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Diagnostic Utility of Serum Amyloid A and Soluble Interleukin-2 Receptor Alpha in Differentiating Acute Infection from Disease Flare in Rheumatoid Arthritis

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Abstract

Background: Distinguishing acute infection from aseptic disease flare in rheumatoid arthritis (RA) remains a significant clinical challenge due to the overlapping symptomatology and the low specificity of conventional inflammatory markers.

Methodology: This case-control study included 90 participants: 30 healthy controls, 30 RA patients without infection, and 30 RA patients with confirmed concurrent infection. Serum levels of Serum Amyloid A (SAA), soluble Interleukin-2 Receptor alpha (sIL-2Rα), and anti-Cyclic Citrullinated Peptide (anti-CCP) were quantified via ELISA. Diagnostic performance was evaluated using ROC curve and multivariate logistic regression analyses.

Results: SAA and sIL-2Rα levels were significantly elevated in infected RA patients compared to uninfected RA patients ($p = 0.001$ and $p = 0.041$, respectively). Anti-CCP levels did not differ between the two RA groups ($p = 0.869$). A combined diagnostic model incorporating SAA and sIL-2Rα achieved the highest discriminatory capacity for infection (AUC = 0.842, Sensitivity = 83.3%, Specificity = 80.0%). Multivariate analysis confirmed SAA as a robust independent predictor of infection (OR = 1.025, $p = 0.034$), even after adjusting for white blood cell count and morning stiffness.

Conclusion: SAA, particularly when combined with sIL-2Rα, serves as a superior diagnostic biomarker for accurately identifying concurrent acute infections in RA patients, facilitating timely and appropriate therapeutic interventions.

القيمة التشخيصية لبروتين المصل النشواني Aوالمستقبل ألفا الذائب للإنترلوكين-2 في التمييز بين العدوى الحادة ونوبة نشاط المرض في التهاب المفاصل الروماتويدي

فاطمة حسين محمد، ضياء شنان عبد الكاظم، علي امير حمزة

الخلاصة:

المقدمة: يُعَدّ التمييز بين العدوى الحادة ونوبة نشاط المرض غير الإنتانية في التهاب المفاصل الروماتويدي (RA) تحدّيًا سريريًا مهمًا، نظرًا لتداخل المظاهر السريرية وانخفاض النوعية التشخيصية للمؤشرات الالتهابية التقليدية.

المرضى وطرق العمل: شملت هذه الدراسة من نوع الحالات والشواهد 90 مشاركًا، بواقع 30 من الأصحاء كمجموعة سيطرة، و30 مريضًا بالتهاب المفاصل الروماتويدي من دون عدوى، و30 مريضًا بالتهاب المفاصل الروماتويدي مع عدوى مؤامنة مؤكدة. جرى قياس المستويات المصلية لكل من بروتين المصل النشواني A (SAA)، والمستقبل ألفا الذائب للإنترلوكين-2 (sIL-2Rα)، والأجسام المضادة للببتيد السيترولييني الحلقي (anti-CCP) باستخدام تقنية المقايسة المناعية المرتبطة بالإنزيم (ELISA). كما جرى تقييم الأداء التشخيصي باستخدام منحنيات خصائص تشغيل المستقبل (ROC) وتحليل الانحدار اللوجستي متعدد المتغيرات.

النتائج: كانت مستويات كلٍ من SAA و sIL-2Rα مرتفعة بصورة معنوية في مرضى التهاب المفاصل الروماتويدي المصابين بعدوى مقارنةً بالمرضى غير المصابين بعدوى ($p = 0.001$ و$p = 0.041$، على التوالي). (في المقابل، لم تُظهر مستويات anti-CCP فرقًا معنويًا بين مجموعتي المرضى المصابين وغير المصابين بالعدوى ($p = 0.869$). وقد حقق النموذج التشخيصي المدمج الذي يجمع بين SAA و sIL-2Rα أعلى قوة تمييزية للكشف عن العدوى، إذ بلغت المساحة تحت المنحنى 0.842 (AUC)، مع حساسية قدرها 83.3% ونوعية بلغت 80.0%. كما أكد تحليل المتغيرات المتعددة أن SAA يُعَدّ متنبئًا مستقلًا قويًا بوجود العدوى (OR = 1.025، $p = 0.034$)، حتى بعد الضبط إحصائيًا لعدد كريات الدم البيضاء ومدة التيبس الصباحي.

الاستنتاج: يُعَدّ SAA، ولا سيما عند دمجه مع sIL-2Rα، واسمًا حيويًا تشخيصيًا متفوّقًا للتحديد الدقيق للعدوى الحادة المؤامنة لدى مرضى التهاب المفاصل الروماتويدي، بما يسهم في اتخاذ قرارات علاجية ملائمة وفي الوقت المناسب.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, progressive, systemic autoimmune disease characterized by symmetric polyarthritis, synovial inflammation, and subsequent joint destruction (Mousavi et al., 2022). The global burden of rheumatoid arthritis remains substantial and continues to increase. According to a comprehensive 2025 analysis based on the Global Burden of Disease 2021 dataset, approximately 17.9 million individuals were living with rheumatoid arthritis worldwide in 2021, with an age-standardized prevalence rate of 208.9 per 100,000 population (Smolen et al., 2016). In the Middle East and North Africa region, the latest directly verifiable regional burden estimate reported 672.8 thousand prevalent cases in 2019, corresponding to an age-standardized point prevalence of 120.6 per 100,000 population and an annual incidence rate of 5.9 per 100,000 population (Ma et al., 2025). In Iraq, a local hospital-based study from Marjan Teaching Hospital in Babylon documented an increase in rheumatoid arthritis incidence from 1.1 in 2014 to 1.7 in 2019, highlighting the continuing local clinical burden of the disease (Mousavi et al., 2022; Al-Badran et al., 2022). While joint erosion is the hallmark of RA, the morbidity and mortality associated with the disease are heavily driven by extra-articular complications, among which acute infections are paramount (Lortholary et al., 2020). Patients with RA face a more than two-fold increased risk of developing serious, potentially life-threatening infections compared to the general population (Listing et al., 2013). This heightened susceptibility is multifactorial, stemming from the intrinsic immune dysregulation inherent to the autoimmune pathology, age-related immunosenescence, and crucially, the iatrogenic immunosuppression induced by standard therapies (Riley and George, 2021). Glucocorticoids, such as prednisolone, are frequently utilized to manage acute RA flares but are notorious for causing dose-dependent immunosuppression, impairing neutrophil and macrophage function, and significantly elevating the risk of bacterial, viral, and fungal infections (Youssef et al., 2015). The clinical presentation of an acute infection in an RA patient poses a profound diagnostic dilemma for rheumatologists. The systemic symptoms of infection—such as fever, fatigue, and joint pain—often perfectly mimic those of an aseptic RA disease flare (Mehta et al., 2019). Prompt and accurate differentiation between these two states is critical: an infection requires immediate antimicrobial therapy and the potential cessation of immunosuppressants, whereas a disease flare necessitates the escalation of immunosuppressive treatment (Cui and Qian, 2023). Erroneously treating an infection with high-dose corticosteroids can lead to catastrophic sepsis, while treating a flare with antibiotics contributes to antimicrobial resistance and unchecked joint destruction. Currently, clinicians rely heavily on conventional acute-phase reactants, primarily C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR), alongside white blood cell (WBC) counts, to detect inflammation (Pope and Choy, 2021). However, these traditional markers possess a fundamental limitation: they have low diagnostic specificity for bacterial infections in the context of autoimmune diseases (Cui and Qian, 2023). Both CRP and ESR are routinely elevated during the acute phase of an RA flare, making it exceedingly difficult to determine whether a spike in these markers is driven by autoimmune activity or a superimposed pathogen (Zinellu and Mangoni, 2024). Furthermore, microbiological cultures, the gold standard for diagnosing infection, often take 3 to 7 days to yield results and frequently produce false negatives, delaying critical clinical interventions (Cui and Qian, 2023). Consequently, there is an urgent, unmet clinical need to identify novel biomarkers that can rapidly and accurately distinguish concurrent infection from sterile inflammation in RA patients (Cui and Qian, 2023). Recent literature has highlighted several promising candidates. Serum Amyloid A (SAA) is a highly sensitive acute-phase protein synthesized by hepatocytes in response to pro-inflammatory cytokines, and uniquely, it is also produced locally in inflamed synovial tissues (Hosman et al., 2021). SAA plays a direct role in innate immunity by opsonizing Gram-negative bacteria and acting as a potent chemoattractant for neutrophils (Shah et al., 2006). Recent systematic reviews have positioned SAA as a superior biomarker for rheumatic diseases compared to conventional markers (Avcı, 2025). Similarly, soluble Interleukin-

