05) as well as in the remission RA group, except for IL8 and IL17 levels (z= 2.837~6.071, all $Padj$<0.05). Furthermore, when comparing the non-remission and remission RA groups, significant increases were observed in the levels of all cytokines (z= 3.338~6.600, all $Padj$<0.05), whereas no differences were observed in any of the immunoinflammatory biomarkers (all $Padj$>0.05).

Prevalence of Various MCR Patterns and Non-Remission RA in Patients with RA

Among all the patients with RA included (n=164), the prevalence of non-remission RA was 22.0% and the occurrence of 3-MCR to 12-MCR ranged degressively from 46.3% to 2.4% (Figure 1A). McNemar's test revealed no differences in the prevalence between 6-MCR/7-MCR and non-remission RA (P=0.359 and 0.332, respectively); however, significant differences were observed between other MCR patterns and non-remission RA (all P<0.05). The Kappa test revealed significant consistency in the prevalence between various MCR patterns and non-remission RA ($Kappa$=0.163~0.682, all P<0.001), with nearly the highest consistency between 6-MCR/7-MCR and non-remission RA ($Kappa$=0.678 with 95% CI of 0.545~0.811 and 0.682 with 95% CI of 0.543~0.821, respectively; both P<0.001) (Figure 1B). These findings suggest that MCRs are common among patients with RA and may be causally linked to RA remission.

Bivariate Correlation Among Cytokines, Immunoinflammatory Biomarkers, 6-MCR/7-MCR, and Non-Remission RA

Using the data from the patients with RA (n=164), the bivariate Spearman correlation plot with a Bonferroni correction of significance level α=0.05/3≈0.017 was used to analyze the correlations among cytokines and immunoinflammatory biomarkers, as well as their association with the prevalences of 6-MCR/7-MCR and non-remission RA (Figure 2). Significant correlations were observed among all the immunoinflammatory biomarkers (ρ=0.33~0.83, all P<0.017), as well as among all the cytokines (ρ=0.20~0.96, all P<0.017). As for the correlations between cytokines and immunoinflammatory biomarkers, apart from a significant correlation observed between IL-6 and MLR (ρ=0.25, P<0.017), no other significant correlations were found between these biomarkers and cytokines (all P>0.017). In addition, all cytokines were significantly correlated with 6-MCR/7-MCR (ρ=0.38~0.72/0.34~0.68, all P<0.017) and non-remission RA (ρ=0.28~0.58, P<0.017), whereas none of the immunoinflammatory biomarkers showed such association (all P>0.017). These findings suggested a significant association between MCR and non-remission RA.

Independent Correlation of Cytokines to Non-Remission RA

Univariate logistic regression analysis (Table 2) revealed a positive association between all 12 cytokines and non-remission RA (OR= 1.005~1.546, all P<0.05), whereas none of the immunoinflammatory biomarkers exhibited a significant association with non-remission RA (all P>0.05). Further multivariate logistic regression analysis adjusted for age and gender revealed that only three risky cytokines (IL6, IL12P70, and TNFα; OR=1.003~1.460, all P<0.05) independently correlated to non-remission RA (Table 2). These findings suggested that these three cytokines play crucial roles in the non-remission stage of RA.

Cytokine Release Pattern for Predicting Clinical Remission of RA

Based upon the aforementioned findings, IL6, IL12P70, and TNFα were used for predicting clinical remission of RA. In the modeling cohort (n=114), the predictive powers of TNFα and IL12P70 alone, with AUC of 0.894 and 0.884 respectively, were found to be superior to that of IL6 (z=2.717 and 2.109, P=0.007 and 0.035, respectively). The combined model demonstrated a statistically significant improvement in predictive power, achieving an AUC of 0.941, compared to IL6, IL12P70, and TNFα alone (z=1.990, 2.640, and 4.260, P=0.048, 0.008, and <0.001, respectively; Figure 3A). The optimal sensitivity and specificity of the combined model were 88.9% and 87.5%, respectively, both of which exceeded those of the three cytokines alone (Figure 3B–E). In the validation group (n=50), the combined model

