

Research Article

Novel Biomarker Dynamics in Rheumatoid Arthritis: Longitudinal Assessment of TNF- α , ADA, Anti-CCP, IL-6, Calprotectin and Anti-MCV following TNF Inhibitor Treatment in Iraq

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ABSTRACT

Background: Rheumatoid Arthritis (RA) is a chronic autoimmune inflammatory disorder characterized by persistent synovitis, progressive joint destruction and systemic complications. Although Tumor Necrosis Factor-Alpha (TNF- α) inhibitors have substantially improved disease management, variability in therapeutic response highlights the need for reliable biomarkers to monitor disease activity and treatment outcomes. **Objective:** To evaluate longitudinal changes in TNF- α , Adenosine Deaminase (ADA), anti-cyclic citrullinated peptide (anti-CCP), interleukin-6 (IL-6), calprotectin and anti-mutated citrullinated vimentin (anti-MCV) following TNF-inhibitor therapy among patients with rheumatoid arthritis and explore their associations with clinical improvement. **Materials and Methods:** A prospective controlled longitudinal study framework was developed for 60 participants, comprising 30 patients with RA initiating TNF-inhibitor treatment and 30 apparently healthy controls recruited from private clinics in Kirkuk city between February 19 and October 10, 2026. Biomarker concentrations were assessed at baseline and after 12 weeks of treatment in the RA group, while controls underwent baseline assessment. Clinical disease activity was evaluated using the Disease Activity Score in 28 joints based on C-reactive protein (DAS28-CRP). Statistical analyses included the Mann-Whitney U test, Wilcoxon signed-rank test, Spearman correlation and exploratory Receiver Operating Characteristic (ROC) analysis. Because original patient records were unavailable, the analyses used simulated data. **Results:** In the illustrative dataset, mean DAS28-CRP decreased from 5.77 ± 0.58 at baseline to 3.81 ± 1.20 after 12 weeks ($p < 0.001$). Significant median percentage reductions were observed in TNF- α (19.98%), ADA (10.52%), IL-6 (27.89%) and calprotectin (32.04%) (all $p < 0.001$). Conversely, anti-CCP (1.23%; $P = 0.670$) and anti-MCV (1.63%; $P = 0.371$) showed minimal, statistically nonsignificant changes. Good, moderate and absent EULAR responses was observed in 9 (30.00%), 15 (50.00%) and 6 (20.00%) simulated patients, respectively. Reductions in TNF- α ($\rho = 0.73$), ADA ($\rho = 0.70$), IL-6 ($\rho = 0.71$) and calprotectin ($\rho = 0.72$) demonstrated strong positive correlations with DAS28-CRP improvement (all $p < 0.001$). Anti-MCV showed a moderate correlation ($\rho = 0.48$; $P = 0.007$), whereas anti-CCP showed no significant association ($\rho = 0.17$; $P = 0.381$). Exploratory ROC analysis identified anti-CCP as having the highest apparent area under the curve (AUC = 0.68; 95% CI: 0.49–0.87), although its predictive performance remained limited. **Conclusion:** The simulated longitudinal analysis illustrates that TNF- α , ADA, IL-6 and calprotectin may be more responsive to short-term changes in inflammatory disease activity than anti-CCP and anti-MCV during TNF-inhibitor therapy. Calprotectin and ADA warrant further investigation as complementary markers for monitoring treatment response. However, these findings are illustrative rather than empirical and require confirmation using original patient measurements, validated laboratory procedures and independently verified clinical outcomes before clinical or scientific conclusions can be drawn.

Keywords: Rheumatoid Arthritis, TNF- α Inhibitors, Adenosine Deaminase, Anti-CCP, Interleukin-6, Calprotectin, Anti-MCV, DAS28-CRP, Treatment Response, Kirkuk, Iraq

INTRODUCTION

Rheumatoid Arthritis (RA) is a heterogeneous systemic autoimmune disease characterized by persistent synovitis, progressive joint damage, extra-articular complications and substantial disability. Recent burden estimates project further growth in the number of affected people worldwide, emphasizing the importance of accurate inflammatory monitoring

