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Tumor necrosis factor alpha-induced protein3 rs;10499194 polymorphism enhances presepsin and sCD64 accuracy in differentiating infection from rheumatoid arthritis flare

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Abstract

Differentiating concurrent general microbial infection from disease flare in Rheumatoid Arthritis (RA) remains challenging. This study evaluated the diagnostic performance of Presepsin and soluble cluster of differentiation 64 (sCD64), and their association with the tumor necrosis factor-alpha-induced protein 3 (TNFAIP3) rs10499194 polymorphism. This case-control study included 90 participants: 30 normal controls, 30 RA without infection, and 30 RA with infection. Serum Presepsin and sCD64 were measured by ELISA, and gene detection for SNP TNFAIP3 rs10499194 (C>T) was performed using the tetra-primer amplification refractory mutation system polymerase chain reaction. Presepsin and sCD64 levels were significantly elevated in the RA with infection group compared to uninfected RA patients and controls (p<0.001). Presepsin (area under the curve, AUC=0.910) and sCD64 (AUC=0.870) outperformed conventional markers (CRP, ESR) in diagnosing infection. The combined biomarkers yielded an AUC of 0.956. The TNFAIP3 T allele was significantly associated with RA susceptibility (OR=2.87, p=0.028). Furthermore, T allele carriers exhibited a dose-dependent, significant increase in both Presepsin (p=0.005) and sCD64 (p=0.013) levels, particularly during infectious episodes. In conclusion, Presepsin and sCD64 are highly accurate biomarkers for distinguishing infection from RA flares. The TNFAIP3 rs10499194 T allele not only increases RA risk but also amplifies the innate immune response during concurrent infections.

Keywords: RA; Presepsin; sCD64; TNFAIP3; rs10499194 polymorphism; general microbial Infection; Biomarkers.

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Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by progressive synovial inflammation, cartilage destruction, and subsequent bone erosion.[1] Beyond its articular manifestations, RA may also be associated with neurocognitive impairment, with case-control evidence showing a higher risk of cognitive dysfunction in RA patients than in normal controls.[2] The global burden of RA is

substantial and steadily increasing. According to the Global Burden of Disease (GBD) 2021 study, an estimated 17.6 million individuals were living with RA worldwide in 2020, with an age-standardized prevalence of 208.8 per 100,000 population, representing a 14.1% increase since 1990.[3] In the Middle East and North Africa region, the prevalence mirrors this global trend, while in Iraq, epidemiological data from Babylon indicated a rising incidence of RA over the past decade, underscoring the growing regional health challenge.[4,5]

The pathogenesis of RA is driven by a complex interplay of genetic susceptibility and environmental triggers, culminating in an aberrant immune response mediated by pro-inflammatory cytokines, autoantibodies such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide (Anti-CCP), and the hyper-activation of signaling pathways like nuclear factor-kappa B (NF-κB).[6,7] Hormonal influences may also modulate disease activity, and oral contraceptive use was associated with reduced RA activity in women.[8]

Patients with RA face a significantly elevated risk of developing concurrent systemic infections compared to the general population. This heightened susceptibility is multifactorial, stemming from the intrinsic immune dysregulation characteristic of the disease itself, alongside the iatrogenic immunosuppressive effects of conventional and biological disease-modifying antirheumatic drugs (DMARDs).[9,10] Infections, particularly bacterial infections of the respiratory and urinary tracts, remain a leading cause of morbidity and premature mortality in the RA.[11] However, diagnosing concurrent bacterial infection in RA patients presents a formidable clinical challenge. Acute disease flares and infectious episodes share overlapping clinical manifestations, including fever, joint pain, and generalized fatigue. Furthermore, conventional acute-phase reactants such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell (WBC) counts are inherently elevated during active RA flares, rendering them insufficiently specific to reliably differentiate between autoimmune inflammation and superimposed bacterial infection.[12,13] This diagnostic uncertainty often leads to either delayed antimicrobial therapy or the inappropriate cessation of necessary immunosuppressive treatments, both of which can have deleterious consequences for the patient.

Consequently, there is a critical unmet need for novel, highly specific biomarkers capable of accurately distinguishing infection from disease activity in RA. Presepsin, a soluble subtype of CD14 (sCD14-ST), has recently emerged as a robust biomarker for bacterial sepsis. It is rapidly secreted into the circulation following the phagocytosis of bacterial pathogens by monocytes and macrophages.[14,15] Recent preliminary studies suggested that Presepsin levels are significantly elevated in RA patients with concurrent bacterial infections, remaining relatively unaffected by the underlying autoimmune inflammatory burden.[16,33] Similarly, the high-affinity immunoglobulin G receptor, Fc-gamma receptor I (FcγRI or CD64), which is markedly up-regulated on the surface of neutrophils and monocytes during acute bacterial infections, was shown as a promised diagnostic tool. The soluble form of CD64 (sCD64), shed from the cell surface upon activation, reflects this innate immune response and was proposed as a sensitive marker for systemic infection. [17,18] Despite their individual potential, the combined diagnostic utility of Presepsin and sCD64 in the specific context of infected RA patients remains largely unexplored, particularly within the Middle Eastern populations.

In addition to circulating biomarkers, genetic profiling offers critical insights into RA susceptibility and the individual's inflammatory response trajectory. The tumor necrosis factor alpha-induced protein 3 (TNFAIP3) gene, located on chromosome 6q23, encodes the A20 protein a potent ubiquitin-editing enzyme that acts as a central negative regulator of the NF-κB signaling pathway.[19,20] Genome-wide association studies have robustly linked polymorphisms in the TNFAIP3 gene to an increased risk of RA and other autoimmune diseases, as these variants can impair A20 function, leading to unbridled NF-κB activation and sustained systemic inflammation.[21,22]

Specifically, the TNFAIP3 rs10499194 (C>T) single nucleotide polymorphism has been implicated in RA susceptibility across various ethnic groups.[23] However, it remains unknown whether this genetic variant not only predisposes individuals to RA but also modulates the acute innate immune response—specifically the expression levels of Presepsin and sCD64 during episodes of concurrent infection.

Therefore, by integrating immunological biomarkers with genetic profiling, this study aimed to provide a comprehensive, precision-medicine approach to mitigating the infectious burden in RA. This study was designed to address these critical knowledge gaps. The primary objectives were: (1) to evaluate and compare the diagnostic performance of Presepsin and sCD64 against conventional inflammatory markers in detecting concurrent infections among Iraqi patients with RA; (2) to determine the independent predictive value of these novel biomarkers using multivariate modeling; and (3) to investigate the association of the TNFAIP3 rs10499194 polymorphism with RA susceptibility and its potential modulatory effect on the circulating levels of Presepsin and sCD64 in the presence and absence of infection.

Materials and Methods

Study Design and Ethical Considerations

This case control, hospital-based study was conducted over an eight-month period, from August 25, 2025, and March 30, 2026, at Al-Marjan Teaching Hospital, a tertiary care center in Babylon, Iraq.

Study Population

A total of 90 participants were enrolled and categorized into three equal groups: Normal Controls (n=30), apparently healthy individuals, were selected from hospital staff and patients' relatives, with data on age and sex. They had no history of autoimmune diseases, chronic inflammatory conditions, or recent infections. RA without Infection (n=30); patients with confirmed RA who were presented with active disease but with no clinical or laboratory evidence of concurrent infection. RA with Infection (n=30); patients with confirmed RA who were presented with a documented, concurrent acute infection.

Inclusion Criteria: Patients in the RA groups were required to fulfill the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for Rheumatoid Arthritis.24 For the "RA with Infection" group, concurrent infection was defined by the presence of acute clinical symptoms (e.g., fever, localized pain, purulent discharge) and confirmed by positive microbiological cultures (blood, urine, sputum, or wound swabs) or typical radiological findings (e.g., chest X-ray for pneumonia).

Exclusion Criteria: Individuals were strictly excluded if they had: (a) other concomitant autoimmune or connective tissue diseases (e.g., systemic lupus erythematosus); (b) active malignancies or a history of radiation/chemotherapy; (c) primary or secondary immunodeficiency disorders (including human immunodeficiency virus, HIV); (d) end-stage renal or hepatic failure; or (e) if they were pregnant or lactating.