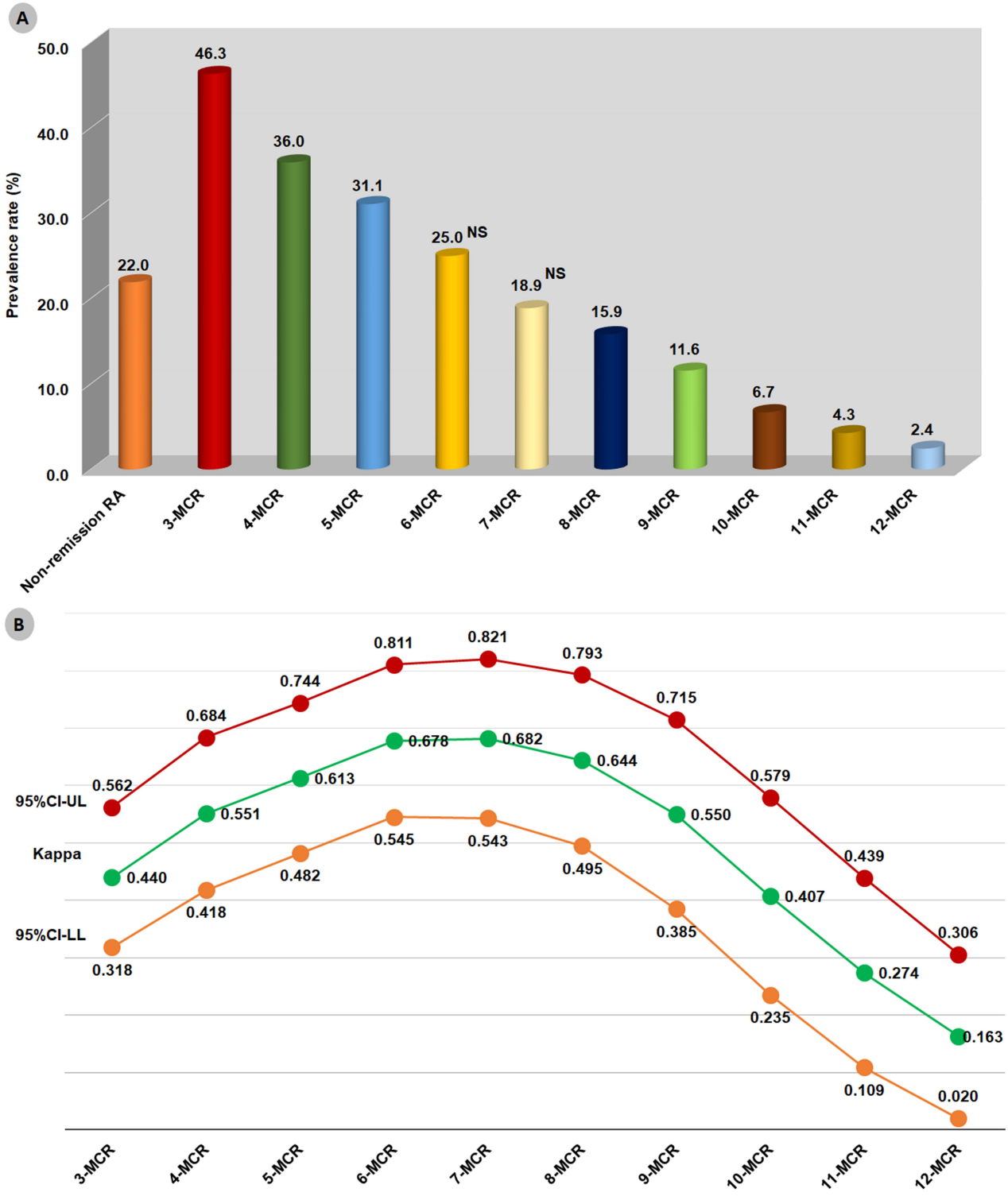

Figure 1 Prevalence of non-remission RA/MCR and their consistency.
Notes: NS, vs the prevalence of non-remission RA, P>0.05. (**A**) Prevalence of non-remission RA and multiple cytokine release; (**B**) Consistency between non-remission RA and various multiple cytokine release patterns. There was a nearly or highest consistency in prevalence between 6-MCR/7-MCR and non-remission RA, with no significant differences observed.