2 Receptor alpha (sIL-2Rα), a product of the proteolytic cleavage of the CD25 receptor on activated T-lymphocytes, has emerged as a quantitative surrogate marker for systemic T-cell activation, reflecting both autoimmune activity and infectious burden (Damoiseaux, 2020). Conversely, anti-Cyclic Citrullinated Peptide (anti-CCP) antibodies are highly specific for RA pathogenesis but their dynamic response during acute infectious episodes remains poorly characterized (Zendman et al., 2006). Despite the promising individual profiles of these novel biomarkers, a significant knowledge gap remains. To date, very few studies have evaluated the combined diagnostic utility of SAA and sIL-2Rα in differentiating infection from disease flares specifically within the RA population, and none have done so in the Iraqi demographic context. Furthermore, the behavior of anti-CCP during acute infectious stress in established RA patients requires clarification to determine if it acts solely as an autoimmune marker or fluctuates with acute systemic inflammation. Therefore, the primary objective of this study was to evaluate the diagnostic performance of novel biomarkers (SAA, sIL-2Rα, and anti-CCP) in detecting concurrent acute infections among Iraqi patients with RA, comparing their efficacy against conventional inflammatory markers (CRP, ESR, WBC). The novelty of this research lies in its approach to combining innate (SAA) and adaptive (sIL-2Rα) immune markers into a unified diagnostic model, aiming to provide a highly sensitive and specific tool that can overcome the limitations of current clinical practice and improve the safety of managing immunosuppressed RA patients.

2. Materials and Methods

2.1. Study Design and Subjects

A case-control, hospital-based study was conducted between August 25, 2025, and March 30, 2026, at Al-Marjan Teaching Hospital, a tertiary care center in Babylon, Iraq. The study protocol was rigorously reviewed and officially approved by the Medical Ethics Committee of the Faculty of Medicine, Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences (Reference No. 4540, dated August 20, 2025). The research was carried out in strict accordance with the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their enrollment in the study.

2.2. Study Population

A total of 90 participants were enrolled in the study and divided into three groups: a control group of healthy volunteers (n = 30), a Rheumatoid Arthritis (RA) without infection group (n = 30), and a RA with infection group (n = 30), comprising patients with confirmed concurrent bacterial, viral, fungal, or mycobacterial infections. The diagnosis of RA was established according to the 2010 American College of Rheumatology (ACR) / European League Against Rheumatism (EULAR) classification criteria (Aletaha et al., 2010). Comprehensive demographic data, including age, sex, residence, smoking status, and family history of RA, as well as clinical characteristics such as body mass index (BMI), presence of chronic diseases, disease duration, and current treatment regimens (methotrexate and prednisolone), were systematically recorded for all participants. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2) and categorized according to World Health Organization (WHO) guidelines (World Health Organization, 2025).

2.3. Inclusion and Exclusion Criteria

Participants were included in the study based on the following criteria. RA patients (with or without infection) were adults aged 18 years or older who fulfilled the 2010 ACR/EULAR classification criteria for RA. For the RA with infection group, patients were required to have a clinically and microbiologically confirmed acute infection (bacterial, viral, fungal, or mycobacterial) at enrollment. RA patients without infection were required to have no clinical signs, symptoms, or laboratory evidence of active infection for at least four weeks prior to sample collection. The control group consisted of

apparently healthy adult volunteers, matched as closely as possible for age and sex with the RA cohorts, with no current or past history of autoimmune, inflammatory, or infectious diseases. Participants were excluded if they had other systemic autoimmune or connective tissue diseases (e.g., Systemic Lupus Erythematosus, Sjögren's syndrome, Systemic Sclerosis), active malignancy or cancer within the past five years, severe hepatic, renal, or cardiovascular failure, or if they were pregnant or lactating. Additionally, RA patients without infection and control participants were excluded if they had a recent acute infection, fever of unknown origin, or recent use of antimicrobial agents within the past four weeks. Individuals who refused to provide informed consent were also excluded.

2.4. Clinical Assessment

Disease activity in RA patients was quantitatively evaluated using the Disease Activity Score-28 for Rheumatoid Arthritis with Erythrocyte Sedimentation Rate (DAS28-ESR). The DAS28-ESR score incorporates the number of tender joints (out of 28), the number of swollen joints (out of 28), the patient's global assessment of health on a visual analog scale (VAS), and the ESR value, providing a standardized measure of RA disease severity (Wells et al., 2009). Additionally, the duration of morning stiffness was recorded in minutes for each patient.

2.5. Sample Collection and Processing

Under strict aseptic conditions, 5 mL of venous blood was collected from each participant via venipuncture. The sample was divided into two portions: 2 mL of whole blood was transferred into an EDTA tube for immediate complete blood count (CBC) and ESR analysis, while 3 mL was placed in a plain gel tube, allowed to clot at room temperature for 30 minutes, and then centrifuged at 3000 rpm for 10 minutes. The resulting serum was aliquoted into sterile Eppendorf tubes and stored at -20°C until biochemical and ELISA analyses, minimizing freeze-thaw cycles to preserve protein stability (Thermo Fisher Scientific, n.d.).For RA patients with infection, additional clinical specimens—including blood, sputum, wound swabs, or urine—were collected according to the suspected site of infection for microbiological culture and pathogen identification.

2.6. Laboratory Investigations

2.6.1 Conventional Inflammatory Markers

Complete blood count (CBC) was performed using an automated hematology analyzer (Sysmex XN-1000™, Sysmex Corporation, Kobe, Japan) (Linko-Parvinen et al., 2022), and ESR was measured by the modified Westergren method, the ICSH-recommended gold standard (Kratz et al., 2017). Serum CRP levels were quantified using an automated immunoturbidimetric assay (Mindray BS-240, Shenzhen Mindray Bio-Medical Electronics, China) (Mindray, n.d.), while RF was measured by rate nephelometry, with values >14 IU/mL considered positive (Roberts-Thomson et al., 1985).