Clinical Assessment and Questionnaire

A structured, pre-tested questionnaire was administered via face-to-face interviews to collect demographic and clinical data. The questionnaire covered: age, sex, smoking status, residential area (urban/rural), family history of RA, and the presence of chronic comorbidities (e.g., hypertension, diabetes mellitus). Anthropometric measurements were taken by trained nursing staff. Body Mass Index (BMI) was calculated by dividing weight in kilograms (measured using a calibrated digital scale, Seca, Germany) by height in meters squared (measured using a wall-mounted stadiometer).

For RA patients, disease duration and the duration of morning stiffness (in minutes) were recorded based on the diagnosis and clinical assessment made by a rheumatologist.[25]

Blood Sampling and Processing

Under strict aseptic precautions, a venous blood sample (5 ml) was collected from the antecubital vein of each participant. The sample

was divided in aliquots as follows: An amount of 2 ml blood was placed into an EDTA tube, and gently inverted to prevent coagulation. A portion was immediately used for complete blood count (CBC), including white blood cell (WBC) count, using an automated hematology analyzer (Sysmex XN-1000, Japan), according to the manufacturer's instructions. The remaining was stored at -20°C for subsequent genomic DNA extraction.

An amount of 3 ml blood was placed into a plain serum separator gel tube, and allowed to clot at room temperature for 30 minutes. The tube was then centrifuged at 1000 ×g for 15 minutes. The serum was carefully divided in aliquots into sterile, labeled Eppendorf tubes and immediately stored at -80°C until used for biochemical and immunological assays. Repeated freeze-thaw cycles were strictly avoided.

Routine Laboratory and Microbiological Investigations

Erythrocyte Sedimentation Rate (ESR) was measured using the standard Westergren method (mm/h). Serum C-reactive protein (CRP) and rheumatoid factor (RF) were quantified using automated turbid-metric assays on a Cobas c311 analyzer (Roche Diagnostics, Germany), according to the manufacturer's instructions.

For the group with RA infection, appropriate clinical specimens (sputum, mid-stream urine, wound swabs, or blood) were collected based on the suspected site of infection before the initiation of new empirical antibiotics. Specimens were cultured on standard microbiological media (Blood agar, MacConkey agar, Chocolate agar, Sabouraud Dextrose Agar). Identification of causative pathogens (bacterial or fungal) was performed using standard biochemical tests and confirmed using a fully automated identification system (VITEK® 2, bioMérieux, France).

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum concentrations of specific biomarkers were quantified using commercially available, sensitive sandwich ELISA kits supplied by Bioassay Technology Laboratory (BT LAB, China) and SunLong Biotech Co., Ltd. (China); the catalogue number, assay range, and analytical sensitivity for each biomarker are detailed below, strictly adhering to the manufacturers' instructions. All assays were performed on a 96-well microplate reader (Bio-Tic, USA) measuring optical density (OD) at 450 nm.

Anti-Cyclic Citrullinated Peptide (Anti-CCP): this was measured using the Quick Step Human anti-CCP ELISA Kits (Cat No: QS0154Hu, SunLong Biotech Co., LTD, China), according to the manufacturer's instructions.

Soluble CD64 (sCD64): this was quantified using the Human Cluster of Differentiation 64 ELISA Kits (Cat No: E4140hu, Bioassay Technology Laboratory [BT LAB], China), according to the manufacturer's instructions. The assay range was 0.5–100 ng/ml with a sensitivity of 0.22 ng/ml. Intra-assay and inter-assay coefficients of variation (CV) were <8% and <10%, respectively.

Presepsin: This was measured using the Human Presepsin (PSPN) ELISA Kits (Cat No: E3754Hu, BT LAB, China), according to the manufacturer's instructions. The assay range was 5–1000 ng/l with a sensitivity of 2.39 ng/l. Intra-assay and inter-assay CVs were <10%.

Genomic DNA Extraction

Genomic DNA was extracted from the stored EDTA-whole blood samples using commercial kits (Magen Hipure blood DNA Mini kit, Magen, China), following the manufacturer's rapid protocol. The concentration and purity of the extracted DNA were assessed using a spectrophotometer (NanoDrop ND-1000, Bio Drop Scientific, USA). DNA samples with an A260/A280 ratio between 1.8 and 2.0 were considered pure and stored at -20°C.

Genotyping of the TNFAIP3 gene single nucleotide polymorphism (SNP) rs10499194 (C>T) was performed using the Tetra-primer Amplification Refractory Mutation System Polymerase Chain Reaction (Tetra-ARMS PCR) technique.[26] The specific primer sequences were:

Primer	Sequence (5' → 3')
Forward outer	ACAGTACCAAGCTGCTTCTCAGGACTAC
Reverse outer	GCAATGAATTGACTCACAGTGTAGCAAT
Forward inner (T allele-specific)	AGAGACAATATTGATTTTCTTCAAATGTGGTC
Reverse inner (C allele-specific)	AAACATGACTACTTTTTGAACAAAAGGGGTA

PCR amplification was performed using a 25 µl reaction volume containing 12.5 µl of Master Mix green(Promega), 3 µl of template DNA, 1 µl of each inner primer, 1µl of each outer primer, and 5.5 µl nuclease-free water. Thermal cycling was conducted in a thermal cycler (Eppendorf, USA) with the following conditions: initial denaturation at 94°C for 5 minutes; 30 cycles each of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 45 seconds; followed by a final extension at 72°C for 3 minutes.

PCR products were resolved by electrophoresis on a 1.5% agarose gel stained with 5 µl ethidium bromide in 1X Tris-borate-EDTA (TBE) buffer at 80 V for 60 minutes. Bands were visualized under a UV transilluminator. The expected amplicon sizes were: 434 bp (outer control band), 285 bp (mutant T allele), and 212 bp (wild-type C allele) (Figure 1).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 26, IBM and GraphPad Prism version 9.0. Data normality was assessed by the Shapiro-Wilk test. Parametric data were expressed as mean ± standard deviation (SD) and analyzed using one-way ANOVA with Tukey's post hoc test or Student's t-test, whereas non-parametric data are presented as median or Interquartile Range (IQR) and compared using the Kruskal-Wallis and Mann-Whitney U tests. Categorical variables are expressed as frequencies and percentages and analyzed using the chi-square test or Fisher's exact test. Spearman's correlation was used to assess associations between continuous variables, and the receiver operating characteristic (ROC) curve analysis was performed to evaluate biomarker diagnostic performance and determine optimal cut-off values by Youden's J index. Univariate and multivariate logistic regression with Firth's penalized maximum likelihood estimation were used to identify independent predictors of infection. For genetic analysis, Hardy-Weinberg equilibrium, Cochran-Armitage trend test, and odds ratios with 95% confidence intervals under allelic and dominant models were applied. A two-tailed $p<0.05$ was considered statistically significant.

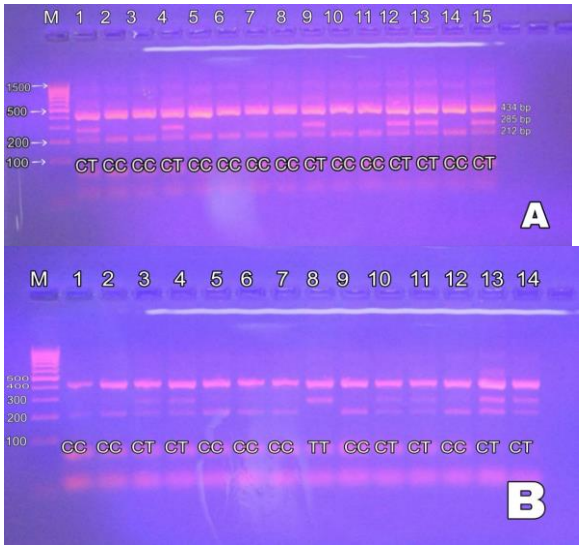

Figure 1. Gel electrophoresis of TNFAIP3 rs10499194 (C>T) polymorphism in RA patients. A: Rheumatoid Arthritis (RA) without infection. B: RA with infection. Genotyping: Amplified PCR products were separated on a 1.5% agarose gel at 80 V for 60 minutes. Lane M contains a 100 bp DNA ladder as a molecular size standard. The observed bands correspond to the expected product sizes of 434 bp, 285bp for the T allele, and 212 bp for C allele.