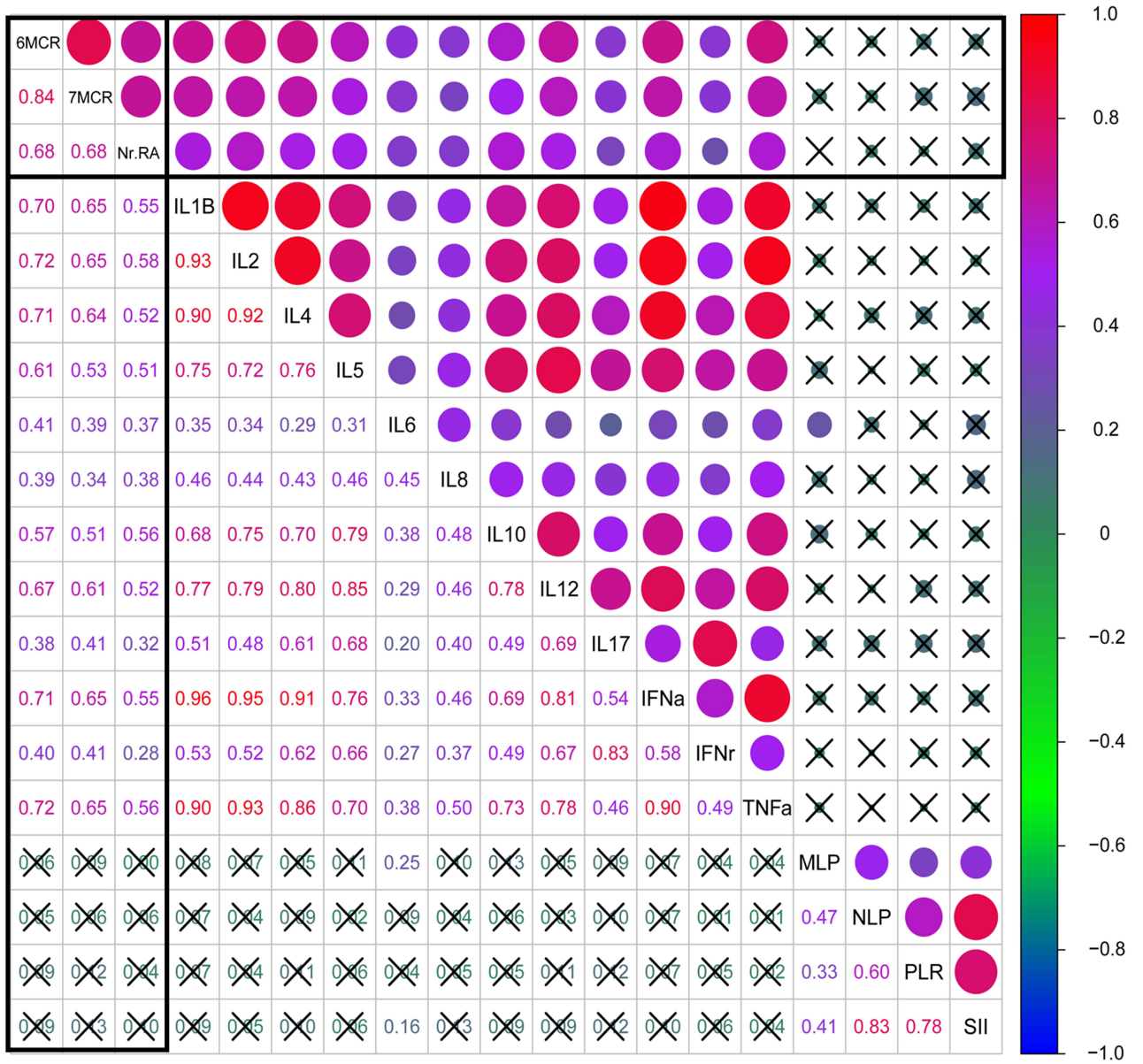

Figure 2 Bivariate Spearman correlation between cytokines/immunoinflammatory biomarkers and non-remission RA/6- or 7-MCR.
Notes: Nr. RA, non-remission RA. The "×" sign indicates a significance greater than 0.017 (a significance level by Bonferroni correction). The upper right and lower left matrix displays the Spearman correlation heatmap and correlation coefficients, respectively. All 12 cytokines demonstrated an evident Spearman correlation with the non-remission RA and 6-MCR/7-MCR, while none of the 4 immunoinflammatory biomarkers did so.

demonstrated a predictive performance comparable to that of the modeling cohort (AUC=0.926 vs 0.941, z=0.369, P=0.712; Figure 3F), with optimal sensitivity and specificity of 87.0% and 87.9%, respectively.