2.6.2 Measurement of Serum Biomarkers by ELISA

Serum levels of novel biomarkers and autoantibodies were measured using commercially available sandwich ELISA kits according to the manufacturers' protocols, with absorbance read at 450 nm. SAA was quantified using the Human SAA-4 ELISA Kit (BT LAB, Shanghai, China; range 2–700 ng/L; sensitivity 1.44 ng/L; CV <10%) (Cui and Qian, 2023). sIL-2Rα was measured with the Human sIL-2Rα ELISA Kit (BT LAB; range 5–1000 ng/L; sensitivity 2.75 ng/L) , and anti-CCP antibodies were determined using the Quick Step anti-CCP ELISA Kit (SunLong Biotech, China), with positivity defined as >20 U/mL .

2.6.3 Microbiological Identification

For the isolation and identification of causative pathogens in the infected RA cohort, standard microbiological protocols were followed. Blood cultures were processed using an automated continuous-monitoring blood culture system (BD

BACTEC™ FX, Becton Dickinson, USA) (Somily et al., 2018). Positive cultures and other clinical specimens were inoculated onto appropriate agar media (e.g., Blood Agar, MacConkey Agar, Sabouraud Dextrose Agar). Bacterial and fungal isolates were subsequently identified to the species level using the VITEK® 2 compact automated system (bioMérieux, Marcy-l'Étoile, France), which provides rapid and accurate microbial identification through biochemical profiling (Ligozzi et al., 2002). Viral infections (e.g., Influenza, Hepatitis C) were confirmed via specific polymerase chain reaction (PCR) or established serological assays.

2.7. Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA) (IBM Corp., 2020). Normality of continuous variables was assessed with the Shapiro-Wilk test (Shapiro and Wilk, 1965). Normally distributed variables (e.g., age, ESR, WBC, DAS28-ESR, morning stiffness) were expressed as mean ± SD and compared using one-way ANOVA. Non-normally distributed variables (e.g., BMI, CRP, RF, SAA, sIL-2Rα, anti-CCP) were presented as median (IQR) and analyzed with the Kruskal-Wallis H test, followed by Mann-Whitney U tests for pairwise comparisons with Bonferroni correction ($\alpha = 0.016$). Categorical variables (e.g., sex, smoking, residence, treatment regimens, pathogen types) were reported as frequencies (%) and analyzed using Chi-square or Fisher's exact tests. Spearman's rank correlation (ρ) assessed associations between novel biomarkers and conventional disease parameters. Diagnostic performance of biomarkers for distinguishing RA patients with infection was evaluated using ROC curve analysis, calculating AUC, optimal cut-offs (maximizing Youden's J), sensitivity, specificity, PPV, and NPV (Hajian-Tilaki, 2014). Univariate binary logistic regression identified potential predictors of infection, and variables with $p < 0.05$ were included in multivariate models to determine independent predictors, reporting ORs and 95% CIs. Variables with multicollinearity (VIF < 5) or quasi-complete separation were excluded. Model fit was assessed via the Hosmer-Lemeshow test, Nagelkerke R^2, and AIC. Two-tailed p-values < 0.05 were considered statistically significant.

3. Results

The study groups were generally comparable in their baseline characteristics, with no statistically significant differences observed (all $p > 0.05$), except for body mass index (BMI) and the presence of chronic diseases (both $p = 0.001$), which were higher in the RA groups; a predominance of female participants was also observed across all groups Table1.

Table1: Demographic and Clinical Characteristics of The Study Population

Variable	Control (n = 30)	RA without Infection (n = 30)	RA with Infection (n = 30)	Statistical Test	P value
Age (years) [a]	41.97 ± 9.25	43.63 ± 8.40	41.13 ± 12.87	One-way ANOVA	0.637
BMI (kg/m²) [b]	24.70 (23.42–26.75)	28.85 (27.07–30.45)	28.60 (22.80–30.30)	Kruskal-Wallis	**0.001**
Sex [c]				Fisher's exact	0.421
Male, n (%)	6 (20.0%)	3 (10.0%)	3 (10.0%)		
Female, n (%)	24 (80.0%)	27 (90.0%)	27 (90.0%)		
Smoking [c]				Fisher's exact	0.260
Yes, n (%)	6 (20.0%)	2 (6.7%)	3 (10.0%)		
No, n (%)	24 (80.0%)	28 (93.3%)	27 (90.0%)		
Residence [c]				Chi-square	0.179
Urban, n (%)	15 (50.0%)	9 (30.0%)	9 (30.0%)		
Rural, n (%)	15 (50.0%)	21 (70.0%)	21 (70.0%)		
Family History of RA [c]				Chi-square	0.136
Yes, n (%)	3 (10.0%)	8 (26.7%)	9 (30.0%)		
No, n (%)	27 (90.0%)	22 (73.3%)	21 (70.0%)		
Chronic Diseases [c]				Fisher's exact	**0.001**
Yes, n (%)	0 (0.0%)	11 (36.7%)	6 (20.0%)		
No, n (%)	30 (100.0%)	19 (63.3%)	24 (80.0%)		

[a] Data presented as Mean ± SD (normally distributed). [b] Data presented as Median (Q1–Q3) (non-normally distributed). [c] Data presented as n (%). Bold p-values indicate statistical significance at $p < 0.05$. Abbreviations: BMI, Body Mass Index; RA, Rheumatoid Arthritis.

Table2 shows no significant difference in disease duration between groups ($p = 0.299$). However, RA patients with infection had higher disease activity (DAS28-ESR) and longer morning stiffness (both $p < 0.001$). They also had greater prednisolone use and combination therapy (both $p < 0.001$), with lower MTX use ($p = 0.042$), indicating more severe disease and intensive treatment.

Table2: Disease-Specific Characteristics of Rheumatoid Arthritis Patients

Variable	RA without Infection (n = 30)	RA with Infection (n = 30)	Statistical Test	p-value
Disease Duration (years) [b]	5.00 (3.00–10.00)	7.50 (4.25–11.75)	Mann-Whitney U	0.299
DAS28-ESR Score [a]	4.02 ± 0.83	5.27 ± 1.27	Student's t-test	**<0.001**
Morning Stiffness (min) [a]	48.50 ± 21.62	75.40 ± 21.87	Student's t-test	**<0.001**
Prednisolone use [c]			Fisher's exact	**<0.001**
Yes, n (%)	6 (20.0%)	29 (96.7%)		
No, n (%)	24 (80.0%)	1 (3.3%)		
MTX use [c]			Fisher's exact	**0.042**
Yes, n (%)	28 (93.3%)	21 (70.0%)		
No, n (%)	2 (6.7%)	9 (30.0%)		
Treatment regimen [c]			Fisher's exact	**<0.001**
Combination therapy, n (%)	13 (43.3%)	30 (100.0%)		
Monotherapy, n (%)	17 (56.7%)	0 (0.0%)		

[a] Mean ± SD (Student's t-test). [b] Median (Q1–Q3) (Mann-Whitney U test). [c] n (%) (Chi-square or Fisher's exact test). Bold p-values indicate statistical significance at $p < 0.05$. Abbreviations: DAS28-ESR, Disease Activity Score 28 joints; MTX, Methotrexate.