Results

Age, sex, smoking status, residence, and family history of RA were similar across all groups, with no statistically significant differences ($p > 0.05$). However, BMI and the prevalence of chronic diseases showed significant differences (p = 0.001 for each), with higher values observed in the RA groups compared to the control group.

Overall, the groups were well matched demographically, with variations confined to BMI and comorbidity burden, as shown in Table1.

Table 1. Demographic and Clinical Characteristics of the Study Groups.

Variable	Normal Controls (n = 30)	RA without Infection (n = 30)	RA with Infection (n = 30)	*p*-value
Age (years) [a]	41.97 ± 9.25	43.63 ± 8.40	41.13± 12.87	NS[*]
BMI (kg/m²) [a]	24.84 ± 3.14	28.90 ± 4.54	27.25 ± 4.30	0.001[H]
Sex, n (%) [b]				NS[F]
Male	6 (20.0%)	3 (10.0%)	3 (10.0%)	
Female	24 (80.0%)	27 (90.0%)	27 (90.0%)	
Smoking Status, n (%) [b]				NS[F]
Yes	6 (20.0%)	2 (6.7%)	3 (10.0%)	
No	24 (80.0%)	28 (93.3%)	27 (90.0%)	
Residence, n (%) [b]				NS[#]
Urban	15 (50.0%)	9 (30.0%)	9 (30.0%)	
Rural	15 (50.0%)	21 (70.0%)	21 (70.0%)	
Family History of RA, n (%)[b]				NS[#]
Yes	3 (10.0%)	8 (26.7%)	9 (30.0%)	
No	27 (90.0%)	22 (73.3%)	21 (70.0%)	
Chronic Diseases, n (%) [b]				0.001[F]
Yes	0 (0.0%)	11 (36.7%)	6 (20.0%)	
No	30 (100.0%)	19 (63.3%)	24 (80.0%)	

[a]Data are presented as Mean ± SD. *:One-way ANOVA was used for normally distributed data; H:Kruskal-Wallis H test for non-normally distributed data. [b] Data are presented as n (%).#:Chi-square test was used when all expected frequencies ≥ 5; F:Fisher's exact test when any expected frequency < 5. * $p > 0.05$ is not significant (NS). Abbreviations: BMI, Body Mass Index; RA, Rheumatoid Arthritis; SD, Standard Deviation. Mean ± SD. b Median (Q1–Q3).

Table 2 shows that disease duration was similar between the RA groups (p=0.299), whereas disease activity was significantly higher in RA with infection, as reflected by increased Disease Activity Score-28 (DAS28)-ESR and longer morning stiffness duration (both p<0.001). CRP, ESR, and WBC differed significantly among the groups and were highest in RA with infection, intermediate in RA without infection, and lowest in the controls (for all p<0.001). In contrast, although RF and anti-CCP were significantly higher in both RA groups than in the controls, they did not differ significantly between RA patients with and without infection.

As showed in Table 3 and Figure 2, there are significant differences in the Presepsin and sCD64 levels, among the study groups (p<0.001 for both). Both biomarkers were highest in RA with infection, followed by RA without infection, and lowest in the controls, with significant pairwise differences between all groups. Also, Anti-CCP differed, but did not distinguish RA patients with and without infection (p<0.001). Overall, Presepsin and sCD64 appear to be strong markers of concurrent infection in RA.

Table 2. Disease-Specific Characteristics and Conventional Inflammatory Markers.

Variable	Control (n=30)	RA w/o Infection (n=30)	RA w/ Infection (n=30)	*p*-value
Disease Duration (years)[b]	N/A	5.00 (3.00–10.00)	7.50 (4.25–11.75)	NS[$]
DAS28-ESR Score a	N/A	4.51 ± 1.10	5.77 ± 1.00	<0.001[t]
Morning Stiffness (min)[a]	N/A	48.50 ± 21.62	75.40 ± 21.87	<0.001
CRP (mg/l)[b]	2.10 (1.60–3.00)	21.70 (17.50–31.02)	46.30 (32.50–64.90)	<0.001[H]
ESR (mm/h)[b]	10.00 (7.00–14.00)	44.00 (34.25–59.25)	62.00 (52.00–75.75)	<0.001[H]
WBC (×10³/µl)[b]	6.15 (5.35–7.57)	9.10 (7.67–10.17)	12.60 (10.83–16.12)	<0.001[H]
RF (IU/ml)[b]	6.05 (3.90–8.05)	51.25 (10.03–83.53)	39.70 (10.38–58.15)	<0.001[H]
Anti-CCP (U/ml)[b]	4.04 (2.50–7.01)	45.47 (12.18–51.60)	33.89 (7.86–48.80)	<0.001[H]

a: Mean ± SD. b: Median (Q1–Q3). $: Mann-Whitney, t: Student's t test. H: Kruskal-Wallis H test. All pairwise comparisons for CRP, ESR, and WBC were significant (p<0.001). RF and Anti-CCP did not differ between RA groups (p=0.474 and p=0.387, respectively). $p > 0.05$ is not significant (NS).

Table 3. Comparison of Novel Biomarkers across the Study Groups.

Biomarker	Control (n=30)	RA w/o Infection (n=30)	RA w/ Infection (n=30)	*p*-value[KW]	η²
Presepsin (ng/l)	129.85 (105.50–183.97)	233.10 (161.40–293.73)	459.10 (356.10–750.43)	<0.001	0.630
sCD64 (ng/ml)	4.07 (2.94–5.62)	8.45 (6.71–14.53)	22.04 (16.23–32.22)	<0.001	0.629
Anti-CCP (U/ml)	4.04 (2.50–7.01)	45.47 (12.18–51.60)	33.89 (7.86–48.80)	<0.001	0.341

Data are presented as Median (Q1–Q3). KW = Kruskal-Wallis. η² = Eta-squared effect size. All pairwise comparisons were significant ($p < 0.05$) for Presepsin, and sCD64.
For Anti-CCP: there was no significant difference between RA groups (p=0.387).

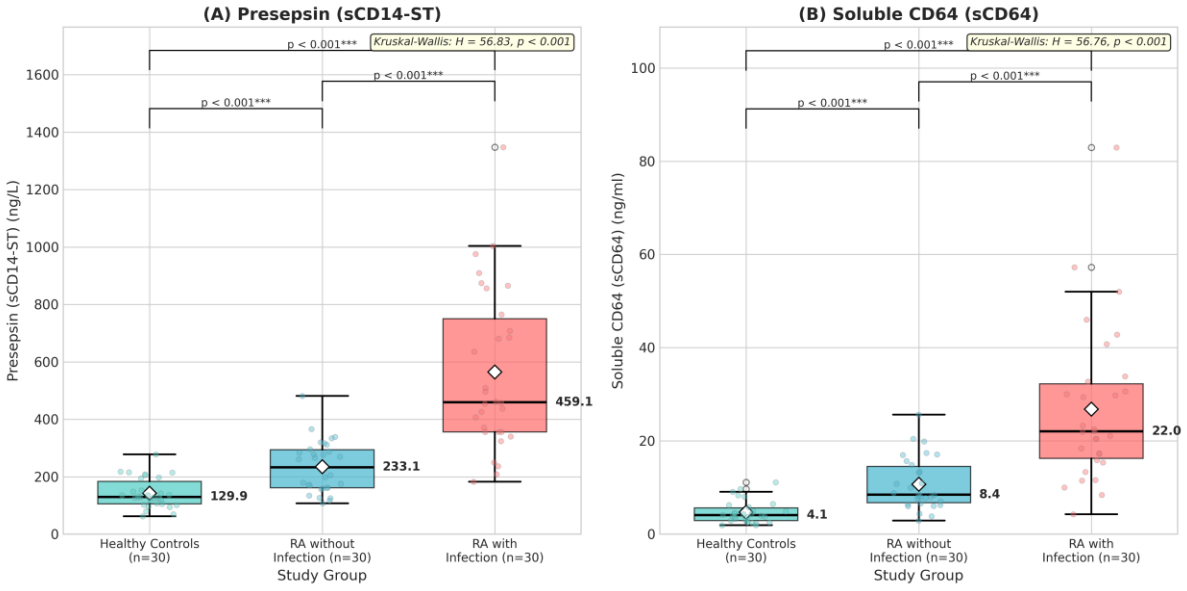

Figure 2. Box plots showing the distribution of (A) Presepsin and (B) sCD64 levels in normal controls, RA patients without infection, and RA patients with infection. The central line represents the median, the diamond marker represents the mean, and the box spans the interquartile range (Q1–Q3). Statistical significance was determined using the Kruskal-Wallis test followed by post-hoc Mann-Whitney U tests. All pairwise comparisons were statistically significant (p<0.001).