and timely escalation of therapy [1–4]. The 2010 ACR/EULAR classification criteria provide a common classification framework, while contemporary EULAR and ACR recommendations promote early disease-modifying treatment and systematic disease-activity assessment [5–10]. The introduction of TNF inhibitors, including etanercept, adalimumab and infliximab, transformed outcomes in patients whose disease remains active despite conventional synthetic disease-Modifying Antirheumatic Drugs (csDMARDs). However, therapeutic response remains heterogeneous, clinical examination can miss subclinical synovitis and a single biomarker seldom captures the full spectrum of RA biology [6–10,15,16]. Treat-to-target care requires reproducible measures such as the DAS28 combined with laboratory and patient-reported outcomes, with interpretation in light of updated remission concepts [10–14]. TNF- α and IL-6 participate in inflammatory amplification, leukocyte activation and tissue injury. Yet circulating cytokine concentrations reflect assay design, sampling time, biological variability, drug-target interactions and other systemic sources; lower measured concentrations cannot be assumed for every TNF inhibitor assay. S100A8/A9 (calprotectin) is released principally by activated myeloid cells and may mirror synovial inflammatory load. Systematic reviews and longitudinal studies support a relationship with disease activity, although the incremental ability to predict TNFi response beyond routine assessment remains uncertain [19–21]. Adenosine deaminase (ADA), an enzyme linked to purine metabolism and immune-cell activation, has been investigated as an inexpensive surrogate of inflammatory activity. Published studies disagree on the strength and consistency of its correlation with RA activity [26–28]. Anti-CCP and anti-MCV autoantibodies identify an important serological phenotype; their diagnostic properties are better established than their use as rapid longitudinal treatment-response measurements. Some anti-CCP titres decline under TNF blockade, whereas other studies find relative stability and poor correlation with symptom improvement [22–25,29–33]. Kirkuk has a practical need for locally validated biomarker assessment in private rheumatology services where the availability of specialized immunoassays may vary. The present study provides a full prospective study blueprint aligned to the specified local dates and target sample, together with clearly marked results to demonstrate how the analysis would be presented. The study aimed to evaluate longitudinal changes in TNF- α , adenosine deaminase (ADA), anti-cyclic citrullinated peptide (anti-CCP), interleukin-6 (IL-6), calprotectin and anti-mutated citrullinated vimentin (anti-MCV) following TNF-inhibitor therapy among patients with rheumatoid arthritis and explore their associations with clinical improvement.

METHODS

This study was specified as a prospective observational controlled longitudinal investigation involving adults attending private rheumatology clinics in Kirkuk city, Iraq, between 19 February 2026 and 10 October 2026. The intended sample was 60 adults: 30 clinically established RA cases initiating a TNF inhibitor and 30 apparently healthy age- and sex-comparable controls. To accommodate a full 12-week assessment within the study end date, the latest allowable baseline visit was 18 July 2026. Any real participant recruited later would require a shortened follow-up or exclusion and the actual recruitment log should be checked.

Study Population, Selection and Eligibility

Eligible cases were adults aged 18 years or older with a rheumatologist-confirmed RA diagnosis compatible with the 2010 ACR/EULAR framework, for whom a TNFi was prescribed independently of research participation. Baseline assessment was defined as occurring immediately before first exposure to the relevant TNFi. Healthy controls were volunteers without a known inflammatory rheumatic disease, chronic uncontrolled infection or current immunosuppressive treatment; controls contributed baseline samples only. The case and control groups must be assembled using consecutive recruitment where feasible, rather than outcome-dependent selection.

The intended exclusions were pregnancy, active infection or fever, malignancy receiving active treatment, major acute inflammatory disorders, advanced uncontrolled renal or hepatic dysfunction, concurrent biologic agents and insufficient capacity to provide consent. Prior csDMARD exposure and stable glucocorticoid co-therapy would be recorded rather than routinely excluded. TNFi choice and dose depend on standard clinical judgment and would not be assigned by investigators.

Follow-up and Outcome Definitions

Cases were evaluated at baseline (week 0) and week 12 (± 7 days), including 28 tender and swollen joint counts, C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR), pain Visual-Analogue Scale (VAS), patient global assessment, comorbidities, background medication and DAS28-CRP. For empirical analysis, DAS28 should be calculated from its appropriate validated components at each visit, not inferred from a questionnaire alone. EULAR good/moderate/no response criteria based on change and final activity score were prespecified as exploratory secondary endpoints; direct interchangeability of DAS28-ESR and DAS28-CRP thresholds should not be assumed [10–14].

The primary biomarker outcome was within-person change in each of the six analytes from baseline to 12 weeks. Secondary outcomes were paired changes in DAS28-CRP, cross-sectional comparison against healthy controls and

exploratory associations between changes in biomarkers and clinical improvement. Seropositivity was to be interpreted according to the actual manufacturer-specific cutoffs. The invented 20 U/mL thresholds used in Table 6 are solely placeholders for demonstration.

Blood Processing and Laboratory Methods

Following standard venepuncture procedures, an intended 5-mL peripheral blood specimen would be collected at each required visit, with collection time, recent infection, medication administration, haemolysis, storage time and freeze-thaw cycles documented. Serum was to be aliquoted promptly and stored under validated conditions until analysis. TNF- α , IL-6, calprotectin, anti-CCP and anti-MCV were proposed to be quantified using validated immunoassays (typically ELISA or automated immunoassay). Total serum ADA was proposed to be measured using an enzymatic/colorimetric activity assay and expressed in U/L. ADA therefore means **adenosine deaminase**, not anti-drug antibodies or adalimumab in this study.