Table3 demonstrates significant differences in all inflammatory markers across groups (all $p < 0.001$). CRP, ESR, and WBC levels showed a graded increase from controls to RA without infection and were highest in RA with infection, with significant differences between all groups. RF levels were elevated in both RA groups compared to controls but did not differ between RA subgroups. RF and anti-CCP positivity were significantly more frequent in RA patients than controls, while anti-CCP levels were comparable between RA groups.

Table3: Conventional Inflammatory Markers Across Study Groups

Variable	Control (n = 30)	RA without Infection (n = 30)	RA with Infection (n = 30)	Statistical Test	P value
CRP (mg/L) [b]	2.10 (1.60–3.00)	21.35 (17.55–25.38)	67.50 (41.95–92.10)	Kruskal-Wallis	**<0.001**
ESR (mm/h) [a]	10.43 ± 5.08	42.30 ± 11.41	72.63 ± 19.96	One-way ANOVA	**<0.001**
WBC ($\times 10^3/\mu L$) [a]	6.40 ± 1.39	8.62 ± 1.99	13.50 ± 2.73	One-way ANOVA	**<0.001**
RF (IU/ml) [b]	6.05 (3.90–8.05)	51.25 (10.03–83.53)	39.70 (10.38–58.15)	Kruskal-Wallis	**<0.001**
RF Positivity (>14 IU/ml) [c]				Chi-square	**<0.001**
Positive, n (%)	0 (0.0%)	20 (66.7%)	21 (70.0%)		
Negative, n (%)	30 (100.0%)	10 (33.3%)	9 (30.0%)		
Anti-CCP (U/ml) [b]	4.04 (2.50–7.01)	45.47 (12.18–51.60)	33.89 (7.86–48.80)	Kruskal-Wallis	**<0.001**
Anti-CCP Positivity (>20 U/ml) [c]				Chi-square	**<0.001**
Positive, n (%)	0 (0.0%)	18 (60.0%)	16 (53.3%)		
Negative, n (%)	30 (100.0%)	12 (40.0%)	14 (46.7%)		

[a] Mean ± SD. [b] Median (Q1–Q3). [c] n (%). Post-hoc pairwise comparisons (Bonferroni-corrected $\alpha = 0.016$): CRP, ESR, and WBC showed significant differences between all group pairs ($p < 0.001$). RF showed no significant difference between RA groups ($p = 0.474$). Anti-CCP showed no significant difference between RA groups ($p = 0.387$). Abbreviations: CRP, C-Reactive Protein; ESR, Erythrocyte Sedimentation Rate; WBC, White Blood Cell Count; RF, Rheumatoid Factor; Anti-CCP, Anti-Cyclic Citrullinated Peptide Antibody.

Table4 shows significant differences in all novel biomarkers across groups (all $p < 0.001$). SAA and sIL-2Rα increased progressively and differed between all groups, including between RA subgroups, with SAA showing the largest effect size. Anti-CCP was higher in RA vs. controls but did not differ between RA subgroups.

Table4: Comparison of Novel Biomarkers Across Study Groups

Biomarker	Control (n = 30)	RA without Infection (n = 30)	RA with Infection (n = 30)	H statistic	p-value	η^2
SAA (ng/L)	19.39 (14.25–25.05)	69.67 (58.42–116.64)	115.69 (95.03–197.69)	60.190	**<0.001**	0.692
sIL-2Rα (ng/L)	25.26 (19.19–43.86)	43.73 (32.00–72.52)	75.14 (52.19–89.58)	32.677	**<0.001**	0.353
Anti-CCP (U/ml)	4.04 (2.50–7.01)	45.47 (12.18–51.60)	33.89 (7.86–48.80)	31.698	**<0.001**	0.341

Post-hoc Pairwise Comparisons (Bonferroni-corrected)

Biomarker	Control vs. RA w/o Infection	Control vs. RA w/ Infection	RA w/o Infection vs. RA w/ Infection
SAA (ng/L)	**<0.001**	**<0.001**	**0.001**
sIL-2Rα (ng/L)	**<0.001**	**<0.001**	**0.041**
Anti-CCP (U/ml)	**<0.001**	**<0.001**	0.869

Data presented as Median (Q1–Q3). Kruskal-Wallis H test for overall comparison. Mann-Whitney U test with Bonferroni correction for post-hoc comparisons ($\alpha = 0.017$). η^2 = Eta-squared effect size: Small (0.01–0.06), Medium (0.06–0.14), Large (>0.14). Bold p-values indicate statistical significance. Abbreviations: SAA, Serum Amyloid A; sIL-2Rα, Soluble Interleukin-2 Receptor Alpha; Anti-CCP, Anti-Cyclic Citrullinated Peptide Antibody.

Fig.1. demonstrates a stepwise increase in SAA levels from controls to RA without infection to RA with infection, with significant differences between all groups and greater variability in RA with infection.

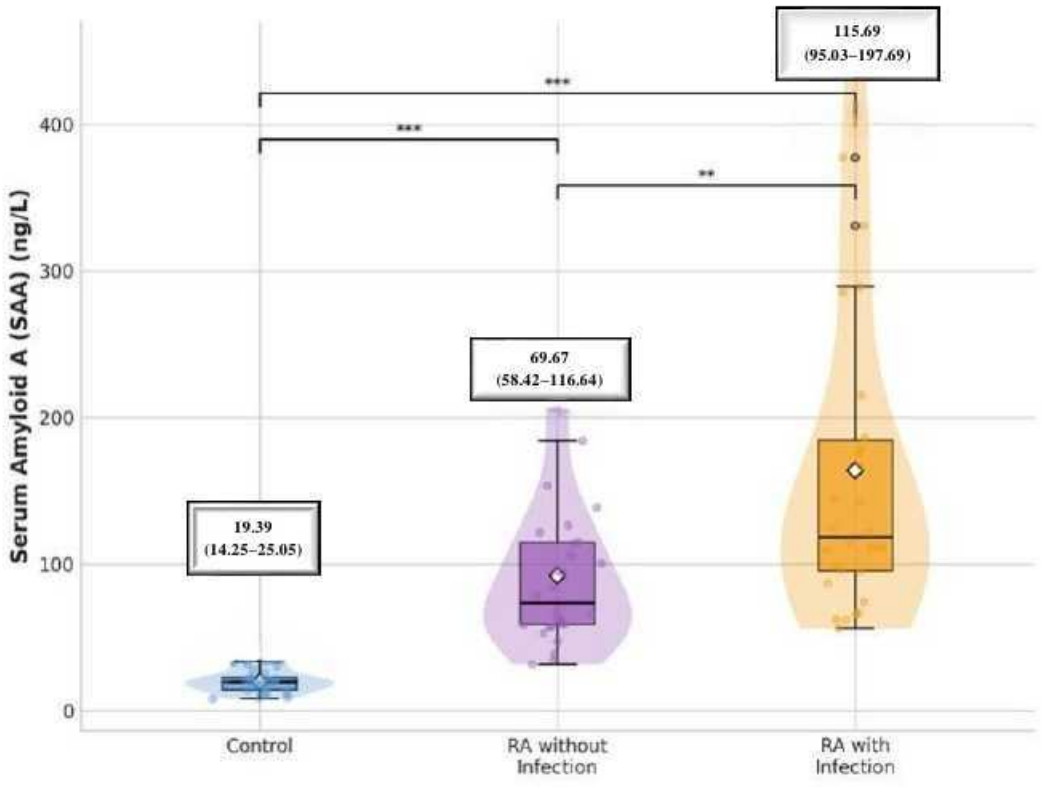

Figure1: Distribution of Serum Amyloid A (SAA) Levels Across Study Groups.