Discussion

The simultaneous detection technique offers an efficient and reliable method for investigating the critical role of the intricate cytokine network in the clinical remission of RA. Our findings revealed a high prevalence of MCR in patients with RA, which is strongly associated with non-remission RA, thereby suggesting that MCR may serve as an immunopathological foundation for disease progression. Our subsequent analysis revealed that the combination of IL6, IL12P70, and TNFα demonstrated an exceptional predictive power for clinical remission of RA. However, immunoinflammatory markers (such as MLR, NLR, PLR, and SII), which have been a prominent focus in the past decade, did not

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Table 2 Univariate or Multivariate Association Between Cytokines/Immunoinflammatory Biomarkers and Active RA

Variables	Univariate analysis			Multivariate analysis			
	***OR* (95% CI)**	***Wald***	***P***	***OR* (95% CI)**	***Wald***	***P***	***VIF***
IL1β	**1.086 (1.051 to 1.122)**	**24.753**	**<0.001**	0.917 (0.760, 1.106)	0.825	0.364	7.341
IL2	**1.108 (1.064 to 1.154)**	**24.258**	**<0.001**	1.059 (0.872, 1.287)	0.337	0.562	3.013
IL4	**1.164 (1.093 to 1.239)**	**22.604**	**<0.001**	0.947 (0.712, 1.260)	0.139	0.709	8.490
IL5	**1.387 (1.146 to 1.678)**	**11.297**	**<0.001**	1.100 (0.925, 1.310)	1.159	0.282	1.478
IL6	**1.005 (1.002 to 1.007)**	**9.511**	**0.002**	**1.003 (1.001, 1.006)**	**5.434**	**0.020**	1.047
IL8	**1.023 (1.010 to 1.035)**	**12.993**	**<0.001**	1.010 (0.997, 1.023)	2.275	0.132	1.328
IL10	**1.313 (1.167 to 1.477)**	**20.561**	**<0.001**	1.020 (0.825, 1.261)	0.032	0.858	3.248
IL12P70	**1.546 (1.321 to 1.809)**	**29.533**	**<0.001**	**1.460 (1.207, 1.765)**	**15.205**	**<0.001**	2.006
IL17	**1.025 (1.004 to 1.047)**	**5.660**	**0.017**	0.998 (0.927, 1.075)	0.002	0.961	1.430
IFNα	**1.192 (1.115 to 1.274)**	**26.872**	**<0.001**	1.053 (0.757, 1.463)	0.093	0.760	4.663
IFNγ	**1.126 (1.023 to 1.240)**	**5.871**	**0.015**	0.928 (0.778, 1.106)	0.697	0.404	1.630
TNFα	**1.122 (1.077 to 1.168)**	**31.402**	**<0.001**	**1.070 (1.026, 1.116)**	**9.765**	**0.002**	2.999
MLP	0.487 (0.078 to 3.040)	0.593	0.441	0.017 (0.001, 3.606)	2.227	0.136	2.022
NLP	0.996 (0.922 to 1.075)	0.013	0.909	0.937 (0.686, 1.280)	0.166	0.684	3.957
PLR	1.001 (0.986 to 1.004)	0.408	0.523	0.995 (0.985, 1.004)	1.243	0.265	2.876
SII	1.000 (0.999 to 1.001)	0.722	0.395	1.002 (0.999, 1.004)	2.217	0.137	5.424

Notes: Adjusted by age and gender. Significance values are shown in bold. In the univariate analysis, all cytokines were observed to be positively associated with the occurrence of active RA. However, only IL6, IL12P70, and TNFα were retained in the stepwise multivariate analysis, and the results of the other variables were extracted from the enter multivariate analysis. None of immunoinflammatory biomarkers showed such association.

demonstrate a significant association. Therefore, cytokine detection can provide a straightforward and feasible evidence for predicting clinical remission in patients with RA.