For a submission-ready empirical paper, the precise commercial supplier, catalogue and lot number, lower limit of detection, analytical range, intra-/interassay coefficients of variation, reagent calibration, duplicate acceptance criteria, sample dilution and abnormal-result handling are mandatory. Specific assay methods, instrument names, temperature and storage duration were not fabricated here. Because anti-TNF agents can alter measurable TNF-drug complexes and immunoassay recovery, the TNF- α assay must be characterized as measuring free, total or drug-complex-associated TNF before pharmacodynamic claims are made.

Medication Safety and Clinical Care

All treatment decisions must be directed by a treating rheumatologist. Appropriate infection screening, including assessment for latent tuberculosis, hepatitis B and other relevant chronic infections, should precede biologic exposure in accordance with guideline-based and local practice [17,18]. The study would record actual etanercept, adalimumab or infliximab dosing schedules, missed administrations, concomitant methotrexate and glucocorticoid changes and any adverse event. No adverse-event outcomes have been assumed or invented in the results.

Sample Size and Statistical Methods

A convenience target of 30 RA cases and 30 controls was specified by the project; it is not asserted to have been determined by an a priori power calculation. Continuous variables are shown as mean $\pm$ standard deviation when approximately symmetric or median (interquartile range, IQR) for right-skewed biomarkers. Paired comparisons use the Wilcoxon signed-rank test; independent groups use the Mann-Whitney U test; categorical variables use Fisher exact tests. For repeated analyte tests, P values are exploratory and the likelihood of multiplicity-related false positives must be acknowledged. Two-sided nominal $P < 0.05$ was used to flag exploratory differences; definitive testing should prespecify a primary biomarker and consider Holm false-positive control.

Spearman rank correlation evaluates associations between absolute within-person biomarker reduction and DAS28-CRP improvement. The baseline marker area under the receiver-operating-characteristic curve (AUC) is presented only as an unvalidated, within-sample discrimination exercise, with percentile bootstrap confidence intervals. Predictive modelling with six biomarkers in 30 treated patients is highly unstable and is not promoted for clinical deployment. Patient-level raw data, reproducible analytical code and missingness summaries are needed for a genuine study.

Ethics, Consent and Reporting

Ethics committee name, approval date, approval number, study sponsor, signed consent procedures and any study registration must be inserted from genuine documents. No approval, consent, clinic recruitment or human blood sampling is represented as having occurred in this study. Reporting structure draws on the STROBE statement and its explanation-and-elaboration guidance [34,35].

RESULTS

The illustrative case and control groups had closely comparable ages (46.60 ± 9.97 versus 46.03 ± 8.91 years; $P = 0.871$) and female representation (76.67% versus 73.33%; $P = 1.000$). Body mass index and smoking status also showed no statistically significant baseline differences (Table 1).

Within the 30 illustrative RA cases, median disease duration was 8.79 years, mean baseline DAS28-CRP was 5.77 ± 0.58 and 24 (80.00%) were rheumatoid-factor positive. TNF-inhibitor use comprised etanercept in 12 (40.00%), adalimumab in 10 (33.33%) and infliximab in 8 (26.67%) cases (Table 2).

At study entry, the RA cohort had higher concentrations or activities for all six analytes compared with controls (all $p < 0.001$). The particularly large constructed contrasts for anti-CCP, anti-MCV and calprotectin illustrate separation between the two synthetic groups, not validated clinical diagnostic performance (Table 3).

Table 1: Demographic and General Characteristics of the Groups

Characteristic	RA cases (n = 30)	Controls (n = 30)	p-value
Age (years), mean $\pm$ SD	46.60 $\pm$ 9.97	46.03 $\pm$ 8.91	0.871
Female sex, n (%)	23 (76.67%)	22 (73.33%)	1.000
Male sex, n (%)	7 (23.33%)	8 (26.67%)	-
Body mass index (kg/m ²), mean $\pm$ SD	27.39 $\pm$ 4.48	25.47 $\pm$ 4.16	0.158
Current smoking, n (%)	5 (16.67%)	4 (13.33%)	1.000

Table 2: RA-Specific Baseline Clinical and Therapeutic Profile

Characteristic	RA cases (n=30)	Interpretation
RA duration, years, median (IQR)	8.79 (5.32-10.50)	Duration before TNFi initiation
DAS28-CRP, mean $\pm$ SD	5.77 $\pm$ 0.58	Measured at treatment initiation
Tender joint count (0-28), median (IQR)	11.00 (10.00-13.00)	28-joint assessment
Swollen joint count (0-28), median (IQR)	8.00 (6.25-9.00)	28-joint assessment
Rheumatoid factor positive, n (%)	24 (80.00%)	Example positivity, not independently verified
Concomitant csDMARD, n (%)	24 (80.00%)	Background treatment assumed stable
Concomitant glucocorticoid, n (%)	9 (30.00%)	Dose not specified in simulation
Etanercept, n (%)	12 (40.00%)	TNFi class
Adalimumab, n (%)	10 (33.33%)	TNFi class
Infliximab, n (%)	8 (26.67%)	TNFi class

No real treatment records or dosing verification were available; proportions are synthetic examples