Table 4 and Figure 3 showed that Presepsin and sCD64 had good diagnostic performance for identifying infection in RA, exceeding that of WBC, CRP, and ESR. Presepsin showed an area under the curve (AUC) of 0.910 and for sCD64 an AUC of 0.870, while their combination achieved the highest accuracy, with an AUC of 0.956, with 83.3% sensitivity, and 96.7% specificity. Overall, the combined Presepsin–sCD64 model provided the best discrimination between RA patients with and without infection, (Figures 4 and 5).

Table 4. Diagnostic Performance of Biomarkers based on ROC Analysis.

Biomarker	AUC	Cut-off	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	Acc. (%)	Youden's J
Combined (Presepsin + sCD64)	0.956	0.672 a	83.3	96.7	96.2	85.3	90.0	0.800
Presepsin (sCD14-ST)	0.910	339.80 ng/l	83.3	93.3	92.6	84.8	88.3	0.767
Soluble CD64 (sCD64)	0.870	17.26 ng/ml	73.3	86.7	84.6	76.5	80.0	0.600
WBC	0.833	10.80 ×10³/µl	76.7	83.3	82.1	78.1	80.0	0.600
CRP	0.823	32.30 mg/L	76.7	76.7	76.7	76.7	76.7	0.533
ESR	0.746	50.00 mm/h	83.3	60.0	67.6	78.3	71.7	0.433

ROC curve analysis comparing RA patients with infection (n=30) vs. without infection (n=30). a Predicted probability threshold from logistic regression. Sens. = Sensitivity; Spec. = Specificity; PPV = Positive Predictive Value; NPV = Negative Predictive Value; Acc. = Accuracy.

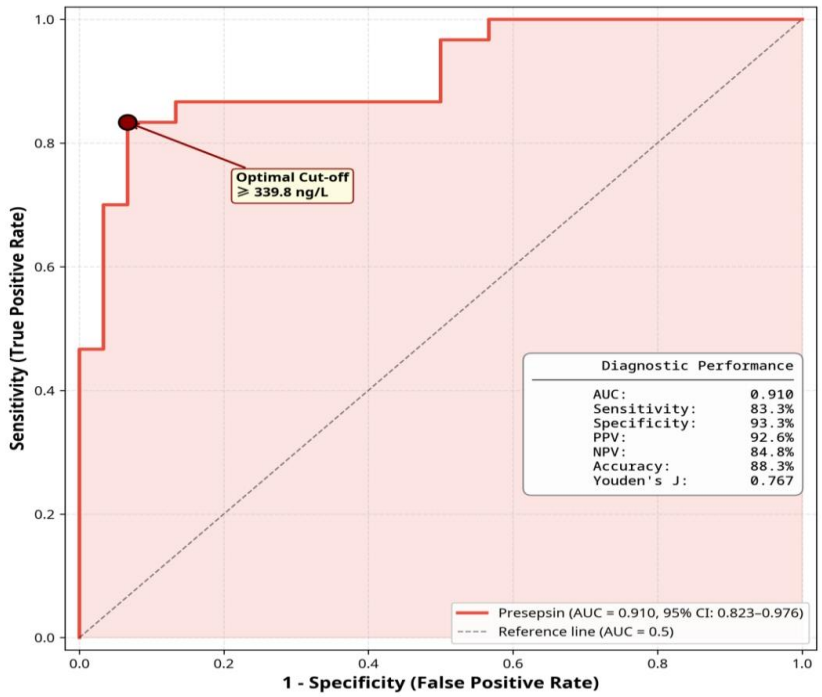

Figure 3. Receiver operating characteristic (ROC) curve analysis of presepsin for discrimination of infection in patients with rheumatoid arthritis.

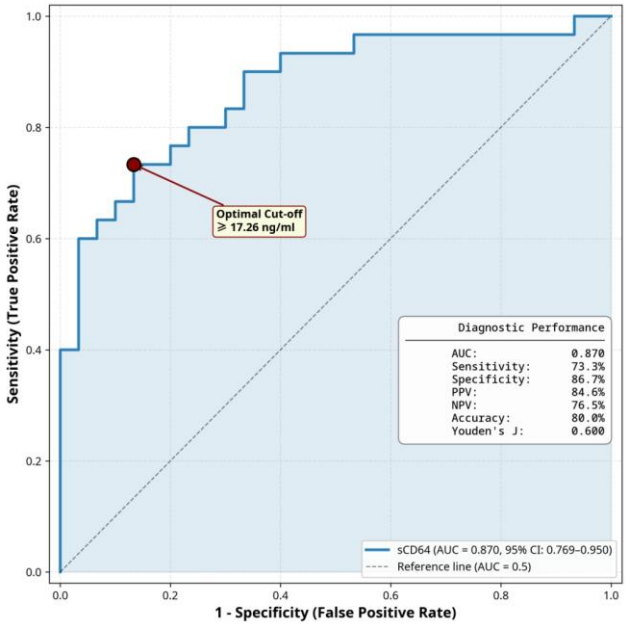

Figure 4. Receiver operating characteristic (ROC) curve analysis of sCD64 for discrimination of infection in patients with rheumatoid arthritis.

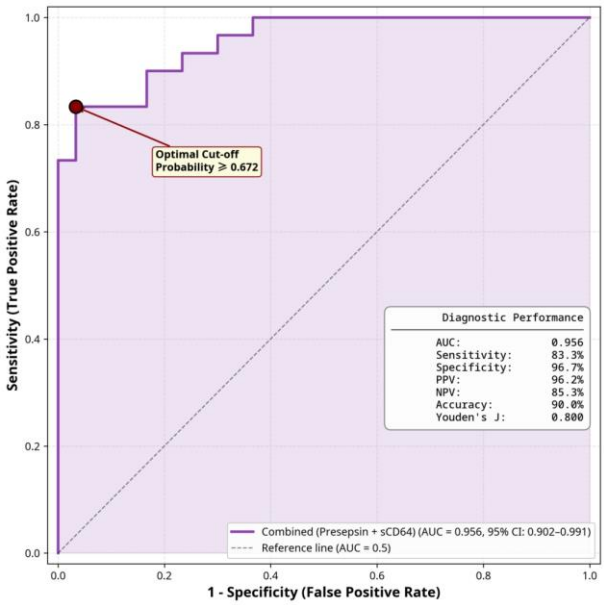

Figure 5. Receiver operating characteristic (ROC) curve analysis of the combined presepsin and sCD64 model for discrimination of infection in patients with rheumatoid arthritis.

Figure 6 showed that Presepsin and sCD64 were positively correlated with DAS28-ESR and inflammatory markers, including CRP, ESR, and WBC count, supporting their association with disease activity and systemic inflammation. In contrast, neither biomarker was significantly correlated with RF. Anti-CCP strongly correlated with RF, but did not correlate with clinical or inflammatory parameters, indicating that Presepsin and sCD64 reflect inflammatory burden rather than autoantibody status.