Unlike most previous studies that focused on one or a few cytokines,[26–28] we measured 12 crucial cytokines that are commonly involved in immune response. Studies focusing on a single or few cytokines cannot account for the synergistic or antagonistic interactions among the complex and extensive network of cytokines, potentially overemphasizing their impact on disease remission in patients with RA. Therefore, a comprehensive analysis of the cytokine network is necessary. Although not all, the vast majority of the 12 cytokines investigated in this study play significant roles in the cytokine network, disease development, and disease outcome. A comprehensive analysis of these cytokines may reveal important cytokine signatures involved in disease remission in patients with RA. Our findings confirmed that a higher prevalence of the 6-MCR or 7-MCR pattern was almost consistent with the prevalence of non-remission RA. Although all 12 examined cytokines were associated with non-remission RA in the univariate analysis, only three cytokines (IL-6, IL-12p70, and TNFα) remained significant in the multivariate analysis. Therefore, in terms of immune dysregulation, this cytokine release pattern may have significant potential for predicting clinical remission of RA.

The cytokine network in RA pathogenesis have been extensively reviewed, highlighting the important roles of IL6 and TNFα.[29,30] Additionally, the development of biological anti-rheumatoid or anti-rheumatic drugs, such as TNFα and IL6 inhibitors and their receptor inhibitors, has made significant advancements in targeting synovial inflammation and preventing further cartilage destruction and bone erosion.[29–31] Therefore, these two cytokines were included in our prediction model as feature variables, as expected. However, our study also noticed that the predictive power of IL6 for