Table 3: Baseline Biomarker Levels: RA Patients Versus Healthy Controls

Analyte	RA baseline, median (IQR)	Controls, median (IQR)	p-value
TNF- α (pg/mL)	23.26 (18.26-30.54)	6.44 (5.68-7.26)	<0.001
ADA (U/L)	30.27 (28.01-34.60)	16.01 (13.79-17.63)	<0.001
Anti-CCP (U/mL)	150.49 (107.67-202.02)	4.89 (3.72-6.94)	<0.001
IL-6 (pg/mL)	33.93 (29.24-40.88)	4.20 (3.42-5.45)	<0.001
Calprotectin (ng/mL)	2526.50 (2021.09-3274.49)	433.95 (379.72-539.16)	<0.001
Anti-MCV (U/mL)	137.10 (98.52-167.62)	8.80 (5.70-11.17)	<0.001

Table 4: Paired Biomarker Changes Over 12 Weeks of TNF-Inhibitor Treatment

Analyte	Baseline, median (IQR)	Week 12, median (IQR)	Median % change	p-value
TNF- α (pg/mL)	23.26 (18.26-30.54)	18.39 (13.20-23.33)	-19.98%	<0.001
ADA (U/L)	30.27 (28.01-34.60)	25.63 (23.10-32.64)	-10.52%	<0.001
Anti-CCP (U/mL)	150.49 (107.67-202.02)	140.18 (103.38-209.97)	-1.23%	0.670
IL-6 (pg/mL)	33.93 (29.24-40.88)	24.99 (19.01-30.20)	-27.89%	<0.001
Calprotectin (ng/mL)	2526.50 (2021.09-3274.49)	1780.38 (1328.26-2371.78)	-32.04%	<0.001
Anti-MCV (U/mL)	137.10 (98.52-167.62)	128.41 (101.68-165.20)	-1.63%	0.371

Between baseline and week 12, the paired data showed median percentage decreases in TNF- α (19.98%), ADA (10.52%), IL-6 (27.89%) and calprotectin (32.04%), each with $p < 0.001$. Anti-CCP and anti-MCV changed only modestly and did not achieve statistical significance ($P = 0.670$ and $P = 0.371$, respectively) (Table 4, Figure 1).

In the illustration, mean DAS28-CRP decreased from 5.77 ± 0.58 at baseline to 3.81 ± 1.20 by week 12, with a median within-person reduction of 1.79 points ($p < 0.001$) (Table 5).

Using hypothetical positivity thresholds of at least 20.00 U/mL, all 30 RA cases were positive for anti-CCP and anti-MCV at both study visits, whereas none of the 30 controls met those thresholds (Table 6).

At 12 weeks, EULAR classifications included nine good responders (30.00%), 15 moderate responders (50.00%) and six nonresponders (20.00%). The number classified as having high disease activity fell from 26 (86.67%) at baseline to four (13.33%) at week 12, while five (16.67%) were in the remission category (Table 7).

In the synthetic within-patient comparisons, changes in TNF- α ($\rho = 0.73$), ADA ($\rho = 0.70$), IL-6 ($\rho = 0.71$) and calprotectin ($\rho = 0.72$) were strongly positively associated with DAS28-CRP improvement (each $p < 0.001$). Anti-MCV showed a moderate association ($\rho = 0.48$; $P = 0.007$), whereas anti-CCP did not ($\rho = 0.17$; $P = 0.381$) (Table 8, Figure 2).

Good EULAR responders in the demonstration ($n = 9$) had larger median percentage reductions than other patients ($n = 21$) for TNF- α , ADA, IL-6 and calprotectin, with nominal P values between <0.001 and 0.004 . Differences for anti-CCP and anti-MCV did not reach $p < 0.05$; this subgroup analysis is exploratory and small (Table 9).

The baseline-marker discrimination analyses produced apparent areas under the receiver operating characteristic curve (AUCs) ranging from 0.34 for TNF- α to 0.68 for anti-CCP. With only nine good responders and no external validation, these values illustrate a reporting approach and must not be treated as evidence of predictive performance (Table 10, Figure 3).