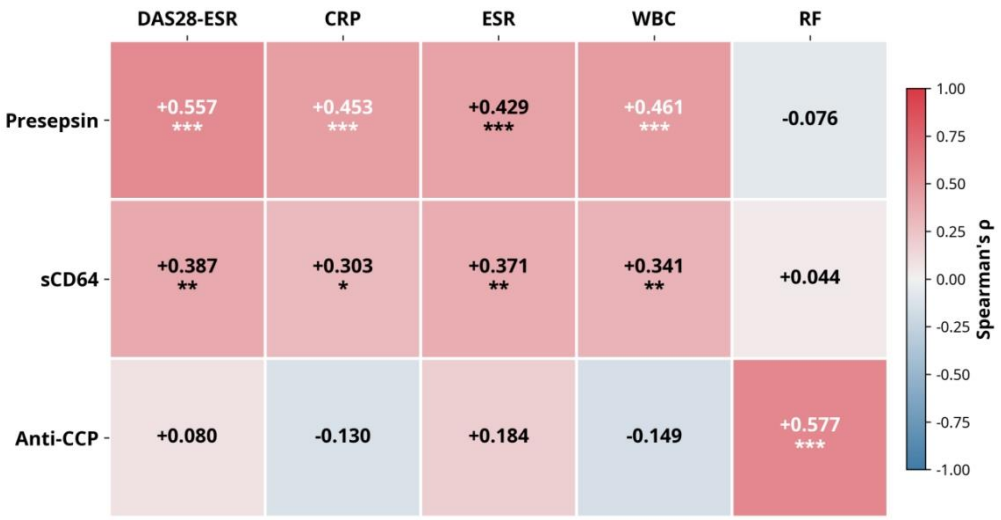

Figure 6. Spearman correlation matrix of presepsin, soluble CD64, anti-CCP, and clinical inflammatory parameters in the study population.

Table 5 Univariate analysis showed that Presepsin, sCD64, WBC, CRP, ESR, DAS28-ESR, and morning stiffness were significant predictors of infection, whereas anti-CCP was not. In multivariate analysis, only Presepsin, sCD64, and WBC remained independent predictors. The final model showed excellent discriminatory performance, with an AUC of 0.978 and an accuracy of 90.0%.

Table 5. Logistic Regression Analysis for Predictors of Infection in RA Patients.

A: Univariate Analysis					
Variable	β	SE	Wald χ^2	OR (95% CI)	*p*-value
Presepsin (ng/l)	0.0153	0.0043	12.84	1.015 (1.007–1.024)	<0.001
sCD64 (ng/ml)	0.2060	0.0519	15.77	1.229 (1.110–1.360)	<0.001
Anti-CCP (U/ml)	-0.0080	0.0117	0.47	0.992 (0.969–1.015)	NS
CRP (mg/l)	0.0678	0.0195	12.11	1.070 (1.030–1.112)	<0.001
ESR (mm/h)	0.0485	0.0160	9.22	1.050 (1.017–1.083)	0.002
WBC (×10³/µl)	0.4809	0.1384	12.06	1.617 (1.233–2.122)	<0.001
DAS28-ESR	0.0743	0.0222	11.21	1.077 (1.031–1.125)	<0.001
Morning Stiffness	0.0567	0.0158	12.89	1.058 (1.026–1.092)	<0.001
B: Multivariate Analysis (Final Model)					
Variable	β	SE	Wald χ^2	Adjusted OR (95% CI)	*p*-value
Presepsin (ng/l)	0.0104	0.0048	4.74	1.010 (1.001–1.020)	<0.029
sCD64 (ng/ml)	0.1910	0.0712	7.19	1.210 (1.053–1.392)	<0.007
WBC (×10³/µl)	0.4490	0.2159	4.32	1.567 (1.026–2.392)	<0.038

Firth's penalized maximum likelihood estimation. Model AUC = 0.978, Hosmer-Lemeshow $p = 0.987$, Classification accuracy = 90.0%. $p > 0.05$ is not significant (NS).

Table 6 and Figure 7 showed that distribution of the TNFAIP3 rs10499194 genotype did not differ significantly among the three study groups. There was a significant decreasing trend in the frequency of CC genotype observed from controls to RA without infection and RA with infection. The T allele was significantly associated with RA susceptibility under the allelic and dominant models, particularly in RA patients with infection compared with normal controls. However, no significant association was found between the two RA subgroups, indicating that this variant may contribute to RA susceptibility rather than specifically to infection status within RA.

Table 6. Distribution and pairwise comparisons of TNFAIP3 rs10499194 (C>T) genotype alleles.

Section	Variable/ Comparison	Genetic model / Category	Controls (n = 30)	RA w/o Inf. (n = 30)	RA w/ Inf. (n = 30)	OR (95% CI)	*p*-value
Genotype distribution	Genotype, n (%)	CC (Wild-type)	25 (83.3%)	19 (63.3%)	17 (56.7%)	—	NS[FF]
		CT (Heterozygous)	4 (13.3%)	9 (30.0%)	10 (33.3%)	—	
		TT (Homozygous variant)	1 (3.3%)	2 (6.7%)	3 (10.0%)	—	
Dominant model	Genotype, n (%)	CT + TT vs CC	5 (16.7%)	11 (36.7%)	13 (43.3%)	—	NS[FF]
Allele frequency	Allele frequency	C allele	54 (90.0%)	47 (78.3%)	44 (73.3%)	—	NS[FF]
		T allele	6 (10.0%)	13 (21.7%)	16 (26.7%)	—	
Pairwise comparisons	All RA vs. Controls	Allelic (T vs. C)	—	—	—	2.87 (1.12–7.35)	0.028*
	All RA vs. Controls	Dominant (CT + TT vs. CC)	—	—	—	3.33 (1.12–9.92)	0.032*
	RA w/ Inf. vs. Controls	Allelic (T vs. C)	—	—	—	3.27 (1.18–9.07)	0.032*
	RA w/ Inf. vs. Controls	Dominant (CT + TT vs. CC)	—	—	—	3.82 (1.15–12.71)	0.047*
	RA w/o Inf. vs. Controls	Allelic (T vs. C)	—	—	—	2.49 (0.88–7.07)	NS
	RA w/o Inf. vs. Controls	Dominant (CT + TT vs. CC)	—	—	—	2.89 (0.86–9.74)	NS
	RA w/ Inf. vs. RA w/o Inf.	Allelic (T vs. C)	—	—	—	1.31 (0.57–3.04)	NS
	RA w/ Inf. vs. RA w/o Inf.	Dominant (CT + TT vs. CC)	—	—	—	1.32 (0.47–3.72)	NS

Cochran–Armitage trend test: z = 2.307, $p = 0.021$ *. All groups were in Hardy–Weinberg equilibrium ($p>0.05$).
* $p > 0.05$ is not significant (NS). FF:FFH exact.

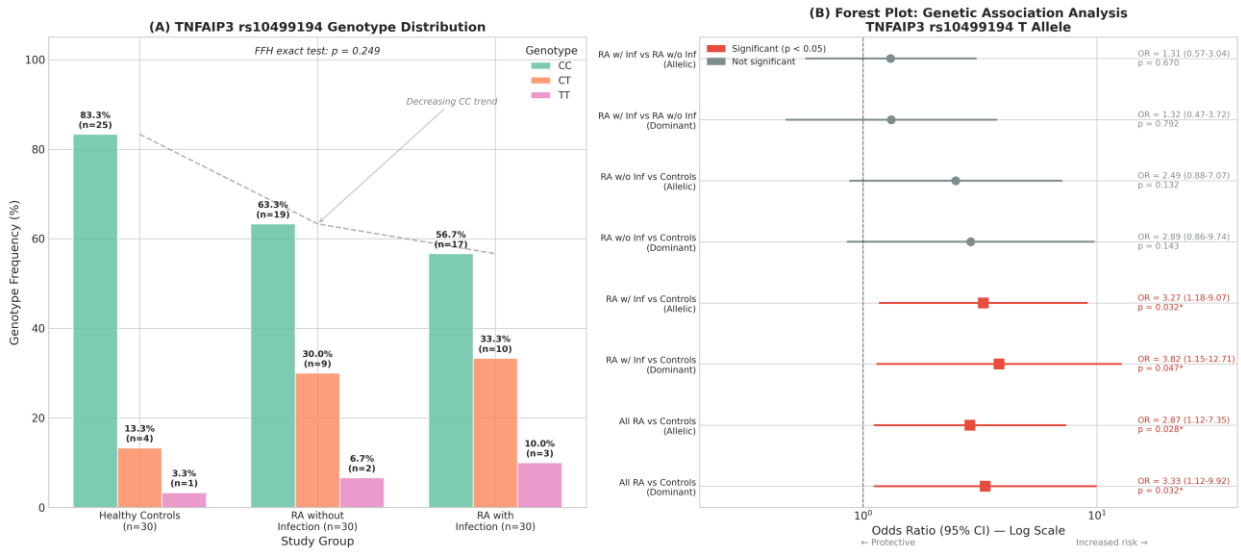

Figure 7. (A) Grouped bar chart showing the frequency of CC, CT, and TT genotypes across the three study groups, with a dashed line indicating the decreasing trend of the wild-type CC genotype from controls (83.3%) to RA without infection (63.3%) to RA with infection (56.7%). (B) Forest plot illustrating the odds ratios (OR) and 95% confidence intervals for various group comparisons under allelic and dominant genetic models. Significant associations (p < 0.05) are highlighted in red, including All RA vs Controls under both allelic (OR = 2.87, p = 0.028) and dominant (OR = 3.33, p = 0.032) models.