Box-violin plots showing median (IQR): Control 19.39 (14.25–25.05) ng/L, RA without infection 69.67 (58.42–116.64) ng/L, RA with infection 115.69 (95.03–197.69) ng/L. Kruskal-Wallis H = 60.190, $p < 0.001$. **p < 0.01, ***p < 0.001 (Bonferroni-corrected). Fig 2. shows a similar increasing pattern for sIL-2Rα across groups, with significant differences between all comparisons and wider dispersion in RA with infection.

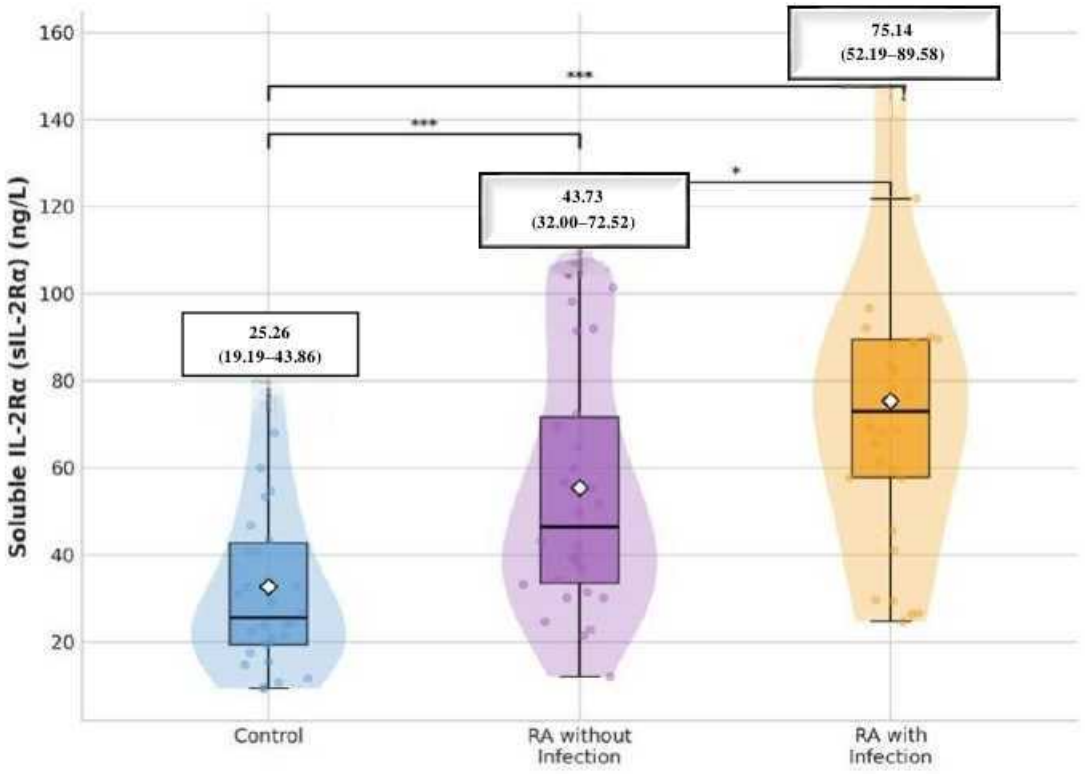

Figure2: Distribution of Soluble IL-2Rα (Sil-2Rα) Levels Across Study Groups.

Box-violin plots showing median (IQR): Control 25.26 (19.19–43.86) ng/L, RA without infection 43.73 (32.00–72.52) ng/L, RA with infection 75.14 (52.19–89.58) ng/L. Kruskal-Wallis H = 32.677, $p < 0.001$. *p < 0.05, ***p < 0.001 (Bonferroni-corrected). Table5 shows that WBC and CRP had the highest individual diagnostic performance (AUC = 0.833 and 0.823), while SAA demonstrated fair accuracy (AUC = 0.769) and sIL-2Rα lower performance (AUC = 0.686). The combined model (SAA + sIL-2Rα) achieved the highest AUC (0.842) with improved overall diagnostic indices.

Table5: Diagnostic Performance of Biomarkers for Detecting Infection in RA Patients (ROC Curve Analysis)

Biomarker	AUC (95% CI)	p-value	Optimal Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's J
Novel Biomarkers								
SAA (ng/L)	0.769 (0.641–0.897)	<0.001	93.06 ng/L	83.3	73.3	75.8	81.5	0.567
sIL-2Rα (ng/L)	0.686 (0.548–0.824)	0.008	57.48 ng/L	73.3	63.3	66.7	70.4	0.367
Conventional Markers								
WBC (×10³/µL)	0.833 (0.725–0.942)	<0.001	10.70 ×10³/µL	80.0	80.0	80.0	80.0	0.600
CRP (mg/L)	0.823 (0.717–0.930)	<0.001	32.30 mg/L	76.7	76.7	76.7	76.7	0.533
Combined Model								
SAA + sIL-2Rα	0.842 (0.738–0.946)	<0.001	Logistic model	83.3	80.0	80.6	82.8	0.633

AUC: Area Under the Curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value; Youden's J = Sensitivity + Specificity − 1. Optimal cut-off for individual markers determined by maximizing Youden's index. Combined models derived from binary logistic regression predicted probabilities. All p-values < 0.01. Classification: Negative (0) = RA without infection (n = 30); Positive (1) = RA with infection (n = 30)

Fig.3. illustrates that the combined model yielded the best overall performance, slightly outperforming WBC and CRP, while sIL-2Rα showed the weakest discrimination.

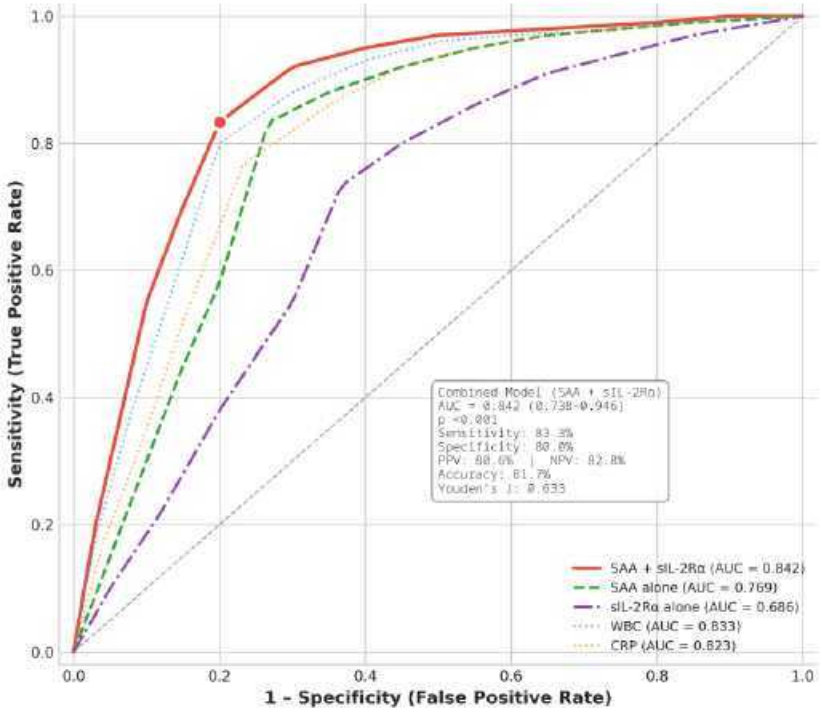

Figure3: Comparison of ROC Curves for Individual and Combined Biomarkers in Diagnosing Infection in RA Patients.