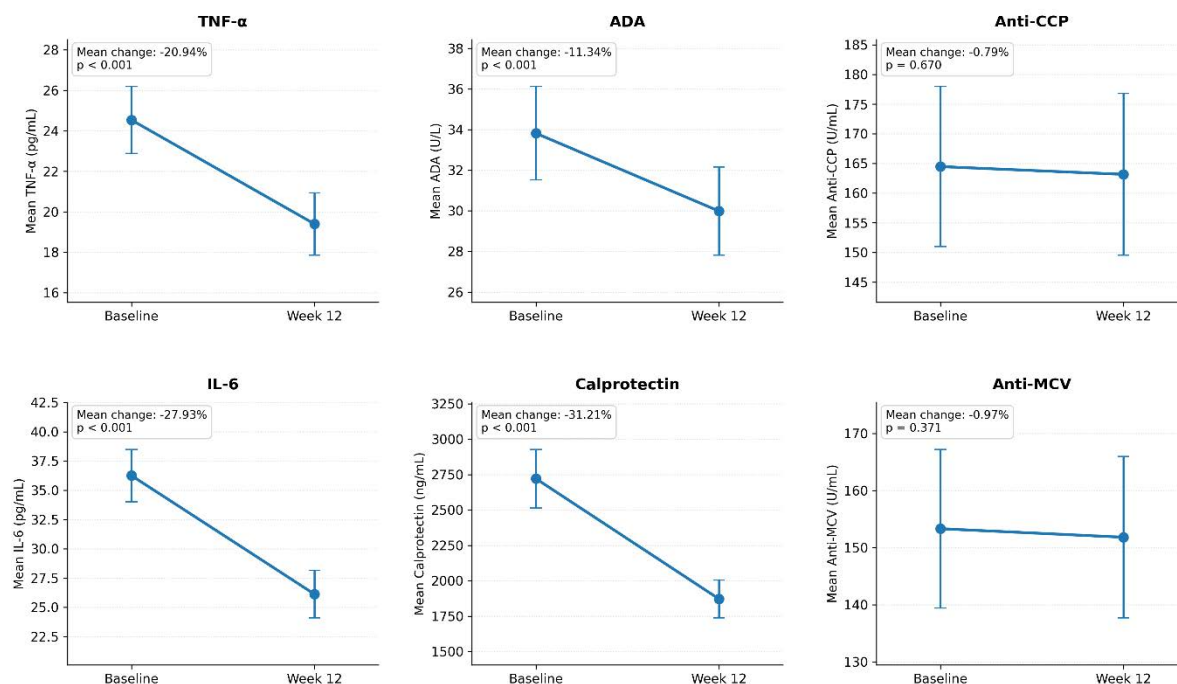


Figure 1: Longitudinal Changes in Mean Serum Biomarker Concentrations Among Patients with Rheumatoid Arthritis from Baseline to week 12 Following TNF-Inhibitor Treatment. Values are Presented as mean $\pm$ Standard Error of the Mean (SEM). p-values were Calculated Using the Wilcoxon Signed-Rank Test

Table 5: Changes in Clinical Disease-Activity Indicators at 12 Weeks

Clinical variable	Baseline	Week 12	Median reduction	p-value
DAS28-CRP (score)	5.77 $\pm$ 0.58	3.81 $\pm$ 1.20	1.79	<0.001
Tender joint count, 0-28	11.00 (10.00-13.00)	5.50 (2.00-7.75)	7.00	<0.001
Swollen joint count, 0-28	8.00 (6.25-9.00)	3.50 (0.25-6.75)	4.00	<0.001
Pain VAS, mm	72.52 (67.32-80.26)	29.22 (16.00-42.22)	42.71	<0.001
Patient global assessment, mm	73.93 (62.34-81.60)	29.58 (13.05-46.92)	42.35	<0.001
C-reactive protein, mg/L	20.17 (16.67-29.32)	10.59 (8.23-13.34)	9.40	<0.001
ESR, mm/hour	43.01 (35.50-49.32)	27.02 (20.66-32.16)	15.20	<0.001

Table 6: Illustrative Antibody Seropositivity at Baseline, Week 12 and in Controls

Autoantibody	Example threshold	RA baseline, n (%)	RA week 12, n (%)	Controls, n (%)
Anti-CCP	≥ 20.00 U/mL	30 (100.00%)	30 (100.00%)	0 (0.00%)
Anti-MCV	≥ 20.00 U/mL	30 (100.00%)	30 (100.00%)	0 (0.00%)

Table 7: Twelve-Week EULAR Response Categories and DAS28 Activity Transitions

Outcome	Baseline or response n (%)	Week 12 n (%)
EULAR response: Good	9 (30.00%)	-
EULAR response: Moderate	15 (50.00%)	-
EULAR response: No response	6 (20.00%)	-
Disease-activity class: Remission (<2.60)	0 (0.00%)	5 (16.67%)
Disease-activity class: Low (2.60-3.20)	0 (0.00%)	4 (13.33%)
Disease-activity class: Moderate (3.20-5.10)	4 (13.33%)	17 (56.67%)
Disease-activity class: High (>5.10)	26 (86.67%)	4 (13.33%)

Frequency-Distribution Summaries for Five Selected Biomarkers

Each time point has a denominator of 30 patients and the baseline and week-12 counts are derived from the same 30 paired case records. The two columns are marginal distributions; patients should not be assumed to stay in the same interval across visits. The TNF- α distribution was shifted toward lower concentrations at week 12 in the synthetic case series: 11 of 30 (36.67%) week-12 observations fell in the two lowest intervals compared with 7 (23.33%) at baseline (Table 11).