Table 7 and Figure 8 showed that carriage of the TNFAIP3 rs10499194 T allele was associated with significantly higher Presepsin and sCD64 levels in the overall RA under the dominant model. This effect was more evident in the RA with infection subgroup, whereas no significant differences were observed in RA without infection. Moreover, both biomarkers showed a significant allele-dose trend across CC, CT, and TT genotypes, indicating that the T allele is associated with enhanced inflammatory biomarker expression in RA.

Table 8 showed that bacterial infections were the predominant type among RA patients with infection, followed by viral and fungal infections, whereas mixed and mycobacterial infections were uncommon. The respiratory tract was the most frequent site of infection, followed by the urinary tract and skin/soft tissue. *S. aureus* was the most commonly isolated pathogen, indicating that bacterial respiratory and urinary infections represent the major infectious burden in this cohort.

Table 7. Association between TNFAIP3 rs10499194 Genotype and Biomarker Levels.

Section / analysis	Biomarker	Group	CC	CT + TT	TT	Statistic	*p*-value	r	Trend p
Panel A. Dominant Model (CC vs. CT+TT)									
Panel A	Presepsin (ng/l)	All RA (n=60)	281.1 (174.8-385.1)	386.7 (284.9-787.6)	-	262	0.011*	0.330	-
		RA w/o Inf. (n=30)	175.4	277.2	-	66	0.102	0.299	-
		RA w/ Inf. (n=30)	426.6	764.7	-	51	0.014*	0.451	-
	sCD64 (ng/ml)	All RA (n=60)	12.5 (7.5-20.4)	17.7 (11.4-35.6)	-	274	0.018*	0.307	-
		RA w/o Inf. (n=30)	7.8	10.9	-	70	NS	0.267	-
		RA w/ Inf. (n=30)	20.4	33.8	-	49	0.011*	0.466	-
Panel B. Dose-response by individual genotypes (All RA, n=60)									
Panel B	Presepsin (ng/l)	All RA	281.1 (174.8-385.1)	355.7 (273.3-810.4)	481.1 (407.1-509.6)	KW H 8.118	0.017*	-	0.005
	sCD64 (ng/ml)	All RA	12.5 (7.5-20.4)	17.1 (11.2-27.5)	42.8 (13.4-52.0)	6.035	0.049*	-	0.013

Data are presented as median (IQR). Panel A: Mann-Whitney U test; Panel B: Kruskal-Wallis test. Trend by Spearman correlation with ordinal coding (CC=0, CT=1, TT=2). r = Z/square root of N. $p > 0.05$ is not significant (NS).

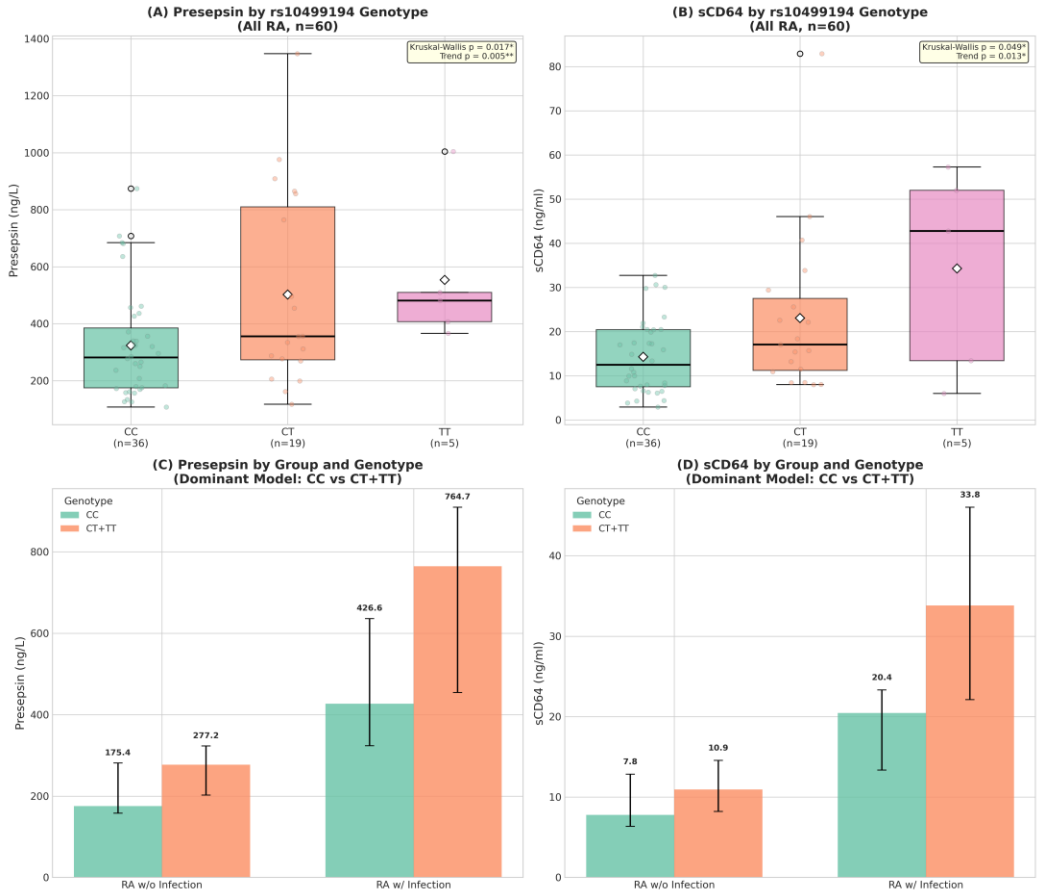

Figure 8. Panels A and B show the dose-dependent increase in Presepsin and sCD64 levels across individual genotypes (CC, CT, TT) in all RA patients (n=60), with significant Kruskal-Wallis tests (Presepsin p = 0.017; sCD64 p = 0.049) and significant dose-response trends (Presepsin trend p = 0.005; sCD64 trend p = 0.013). Panels C and D illustrated the biomarker levels stratified by both infection status and genetic model (dominant model: CC vs CT+TT), demonstrating that T allele carriers consistently exhibit higher biomarker levels, with the most pronounced effect in the RA with infection group.

Table 8. Distribution of Causative Pathogens in the 30 Infected RA Patients.

Section	Category	n	%
By Type of Infection	Bacterial	21	70.0
	Viral	5	16.7
	Fungal	2	6.7
	Mixed (Bacterial + Fungal)	1	3.3
	Mycobacterial	1	3.3
By Site of Infection	Respiratory tract	10	33.3
	Urinary tract	6	20.0
	Skin and soft tissue	6	20.0
	Bone and joint	3	10.0
	Bloodstream	2	6.7
	Gastrointestinal	1	3.3
	Other (oral/dental)	2	6.7

Table 8. Continued.