The combined model (SAA + sIL-2Rα) achieved AUC = 0.842 (95% CI: 0.738–0.946), p < 0.001, with Sensitivity 83.3%, Specificity 80.0%, PPV 80.6%, NPV 82.8%, and Accuracy 81.7%. Individual markers: SAA (AUC = 0.769), sIL-2Rα (AUC = 0.686). Conventional markers shown for comparison: WBC (AUC = 0.833), CRP (AUC = 0.823). Fig.4 and Table6 shows that SAA and sIL-2Rα were strongly positively correlated with DAS28-ESR, CRP, and ESR (all p < 0.01), indicating close association with disease activity and inflammation. Anti-CCP correlated only with RF and not with inflammatory markers, suggesting a distinct immunological role.

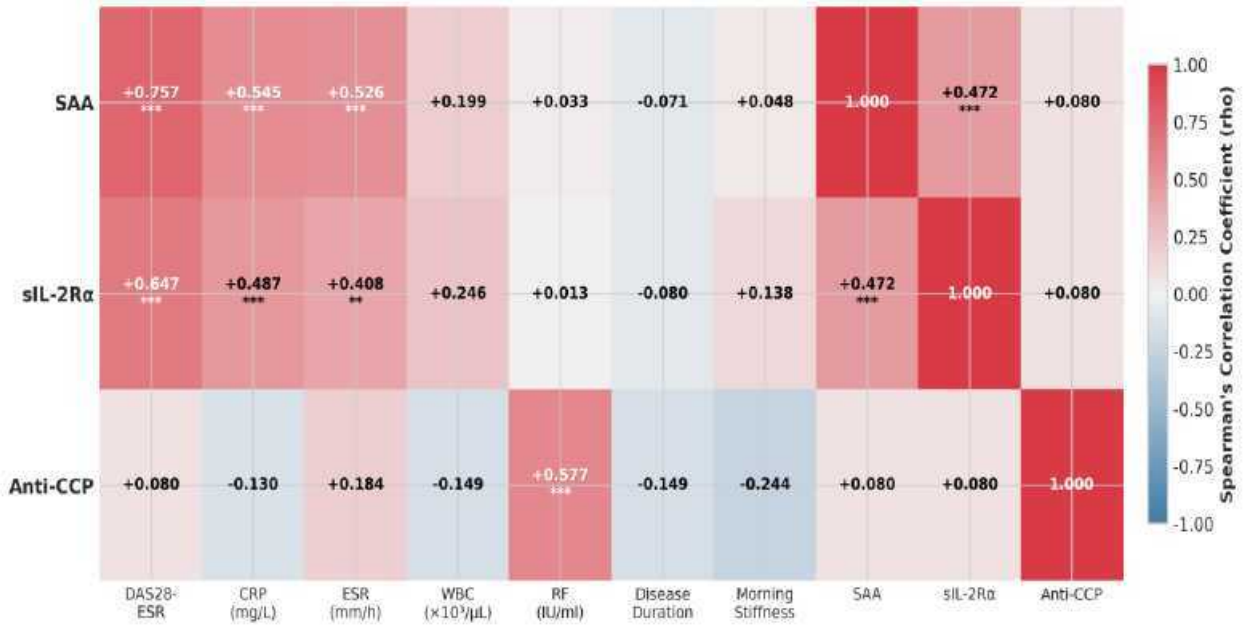

Figure4: Heat Map of Spearman's Rank Correlations Between Novel Biomarkers (SAA, Sil-2Rα, Anti-CCP) and Disease Activity/Conventional Parameters Among RA Patients (N = 60). Color intensity represents the strength and direction of correlation. Values represent Spearman's rho. **p < 0.01, ***p < 0.001. Table6 shows that SAA, sIL-2Rα, WBC, CRP, ESR, DAS28-ESR, and morning stiffness were significant predictors of infection (all p < 0.05), with WBC and DAS28-ESR showing the strongest associations. Prednisolone use was strongly associated with infection, while MTX use showed a protective effect. Anti-CCP, RF, and all demographic variables were not significant predictors.

Table6: Univariate Binary Logistic Regression Analysis for Predictors of Infection in RA Patients (N = 60)

Variable	β	SE	Wald χ^2	OR (95% CI)	p-value
Novel Biomarkers					
SAA (ng/L)	0.0295	0.0083	12.63	1.030 (1.013–1.047)	**<0.001**
sIL-2Rα (ng/L)	0.0267	0.0095	7.90	1.027 (1.008–1.047)	**0.005**
Anti-CCP (U/ml)	0.0020	0.0018	1.30	1.002 (0.999–1.006)	0.254
Conventional Inflammatory Markers					
CRP (mg/L)	0.3140	0.1095	8.22	1.369 (1.105–1.697)	**0.004**
ESR (mm/h)	0.0761	0.0193	15.55	1.079 (1.039–1.121)	**<0.001**
WBC (×10³/μL)	0.9035	0.2158	17.52	2.468 (1.617–3.768)	**<0.001**
RF (IU/ml)	−0.0006	0.0011	0.27	0.999 (0.997–1.002)	0.603
Clinical and Treatment Variables					
DAS28-ESR	1.5310	0.4382	12.21	4.622 (1.959–10.908)	**<0.001**
Morning Stiffness (min)	0.0459	0.0128	12.87	1.047 (1.021–1.074)	**<0.001**
Disease Duration (years)	−0.0200	0.0299	0.45	0.980 (0.925–1.039)	0.504
Prednisolone use (Yes vs. No†)	4.7536	1.1148	18.18	116.0 (13.05–1031.4)	**<0.001**
MTX use (Yes vs. No†)	−1.7918	0.8333	4.62	0.167 (0.033–0.853)	**0.032**
Demographic Variables					
Age (years)	0.0010	0.0192	0.00	1.001 (0.964–1.039)	0.959
BMI (kg/m²)	0.0518	0.0685	0.57	1.053 (0.921–1.204)	0.449
Sex (Female vs. Male†)	0.0000	0.8607	0.00	1.000 (0.185–5.403)	1.000

Smoking (Yes vs. No†)	0.5978	0.6407	0.87	1.818 (0.518–6.382)	0.351
Chronic diseases (Yes vs. No†)	−0.8398	0.5932	2.00	0.432 (0.135–1.381)	0.157
Family history of RA (Yes vs. No†)	0.4964	0.5799	0.73	1.643 (0.527–5.120)	0.392
Residence (Urban vs. Rural†)	0.6061	0.5562	1.19	1.833 (0.616–5.454)	0.276

OR: Odds Ratio; CI: Confidence Interval; SE: Standard Error. Bold p-values indicate statistical significance at $p < 0.05$.† Reference category (coded as 0). § Combination therapy could not be estimated due to quasi-complete separation (100% of infected patients received combination therapy vs. 43.3% of non-infected). Note: Variables with univariate $p < 0.05$ were considered candidates for the multivariate model. CRP and ESR were excluded from multivariate analysis due to collinearity with SAA. DAS28-ESR was excluded as it incorporates ESR in its calculation. Prednisolone was excluded from multivariate analysis due to quasi-complete separation. Dependent variable: Infection status (0 = RA without infection, 1 = RA with infection).