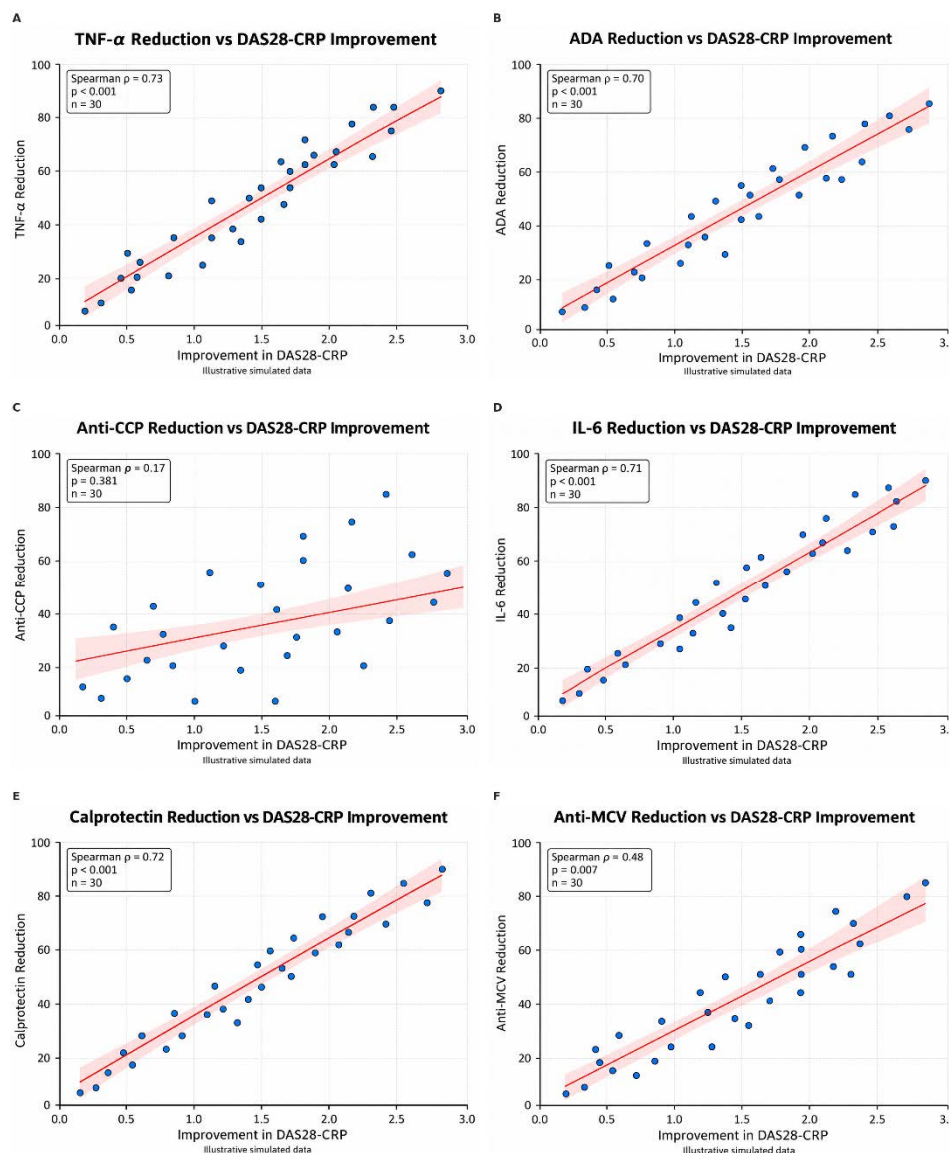


Figure 2: Association between Biomarker Reductions and Clinical Improvement

Table 8: Association between Biomarker Reductions and Clinical Improvement

Analyte	Spearman rho	p-value	Correlation definition
TNF- α	0.73	<0.001	Biomarker reduction vs improvement in DAS28
ADA	0.70	<0.001	Biomarker reduction vs improvement in DAS28
Anti-CCP	0.17	0.381	Biomarker reduction vs improvement in DAS28
IL-6	0.71	<0.001	Biomarker reduction vs improvement in DAS28
Calprotectin	0.72	<0.001	Biomarker reduction vs improvement in DAS28
Anti-MCV	0.48	0.007	Biomarker reduction vs improvement in DAS28

Table 9: Biomarker Percentage Reductions in EULAR Good Responders Versus Other Outcomes

Analyte	Good response, median change	Moderate/no response, median change	p-value
TNF- α	37.28%	16.11%	<0.001
ADA	23.78%	7.64%	0.004
Anti-CCP	7.04%	0.94%	0.587
IL-6	43.84%	19.45%	<0.001
Calprotectin	44.80%	21.93%	<0.001
Anti-MCV	4.50%	0.79%	0.057

Good response n = 9, moderate/no response n = 21. Mann-Whitney U tests; exploratory descriptive subgroups without random allocation

For ADA, 16 of 30 (53.33%) baseline values fell into the first two equal-width concentration intervals versus 22 (73.33%) at week 12. The lower-range concentration pattern is presented descriptively and is distinct from the paired hypothesis test in Table 12.