Section	Category	n	%
Most Common Organisms	*Staphylococcus aureus*	8	25.8
	Escherichia coli	4	12.9
	Streptococcus pneumoniae	3	9.7
	Streptococcus pyogenes	3	9.7
	Candida albicans	3	9.7
	Klebsiella pneumoniae	2	6.5
	Influenza virus	2	6.5
	Herpes zoster virus	2	6.5

Discussion

The present study represented a comprehensive investigation into the diagnostic utility of Presepsin and sCD64 as novel biomarkers for identifying concurrent infections in patients with RA, alongside an exploration of the genetic contribution of the TNFAIP3 rs10499194 polymorphism to disease susceptibility and biomarker modulation. To the best of our knowledge, this is the first study conducted on an Iraqi population that simultaneously evaluated these serological and genetic markers in the context of RA-associated infections. The findings of this study carry significant clinical implications, as the timely differentiation of infection from disease flare in RA remains one of the most formidable diagnostic challenges confronting rheumatologists. [13]

The demographic analysis revealed that the three study groups were well-matched for age, sex, smoking status, residence, and family history of RA, thereby minimizing the influence of potential confounders on subsequent biomarker and genetic analyses. However, BMI was significantly higher in both RA groups compared to normal controls (p = 0.001), which is consistent with the findings of Jalal *et al.,* 2023, who reported a positive association between obesity and impaired quality of life in Iraqi RA patients from Erbil, Iraq.[27] Similarly, an Iraqi study from Babylon province by Jasim et al., 2024, demonstrated that elevated BMI was positively correlated with disease activity markers and DAS28 scores in RA patients.[28] The higher prevalence of chronic diseases in RA groups observed in our study aligns with the well-established literature documenting the increased comorbidity burden in RA, which itself constitutes an independent risk factor for infection.[11]

Our results demonstrated that conventional inflammatory markers, including CRP, ESR, and WBC count, were significantly elevated in RA patients with concurrent infection compared to those without infection and controls. While these markers achieved statistical significance in group comparisons, their diagnostic performance in the ROC analysis was only moderate, with AUC values of 0.823, 0.746, and 0.833 for CRP, ESR, and WBC, respectively. These findings corroborate the observations of Cui and Qian, 2023, who emphasized in their comprehensive review that traditional laboratory markers lack specificity for bacterial infection in RA and cannot reliably distinguish disease flares from superimposed infections.[13] The fundamental limitation lies in the shared inflammatory pathways activated in both RA exacerbation and infection, rendering these conventional markers inherently non-specific in the context of autoimmune disease.[29] Importantly, RF and Anti-CCP levels did not differ significantly between the infected and non-infected RA groups (p = 0.474 and p = 0.387, respectively), confirming that autoantibody titers reflect the underlying autoimmune process rather than the acute infectious burden. This observation is consistent with the established understanding that these autoantibodies are markers of RA-specific immune dysregulation and do not fluctuate in response to intercurrent infections.[24]

A central finding of this study is the demonstration that Presepsin levels were markedly elevated in RA patients with concurrent infection compared to both uninfected RA patients and controls, with a

large effect size ($\eta^2 = 0.630$). The ROC analysis revealed an AUC of 0.910, with an optimal cut-off of 339.80 ng/l providing 83.3% sensitivity and 93.3% specificity. These results are in agreement with the landmark study by Tsujimoto et al., 2018, who first evaluated Presepsin as a biomarker of systemic bacterial infection specifically in RA patients and concluded that Presepsin may be useful marker to identify infection in RA patients and may better reflect infection severity than procalcitonin.[16] Our findings extend their work by providing quantitative cut-off values and demonstrating superior diagnostic performance compared to conventional markers in a Middle Eastern population.

The mechanistic basis for Presepsin's specificity in detecting infection lies in its unique production pathway. Presepsin is a 13-kDa truncated fragment of soluble CD14, generated when monocytes and macrophages phagocytose bacteria. During this process, membrane-bound CD14 binds the lipopolysaccharide-lipopolysaccharide binding protein (LPS-LBP) complex, activating Toll-like receptor 4 (TLR4) signaling and downstream NF-κB pathways. Subsequently, CD14 is internalized and cleaved by lysosomal enzymes, particularly cathepsin D, releasing Presepsin into the circulation. [14,30] This phagocytosis-dependent mechanism renders Presepsin highly specific to bacterial infection, as it is not substantially elevated during sterile inflammatory processes or autoimmune flares alone.[31] A meta-analysis by Zhang et al., 2015, encompassing 11 studies with 3,057 patients, reported a pooled AUC of 0.89 for Presepsin in diagnosing sepsis, which is comparable to our finding of 0.910 in the RA-specific context.[32]

Furthermore, our results are supported by the Egyptian study of Abdul-Hussain et al., 2025, who investigated Presepsin alongside receptor activator of nuclear factor kappa-B ligand (RANKL) in women with RA and reported that Presepsin levels correlated significantly with disease severity stages, proposing it as a viable diagnostic marker.[33] Similarly, AlJarhi et al., 2021, demonstrated that Presepsin was significantly elevated in Egyptian systemic lupus erythematosus patients with bacterial infection compared to those with disease flare alone, further validating its utility across autoimmune diseases.[34] The review by Memar et al., 2019, concluded that in RA cases, Presepsin is a promising novel marker for the diagnosis of bacterial infections regardless of RA disease activity, which directly supports our observation that Presepsin levels were not confounded by autoantibody titers (RF and Anti-CCP).[31]

In our study, sCD64 demonstrated robust diagnostic performance, with an AUC of 0.870, sensitivity of 73.3%, and specificity of 86.7% at a cut-off of 17.26 ng/ml. sCD64 is the high-affinity immunoglobulin G receptor expressed constitutively on monocytes and macrophages, with minimal expression on resting neutrophils. During bacterial infection, interferon-gamma (IFN-γ) and granulocyte colony-stimulating factor markedly up-regulated CD64 expression on neutrophils, while proteolytic cleavage releases soluble CD64 into the circulation.[18] This mechanism provides a pathophysiological rationale for the elevated sCD64 levels observed in our infected RA.

The study by Matsui et al., 2006, was among the first to demonstrate that CD64 expression on neutrophils is a sensitive and specific marker for detecting infection in RA patients, reporting a sensitivity of 92.7% and specificity of 96.5% at a cut-off of 2,000 molecules per cell.[35] While their study measured membrane-bound CD64 via flow cytometry, our study extends these findings to the sCD64 measured by ELISA, which offers practical advantages in terms of sample processing and accessibility in resource-limited settings. Allen et al., 2002, provided early evidence that quantitative CD64 measurement can distinguish between systemic infection and the flare of autoimmune diseases, including RA, reporting that CD64 levels were significantly higher in infected patients than in those experiencing disease exacerbation.[36] More recently, Pandey et al., 2021, confirmed the utility of neutrophil CD64 in distinguishing bacterial infection from disease flare across multiple autoimmune disorders, further supporting our findings.[37]

The study by Matt et al., 2015, reported that early RA patients display increased membrane and sCD64, with sCD64 correlating with joint

disease activity, and proposed sCD64 as an RA-specific biomarker.[17] This is consistent with our observation that even uninfected RA patients had significantly higher sCD64 levels than normal controls, reflecting the chronic immune activation inherent to RA. The critical distinction, however, is that the magnitude of sCD64 elevation in the infected RA group was substantially greater, enabling effective discrimination between infection and disease activity alone.

A particularly noteworthy finding of this study is that the combination of Presepsin and sCD64 achieved the highest diagnostic accuracy, with an AUC of 0.956, sensitivity of 83.3%, specificity of 96.7%, and overall accuracy of 90.0%. This combined approach outperformed each individual biomarker and all conventional markers. The multivariate logistic regression model further confirmed that Presepsin (Adjusted OR = 1.010, *p*=0.029) and sCD64 (Adjusted OR = 1.210, *p*=0.007) were independent predictors of infection, alongside WBC count (Adjusted OR = 1.567, *p* = 0.038), with the final model achieving an AUC of 0.978.

The rationale for the complementary nature of these two biomarkers lies in their distinct but overlapping biological pathways. Presepsin reflects monocyte/macrophage-mediated phagocytic activity in response to bacterial invasion, while sCD64 captures the broader IFN-γ-driven immune activation associated with infection.[14,18] By integrating both markers, the diagnostic algorithm captures multiple facets of the host immune response to infection, thereby reducing false-negative and false-positive rates. This multi-biomarker approach aligns with the growing consensus in the literature that no single biomarker is sufficient for the complex task of differentiating infection from inflammation in immuno-compromised patients, and that combinatorial strategies offer superior clinical utility.[13,38]

The genetic component of this study revealed that the TNFAIP3 rs10499194 T allele was significantly associated with RA susceptibility under both allelic (OR = 2.87, *p*=0.028) and dominant (OR = 3.33, *p*=0.032) models when comparing all RA patients to normal controls. This finding is particularly significant given the established role of TNFAIP3 (also known as A20) as a master regulator of NF-κB signaling and inflammation. The TNFAIP3 gene encodes a ubiquitin-editing enzyme that terminates NF-κB activation by deubiquitinating key signaling molecules such as receptor-interacting protein kinase 1 and TNF receptor-associated factor 6 (TRAF6).[20,22] Functional impairment of A20 due to genetic polymorphisms leads to sustained NF-κB activation, resulting in excessive production of pro-inflammatory cytokines including Tumor Necrosis Factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6, which are central mediators of RA pathogenesis.[18]

Interestingly, our finding that the T allele confers increased RA risk contrasts with the meta-analysis by Wang et al., 2016, which reported that the rs10499194 T allele was associated with a decreased risk of RA in predominantly Caucasian populations.[39] This discrepancy likely reflects ethnic heterogeneity in the genetic architecture of RA susceptibility, as the functional impact of SNPs can vary across populations due to differences in linkage disequilibrium patterns and gene-environment interactions. Indeed, the study by Li et al., 2017, noted that while no association was found between TNFAIP3 rs10499194 and RA in Europeans, a significant association was observed in Asian populations, suggesting population-specific effects.[40] Our findings in an Iraqi Arab population add a novel dimension to this body of literature, representing the first report of this association in a Middle Eastern cohort.