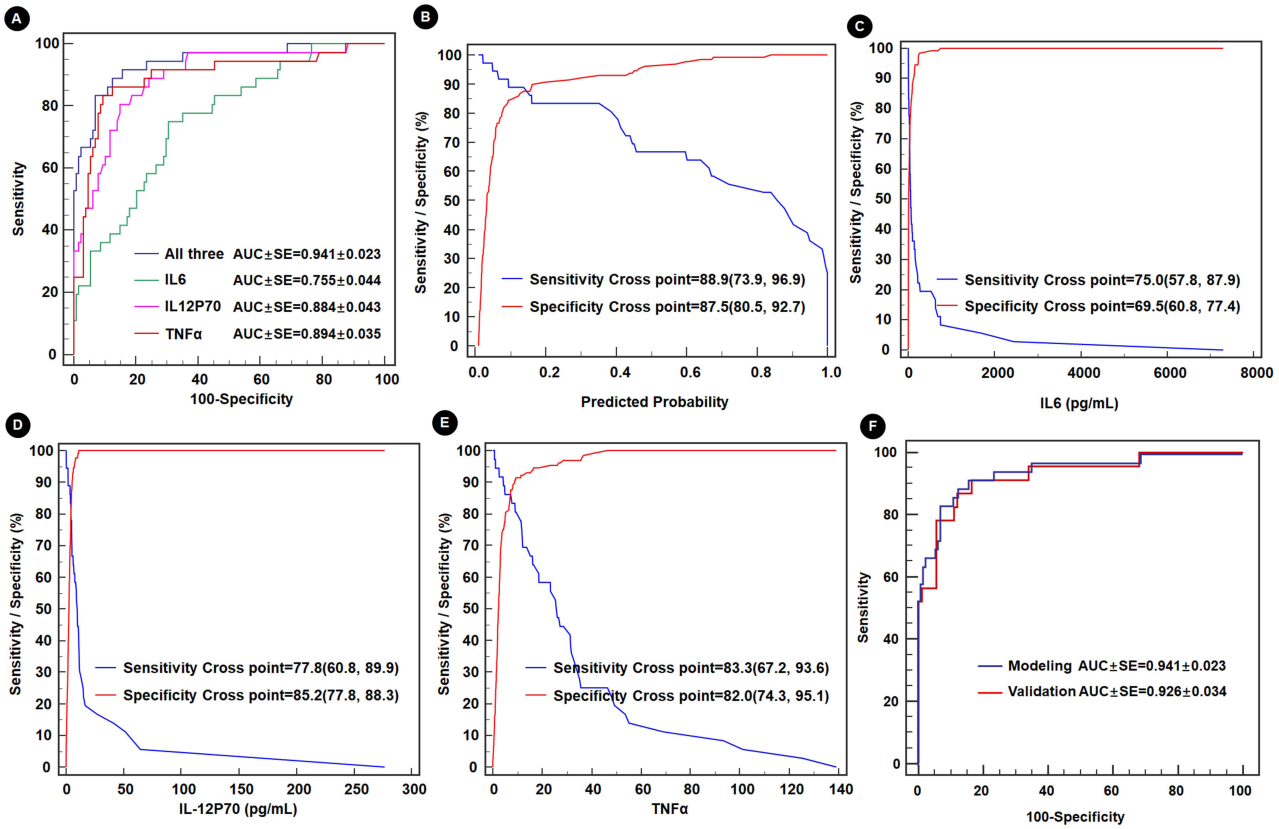

Figure 3 The predictive power of selected feature variables for non-remission RA.
Notes: (**A**) The ROC curve of three cytokines alone and in combination for predicting non-remission RA. (**B**–**E**) The sensitivity/specificity versus threshold plot of three cytokines alone and in combination. The crosspoint yielded the sensitivity and specificity at an optimal balanced trade-off condition. (**F**) The ROC curve of three cytokines combined-test in the modeling and validation groups.

clinical remission was significantly inferior to that of the other two feature variables (IL12P70 and TNFα). These weaknesses may be associated with its position in the cytokine network and interactions with other cytokines. The leading role of IL6 in the cytokine network has been extensively reported; it elicits a cascade of cytokine expression.[32–34] Our study revealed a more prominent and prevailing expression of IL6 during the remission stage of RA compared to other cytokines, which consequently attenuated its diagnostic power for non-remission RA. Nevertheless, the roles of IL6 and TNFα in the pathogenesis of RA cannot be disregarded.

Besides IL6 and TNF-α, this study also identified IL12P70 as an independent risk factor for non-remission RA, with a significant predictive power. IL12P70, a member of the IL12 family along with IL23, IL27, and IL35, consists of p35 and p40 subunits that exhibit unique homology to other members of the IL12 family in terms of subunit composition, receptor interactions, and signaling pathways.[35–37] Furthermore, IL12P70, which is categorized as Th1/Th17-related proinflammatory cytokines together with IL6, TNFα, IL17, and IFNγ, is released in response to stimulation by these cytokines; collectively, they contribute to an inflammatory environment, to synergistically enhance the differentiation of Treg cells into Th-like Treg cells.[38,39] IL12P70 has been proposed to be a crucial mediator of synovial inflammation during RA progression.[40,41]

Taken together, our combined model based on the three cytokines demonstrated high sensitivity and specificity in predicting clinical remission of RA. Among the three cytokines, IL6 can indicate the impending cascade release of cytokines, TNFα focuses on its crucial regulatory role in the cytokine network, and IL12P70 can predict the outcome of synovial inflammation. The three cytokines, so to speak, cover the entire progression of RA, thereby demonstrating the rationality of the prediction model.

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Limitations

The diagnostic differences between current core indicators and the ACR/EULAR criteria may have introduced bias into the results of this study. Treatment with bDMARDs may not be suitable for this cytokine signature, as it typically involves IL6 or TNFα inhibitors. Our conclusions were based on newly diagnosed patients treated with csDMARDs. However, whether these findings can be extrapolated to patients undergoing multiple courses of treatment, particularly those receiving targeted synthetic disease-modifying antirheumatic drugs, remains unclear. In addition, analyzing only 12 cytokines is insufficient to fully capture the overall signatures of disease remission in patients with RA. Therefore, further investigation is required.

Conclusions

Our study identified a composite pattern of three cytokines (IL6, TNFα, and IL12P70) that demonstrates excellent predictive power for the clinical remission of RA. In the cytokine network, IL6 is a vital and central player, TNFα plays a crucial regulatory role, and IL12P70 is proposed as a key mediator of synovial inflammation. Therefore, further exploration of these three cytokines in RA pathogenesis is crucial to establish a theoretical basis for clinical management.

Data Sharing Statement

The datasets in the study are available from the corresponding author upon reasonable request.

Ethics Approval Statement

This study was in accordance with the principles of the Declaration of Helsinki, and approved by the Ethics Committee of Mianyang Central Hospital (approval no. S2022092, date Aug. 27, 2022).

Patient Consent Statement

All participants signed informed consent.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors have declared that no competing interests exist in this work.

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