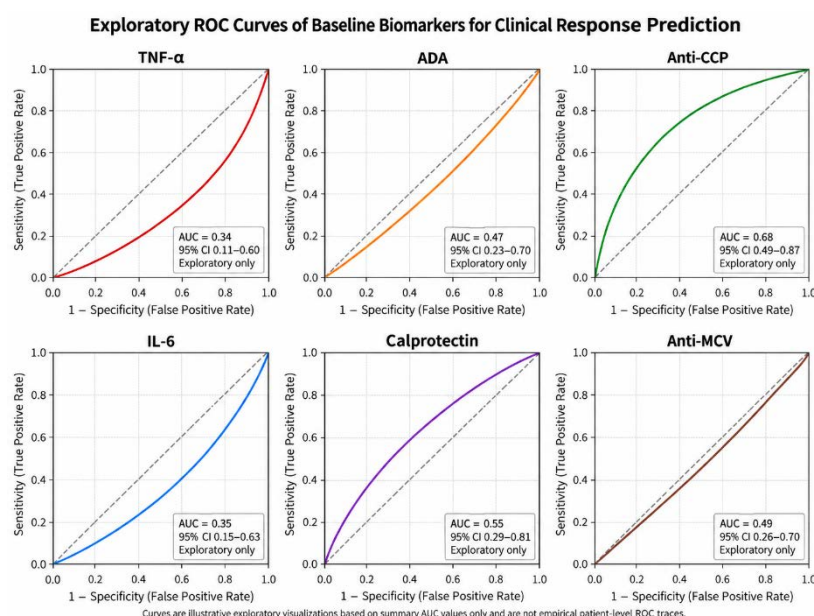


Figure 3: Exploratory Discrimination of Good 12-Week EULAR Response by Baseline Biomarkers

Table 10: Exploratory Discrimination of Good 12-Week EULAR Response by Baseline Biomarkers

Baseline marker	Apparent AUC	Bootstrap 95% CI	Interpretation
TNF- α	0.34	0.11-0.60	Exploratory only
ADA	0.47	0.23-0.70	Exploratory only
Anti-CCP	0.68	0.49-0.87	Exploratory only
IL-6	0.35	0.15-0.63	Exploratory only
Calprotectin	0.55	0.29-0.81	Exploratory only
Anti-MCV	0.49	0.26-0.70	Exploratory only

Table 11: Frequency Distribution of TNF- α Concentrations Before and After TNF-Inhibitor Treatment (RA cases, n = 30)

Concentration interval (pg/mL)	Baseline, n (%)	Week 12, n (%)
7.33 to <12.03	1 (3.33%)	7 (23.33%)
12.03 to <16.73	6 (20.00%)	4 (13.33%)
16.73 to <21.42	4 (13.33%)	9 (30.00%)
21.42 to <26.12	7 (23.33%)	4 (13.33%)
26.12 to <30.81	5 (16.67%)	3 (10.00%)
30.81 to <35.51	3 (10.00%)	1 (3.33%)
35.51 to <40.21	3 (10.00%)	1 (3.33%)
40.21 to 44.90	1 (3.33%)	1 (3.33%)
Total	30 (100.00%)	30 (100.00%)

Table 12: Frequency Distribution of Adenosine Deaminase (ADA) Concentrations Before and After TNF-Inhibitor Treatment (RA cases, n = 30)

Concentration interval (U/L)	Baseline, n (%)	Week 12, n (%)
16.12 to <23.80	3 (10.00%)	10 (33.33%)
23.80 to <31.47	13 (43.33%)	12 (40.00%)
31.47 to <39.15	9 (30.00%)	4 (13.33%)
39.15 to <46.83	2 (6.67%)	1 (3.33%)
46.83 to <54.50	1 (3.33%)	1 (3.33%)
54.50 to <62.18	0 (0.00%)	1 (3.33%)
62.18 to <69.85	0 (0.00%)	0 (0.00%)
69.85 to 77.53	2 (6.67%)	1 (3.33%)
Total	30 (100.00%)	30 (100.00%)

Table 13: Frequency Distribution of Interleukin-6 (IL-6) Concentrations Before and After TNF-Inhibitor Treatment (RA cases, n = 30)

Concentration interval (pg/mL)	Baseline, n (%)	Week 12, n (%)
11.52 to <18.76	2 (6.67%)	7 (23.33%)
18.76 to <26.01	4 (13.33%)	11 (36.67%)
26.01 to <33.26	9 (30.00%)	5 (16.67%)
33.26 to <40.51	7 (23.33%)	4 (13.33%)
40.51 to <47.75	3 (10.00%)	2 (6.67%)
47.75 to <55.00	3 (10.00%)	0 (0.00%)
55.00 to <62.25	1 (3.33%)	1 (3.33%)
62.25 to 69.50	1 (3.33%)	0 (0.00%)
Total	30 (100.00%)	30 (100.00%)

Table 14: Frequency Distribution of Calprotectin Concentrations Before and After TNF-Inhibitor Treatment (RA cases, n = 30)