The Iranian study by Alizadeh et al., 2026, which employed the same Tetra-ARMS PCR primers used in our investigation, reported that the CT and TT genotypes of rs10499194 were significantly associated with an increased risk of thrombocytopenia (OR = 2.5 for CT, OR = 1.8 for TT), with the CC genotype frequency in normal controls being 76.9%.[26] This is remarkably consistent with our observation of 83.3% CC frequency in the controls and supports the notion that the T allele confers susceptibility to immune-mediated conditions in Middle Eastern populations. Furthermore, the study by Xu et al., 2017, demonstrated that the rs10499194 T

allele was associated with increased risk of type-1 autoimmune hepatitis in a Chinese population, reinforcing the cross-disease relevance of this polymorphism in autoimmune pathology.[41]

The Chinese study by Tang et al., 2026, which investigated TNFAIP3, IRAK1, and TLR4 gene polymorphisms in 308 RA patients and 310 controls, reported that certain TNFAIP3 variants (rs5029930 and rs5029939) were significantly associated with RA susceptibility, further confirming the involvement of the TNFAIP3 locus in RA pathogenesis across diverse populations.[42]

Perhaps the most novel contribution of this study is the demonstration that the TNFAIP3 rs10499194 genotype significantly modulates the levels of Presepsin and sCD64 in RA patients. T allele carriers exhibited significantly higher levels of both biomarkers under the dominant model (p=0.011 for Presepsin, p=0.018 for sCD64), with a significant dose-response relationship across CC, CT, and TT genotypes (trend p=0.005 for Presepsin, p=0.013 for sCD64). This genotype-biomarker interaction was most pronounced in the RA with infection subgroup, suggesting that the T allele amplifies the inflammatory response to infection.

The biological plausibility of this finding rests on the functional role of A20/TNFAIP3 in restraining NF-κB signaling. Polymorphisms that compromise A20 function would be expected to result in an exaggerated NF-κB-driven inflammatory response upon bacterial challenge, leading to enhanced production of downstream effectors including the monocyte/macrophage activation products Presepsin and sCD64.[20,22] The comprehensive review study by Wu et al., 2020, demonstrated that A20-deficient mice develop severe arthritis with markedly elevated inflammatory mediators, providing experimental support for the concept that TNFAIP3 dysfunction amplifies inflammatory responses.[18] This genotype-biomarker relationship was not previously reported in the literature and represents a significant advance in understanding the genetic determinants of infection-related biomarker variability in RA.

The microbiological profile of infections in our RA cohort revealed that bacterial infections predominated (70.0%), with the respiratory tract being the most common site (33.3%), followed by the urinary tract and skin/soft tissue (each 20.0%). *S. aureus* was the most frequently isolated organism (25.8%). These findings are consistent with the landmark population-based study by Doran et al., 2002, who reported that RA patients had a significantly increased risk of infections (HR = 1.70, 95% CI: 1.42–2.03), with the highest risk ratios observed for bone, joint, skin, soft tissue, and respiratory tract infections. [11]

Importantly, our findings align closely with several previous Iraqi studies. For instance, the study by Jasim et al., 2023, evaluated urinary tract infections in 50 Iraqi RA patients and reported that *S. aureus* was the most common isolated gram-positive organism, with 88% of patients showing positive cultures.[43] Similarly, an Iraqi study from Baghdad on the effect of bacterial respiratory tract infections in RA patients found that 74.19% of RA patients were infected with pathogenic bacteria, with *S. aureus* being the predominant organism (10 out of 23 infected patients).[44] The predominance of *S. aureus* in our cohort and in these Iraqi studies may reflect the high carriage rates and antimicrobial resistance patterns of this organism in the Iraqi healthcare setting, as documented by Hussein et al., 2024.[45] The increased susceptibility of RA patients to infections is multifactorial, encompassing intrinsic immune dysregulation, immunosuppressive therapy with DMARDs and corticosteroids, and disease-related comorbidities.[29] In parallel, emerging therapeutic strategies such as methotrexate-loaded nanoparticles showed the potential to attenuate arthritis progression and reduce inflammatory mediator levels, highlighting ongoing efforts to optimize RA management in clinically complex settings.[46]

This study added to the growing body of Iraqi literature on RA. Al-Badran et al., 2022, documented the incidence of RA at Marjan Teaching Hospital in Babylon, the same institution where our sample collection was conducted, providing important epidemiological

context for our findings.[47] The study by Awni et al., 2023, investigated the effect of NOD-like receptor pyrin domain-containing 3 (NLRP3) inflammasome gene polymorphisms on disease susceptibility and response to TNF-α inhibitors in Iraqi RA patients, representing another important Iraqi contribution to the understanding of genetic determinants of RA.[48] Hashim et al., 2024, studied the 857 T/C SNP in the TNF-α gene in RA patients from Babylon province, Iraq, and reported significant associations with disease susceptibility, complementing our findings on the TNFAIP3 locus.[49] To our knowledge, the present study is the first to investigate the TNFAIP3 rs10499194 polymorphism specifically in Iraqi RA patients, filling an important gap in the regional genetic literature.

The clinical implications of our findings are substantial. First, the combination of Presepsin and sCD64 offers a rapid, ELISA-based diagnostic tool that can be readily implemented in clinical laboratories to aid in the early identification of concurrent infections in RA patients, potentially reducing diagnostic delays and improving patient outcomes. Second, the identification of the TNFAIP3 rs10499194 T allele as a risk factor for RA and as a modulator of infection-related biomarker levels suggested that genotyping of this polymorphism could help identify RA patients at heightened risk of infection, enabling targeted surveillance strategies. Third, the finding that conventional markers (CRP, ESR) are insufficient for reliably detecting infection in RA underscores the need for incorporating novel biomarkers into clinical practice guidelines for the management of febrile RA patients.

In conclusion, this study showed that integrating novel biomarkers with genetic profiling may improve differentiation between bacterial infection and autoimmune flare in RA. Presepsin and sCD64 were strong independent predictors of infection and outperformed conventional inflammatory markers such as CRP and ESR, which are often influenced by RA activity. Their combined use provided excellent diagnostic accuracy, supporting a multi-marker approach for better clinical decision-making and more timely management. The findings also suggested an important role for the TNFAIP3 rs10499194 polymorphism in RA-related inflammation. Although the T allele was associated with RA susceptibility, it was also linked to higher Presepsin and sCD64 levels, particularly during infection, indicating a possible genotype-related amplification of innate immune responses.

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Author Contributions

FHM, Contributed to the conception and design of the study, laboratory investigations, data collection, biomarker and molecular analyses, interpretation of results, and drafting of the manuscript. She also serves as the corresponding author and accepts responsibility for the integrity of the work as a whole. DSAA, Contributed to the study design, supervision of the microbiological and molecular work, interpretation of the results, and critical revision of the manuscript for important intellectual content. AAH, Contributed to patient recruitment, clinical evaluation, clinical data collection, interpretation of the clinical findings, and critical revision of the manuscript.

Declaration of Conflicting Interests

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethical Committee of the Jabir Ibn Hayyan Medical University, Faculty of Medicine, Iraq (Reference No: 4540, dated August 20, 2025).

Informed consent

A written informed consent was obtained from each individual participant prior to be included in the study.

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