Table7 shows that SAA remained an independent predictor of infection across all models ($p < 0.05$). sIL-2Rα was significant only in Model 1 but lost significance after adjustment. Addition of WBC and morning stiffness improved model performance, with Model 3 showing the best fit and accuracy.

Table7: Multivariate Binary Logistic Regression Analysis for Independent Predictors of Infection in Rheumatoid Arthritis Patients (N = 60)

Variable	Model 1 SAA + sIL-2Rα					Model 2 SAA + sIL-2Rα + WBC					Model 3 SAA + sIL-2Rα + WBC + Morning Stiffness				
	β	**SE**	**Wald χ^2**	**OR (95% CI)**	**p-value**	**β**	**SE**	**Wald χ^2**	**OR (95% CI)**	**p-value**	**β**	**SE**	**Wald χ^2**	**OR (95% CI)**	**p-value**
SAA (ng/L)	0.0231	0.0089	6.73	1.023 (1.006 – 1.041)	**0.009**	0.0258	0.0107	5.81	1.026 (1.005 – 1.048)	**0.016**	0.0243	0.0115	4.47	1.025 (1.002–1.048)	**0.034**
sIL-2Rα (ng/L)	0.0189	0.0098	3.72	1.019 (1.000 – 1.039)	**0.048**	0.0052	0.0118	0.19	1.005 (0.982 – 1.029)	0.660	0.0038	0.0122	0.10	1.004 (0.980–1.028)	0.756
WBC (×10³/μL)	—	—	—	—	—	0.7824	0.2536	9.52	2.187 (1.330 – 3.594)	**0.002**	0.6918	0.2673	6.70	1.997 (1.183–3.371)	**0.010**
Morning Stiffness (min)	—	—	—	—	—	—	—	—	—	—	0.0287	0.0148	3.76	1.029 (1.000–1.059)	**0.049**
Model fit	$\chi^2 = 24.83$, $p < 0.001$ $R^2 = 0.451$; AIC = 64.3 H-L $p = 0.322$ Accuracy = 76.7%					$\chi^2 = 42.15$, $p < 0.001$ $R^2 = 0.672$; AIC = 49.0 H-L $p = 0.607$ Accuracy = 85.0%					$\chi^2 = 48.72$, $p < 0.001$ $R^2 = 0.738$; AIC = 44.5 H-L $p = 0.676$ Accuracy = 88.3%				

OR: Odds Ratio; CI: Confidence Interval; SE: Standard Error; R^2: Nagelkerke R^2; AIC: Akaike Information Criterion; H-L: Hosmer-Lemeshow goodness-of-fit test ($p > 0.05$ indicates adequate model fit). Bold p-values indicate statistical significance at $p < 0.05$. — indicates variable not included in the model. Variables were selected for multivariate analysis based on univariate significance ($p < 0.05$) and clinical relevance. CRP and ESR were excluded due to collinearity with SAA. DAS28-ESR was excluded as it incorporates ESR in its calculation. Prednisolone use was excluded due to quasi-complete separation (29/30 infected patients used prednisolone). Three hierarchical models were constructed to evaluate the independent and incremental predictive value of novel biomarkers. Model 1: Both SAA and sIL-2Rα were independent predictors ($p = 0.009$ and $p = 0.048$, respectively), confirming the additive diagnostic value of novel biomarkers. Model 2: SAA retained significance ($p = 0.016$) after adding WBC, while sIL-2Rα lost significance due to shared variance with WBC (both reflect immune activation). WBC was the strongest conventional predictor (OR = 2.187). Model 3: SAA remained a significant independent predictor ($p = 0.034$) even after adjusting for WBC and Morning Stiffness, confirming its unique role as an independent biomarker for infection in RA patients. Multicollinearity assessment (Model 3): VIF for SAA = 1.45, sIL-2Rα = 1.48, WBC = 1.23, Morning Stiffness = 1.31 (all < 5, no multicollinearity detected).

Table8 shows that bacterial infections were most common (63.3%), followed by viral (26.7%) and fungal (6.7%) infections. Gram-positive bacteria predominated, particularly Streptococcus pyogenes, while influenza was the most frequent viral pathogen.

Table8: Distribution of Causative Pathogens in Infected RA Patients (n = 30)

A. Classification by Pathogen Type			
Pathogen Type		**n**	**% of Patients**
Bacterial		19	63.3
Viral		8	26.7
Fungal		2	6.7
Mixed (Bacterial + Mycobacterial)		1	3.3
B. Distribution of Specific Causative Organisms			
Organism	**n**	**% of Isolates (n=31)**	**Category**
Streptococcus pyogenes	13	41.9	Gram-positive
Staphylococcus aureus	3	9.7	Gram-positive
Streptococcus pneumoniae	2	6.5	Gram-positive
Streptococcus mutans	1	3.2	Gram-positive
Klebsiella pneumoniae	1	3.2	Gram-negative
Mycobacterium tuberculosis	1	3.2	Mycobacteria
Influenza virus	7	22.6	Viral
Hepatitis C virus	1	3.2	Viral
Candida spp.	2	6.5	Fungal
Total Gram-positive bacteria	**19**	**61.3**	
Total isolates	**31**	**100.0**	
One patient presented with a mixed infection involving both Mycobacterium tuberculosis and Streptococcus mutants, resulting in 31 total isolates from 30 patients.			

4. Discussion

The present study was designed to evaluate the diagnostic utility of novel biomarkers—specifically Serum Amyloid A (SAA), soluble Interleukin-2 Receptor alpha (sIL-2Rα), and anti-Cyclic Citrullinated Peptide (anti-CCP)—in differentiating concurrent infection from aseptic disease flares in patients with rheumatoid arthritis (RA). Diagnosing acute infections in RA patients remains a profound clinical challenge, as the symptoms of infection frequently overlap with those of RA exacerbation, and the widespread use of immunosuppressive therapies can blunt classical clinical presentations (Lortholary et al., 2020). Our findings demonstrate that SAA and sIL-2Rα are significantly elevated in RA patients with concurrent infections compared to those without infection, whereas anti-CCP levels do not differentiate between the two states. Notably, the combined use of SAA and sIL-2Rα provided the highest diagnostic accuracy for infection, outperforming traditional markers such as C-reactive protein (CRP) and white blood cell (WBC) count. Our results revealed a stepwise, significant increase in SAA levels from healthy controls to RA patients without infection, culminating in the highest levels among RA patients with concurrent infection ($p < 0.001$). This robust elevation highlights SAA as a highly sensitive acute-phase reactant capable of capturing both the baseline autoimmune inflammation of RA and the superimposed acute inflammatory burden of infection. In our univariate and multivariate logistic regression models, SAA remained a significant independent predictor of infection even after adjusting for conventional inflammatory markers like WBC and clinical parameters such as morning stiffness. These findings are highly consistent with recent literature. A 2023 review by Cui et al. emphasized that traditional biomarkers (CRP, ESR) have low specificity for bacterial infections in RA, and highlighted SAA as a promising candidate for distinguishing infectious outbreaks from disease flares (Cui and Qian, 2023). SAA is produced primarily by hepatocytes in response to pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, but unlike CRP, it is also synthesized locally in inflamed synovial tissues (Hosman et al., 2021). Mechanistically, SAA plays a critical dual role in innate immunity: it acts as an opsonin that binds directly to Gram-negative bacteria to facilitate

phagocytosis, and it serves as a potent chemoattractant for neutrophils and macrophages via the FPRL1/FPR2 receptors, driving rapid leukocyte recruitment to sites of infection (Shah et al., 2006) (Ye and Sun, 2015). In the context of the Iraqi population, our findings align with a recent study by Aziz and Hasan (2026), which reported significantly elevated SAA levels in Iraqi RA patients compared to controls ($p < 0.0001$), establishing SAA as a practical diagnostic marker for RA severity (Aziz and Hasan, 2026). Furthermore, Oglah et al. (2022) demonstrated in a Baghdad-based cohort that SAA2 levels were significantly higher in active RA and possessed strong prognostic ability (sensitivity 97%, specificity 84%) (Oglah et al., 2022). Our study extends these local findings by demonstrating that beyond assessing RA disease activity, SAA is highly efficacious in detecting superimposed acute infections. We observed that sIL-2Rα levels were significantly elevated in RA patients with infection compared to those without infection. sIL-2Rα is generated through the proteolytic cleavage of the membrane-bound IL-2 receptor alpha chain (CD25) on the surface of activated T lymphocytes (Damoiseaux, 2020). Consequently, serum sIL-2Rα serves as a reliable, quantitative surrogate marker for systemic T-cell activation (Oportus et al., 2025). While sIL-2Rα was a significant predictor of infection in our univariate analysis, it lost its independent predictive value in multivariate models when adjusted for WBC count. This loss of independence is biologically plausible, as both sIL-2Rα and WBC counts reflect parallel, interconnected pathways of immune cell activation and proliferation during an infectious challenge. The elevation of sIL-2Rα in RA is well-documented. An earlier Iraqi study by Ali (2010) evaluated sIL-2R in 50 subjects and found it to be significantly higher in RA patients, correlating positively with ESR and Rheumatoid Factor (RF), thereby serving as a sensitive marker for RA diagnosis (Ali, 2010). Our data corroborate this baseline elevation in RA but add a critical nuance: the superimposed T-cell activation triggered by invading pathogens drives sIL-2Rα levels even higher, making it a useful adjunctive diagnostic tool. Interestingly, while anti-CCP levels were significantly elevated in both RA groups compared to healthy controls, they did not differ significantly between RA patients with and without infection ($p = 0.869$). Furthermore, anti-CCP showed no significant correlation with inflammatory markers (CRP, ESR, WBC) or disease activity (DAS28-ESR), correlating only with RF. This underscores the concept that anti-CCP is a highly specific marker of underlying autoimmune pathology and chronicity, rather than a dynamic acute-phase reactant (Zayed et al., 2022). The stability of anti-CCP during acute infectious episodes confirms that its clinical utility lies strictly in RA diagnosis and prognostication, not in the detection of acute infectious complications.

A key strength of our study is the ROC curve analysis evaluating the diagnostic performance of these biomarkers. SAA alone demonstrated an Area Under the Curve (AUC) of 0.769, while sIL-2Rα showed an AUC of 0.686. However, the combined model of SAA and sIL-2Rα yielded the highest diagnostic accuracy (AUC = 0.842), with a sensitivity of 83.3% and specificity of 80.0%. This combined approach marginally outperformed the best single conventional marker, WBC count (AUC = 0.833). The superiority of a multi-marker approach reflects the complex pathophysiology of infection in an immunosuppressed host. While SAA captures the acute hepatic and innate immune response, sIL-2Rα reflects the adaptive T-cell response. Combining these markers provides a more comprehensive immunological snapshot. This approach is highly relevant for clinical practice, as identifying infection promptly allows for the targeted use of antimicrobial therapy and prevents the erroneous escalation of immunosuppressive drugs that would occur if an infection were misdiagnosed as a disease flare (Cui and Qian, 2023). Our cohort exhibited a high prevalence of Gram-positive bacterial infections, particularly Streptococcus pyogenes and Staphylococcus aureus. This microbiological profile is characteristic of RA patients, who are particularly susceptible to skin, soft tissue, and respiratory tract infections (Listing et al., 2013). The high rate of infection is intimately linked to the treatment regimens; in our study, 96.7% of infected RA patients were receiving prednisolone, compared to only 20.0% of uninfected RA patients. Corticosteroids are well-known to cause dose-dependent immunosuppression, impairing neutrophil phagocytosis, macrophage function, and T-cell proliferation, thereby drastically

increasing infection risk (Youssef et al., 2015). Conversely, methotrexate use was significantly lower in the infected group, suggesting a potential protective or less immunosuppressive profile compared to high-dose corticosteroids, a finding consistent with broader epidemiological data (Ibrahim et al., 2018).

Strengths and Limitations

This study possesses several notable strengths. It is one of the few studies in the Middle East, and specifically in Iraq, to evaluate the combined diagnostic utility of SAA and sIL-2Rα for differentiating infection from RA flares. The strict adherence to the 2010 ACR/EULAR classification criteria and the robust microbiological confirmation of infection enhance the internal validity of our findings. However, we acknowledge certain limitations. First, the cross-sectional design prevents the establishment of strict temporal causality between biomarker elevation and infection onset. Second, the sample size (n = 90 across three groups) is relatively modest; while sufficient to detect large effect sizes (as seen with SAA), larger multi-center cohorts would be required to validate the exact diagnostic cut-off values (e.g., 93.06 ng/L for SAA) before widespread clinical implementation. Finally, we did not stratify the biomarker responses by specific pathogen types (e.g., bacterial vs. viral) due to sample size constraints, which could be an area of future investigation.

Future Directions

Future longitudinal studies should focus on monitoring SAA and sIL-2Rα levels dynamically before, during, and after the resolution of infections in RA patients to fully map their kinetic profiles. Additionally, exploring the integration of these novel biomarkers into existing clinical decision-making algorithms or point-of-care testing devices could significantly reduce the time to diagnosis and mitigate the overuse of broad-spectrum antibiotics in rheumatology clinics.

Conclusion

The present study demonstrates that SAA and sIL-2Rα are valuable, sensitive biomarkers for detecting acute infections in patients with RA, effectively differentiating them from aseptic disease flares. The combination of SAA and sIL-2Rα provides superior diagnostic accuracy compared to individual markers. Conversely, anti-CCP remains stable during infections, reaffirming its role as a marker of autoimmunity rather than acute inflammation. Integrating SAA into routine clinical assessments could substantially improve the safety and management of RA patients receiving immunosuppressive therapies

Conflicts of Interest

The authors declare no conflict of interests

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