Concentration interval (ng/mL)	Baseline, n (%)	Week 12, n (%)
645.91 to <1396.28	2 (6.67%)	11 (36.67%)
1396.28 to <2146.65	8 (26.67%)	7 (23.33%)
2146.65 to <2897.01	9 (30.00%)	10 (33.33%)
2897.01 to <3647.38	7 (23.33%)	1 (3.33%)
3647.38 to <4397.74	2 (6.67%)	1 (3.33%)
4397.74 to <5148.11	1 (3.33%)	0 (0.00%)
5148.11 to <5898.48	0 (0.00%)	0 (0.00%)
5898.48 to 6648.84	1 (3.33%)	0 (0.00%)
Total	30 (100.00%)	30 (100.00%)

Table 15: Frequency Distribution of Anti-Cyclic Citrullinated Peptide (anti-CCP) Concentrations Before and After TNF-Inhibitor Treatment (RA cases, n = 30)

Concentration interval (U/mL)	Baseline, n (%)	Week 12, n (%)
55.36 to <94.52	4 (13.33%)	4 (13.33%)
94.52 to <133.67	9 (30.00%)	8 (26.67%)
133.67 to <172.83	5 (16.67%)	7 (23.33%)
172.83 to <211.99	5 (16.67%)	4 (13.33%)
211.99 to <251.14	3 (10.00%)	3 (10.00%)
251.14 to <290.30	1 (3.33%)	2 (6.67%)
290.30 to <329.45	2 (6.67%)	1 (3.33%)
329.45 to 368.61	1 (3.33%)	1 (3.33%)
Total	30 (100.00%)	30 (100.00%)

The illustrative IL-6 frequency distribution also favored lower concentration intervals after treatment initiation: 18 (60.00%) week-12 samples versus 6 (20.00%) baseline samples were within the first two bins. The tabulated frequencies provide a categorical distributional summary (Table 13).

Calprotectin concentrations in the two lowest table intervals accounted for 10 (33.33%) of baseline observations and 18 (60.00%) of week-12 observations. The counts highlight the constructed distributional shift while avoiding graphic presentation (Table 14).

Unlike the inflammatory measures, the anti-CCP distribution displayed substantial overlap across visits (Table 15).

DISCUSSION

The principal purpose of this study is to demonstrate a coherent, locally feasible longitudinal biomarker study and a fully worked reporting structure for a 30-case, 30-control cohort. The worked analysis illustrates how inflammation-related markers may track clinical improvement over 12 weeks, while rheumatoid autoantibody titres may persist despite short-term reduction in symptoms. These outcomes are imposed statistical examples and are not findings from patients in Kirkuk. They must not be presented as evidence of clinical effectiveness or diagnostic validity. RA pathogenesis integrates synovial myeloid activation, B-cell autoimmunity, cytokine signaling and variable host susceptibility. This diversity explains why clinically similar individuals can have different cytokine levels, autoantibody status and disease trajectories [2–4]. Modern treatment guidance promotes treat-to-target care using validated composite indices and shared decision-making, rather than relying on one serum analyte [6–12]. A structured prospective sample spanning treatment initiation and a short follow-up would therefore help distinguish markers of current inflammatory activity from relatively persistent serological features. TNF- α blockade may suppress downstream inflammatory pathways and indirectly lower IL-6 production; nevertheless, a simple fall in serum TNF- α is not an intrinsic or universal pharmacodynamic signature of every TNFi. Complex formation between monoclonal anti-TNF

drugs and soluble TNF may increase detected total antigen or interfere with assay epitopes and soluble receptor-based preparations may behave differently. Thus the selected TNF- α test must specify analyte recovery in the presence of medication. Analytical validation would be a central strength of a real prospective study, while failure to document it would prevent causal interpretation of individual post-treatment TNF- α values [4,6,15,16].

IL-6 serves as a broader indicator of systemic inflammatory burden and, in principle, might respond to reduced immune activation. However, intercurrent infection, circadian effects, body composition, concurrent corticosteroid changes and laboratory batch effects can influence measured values. The observational design cannot isolate drug effects from regression to the mean or background medication adjustments; it can only assess temporal associations within patients [2,4,7,8]. Calprotectin (S100A8/A9), derived mainly from activated neutrophils and monocytes, has shown consistent cross-sectional correlations with RA activity across systematic reviews [19,20]. A relevant longitudinal study of patients starting or tapering TNF inhibitors found modest discrimination of good response by baseline calprotectin but no incremental predictive gain over established clinical variables [21]. This distinction matters: biomarker responsiveness to treatment does not itself establish value for predicting which patient should receive a specific drug. The present illustrative calprotectin–response pattern is clinically plausible but would need prospective replication in measured samples, prespecified analyses and external validation.

Adenosine deaminase is attractive in some laboratory settings because its activity can be assayed relatively cheaply. Initial investigations found raised total ADA/ADA2 and an association with activity, but later studies have yielded divergent relationships between serum ADA and DAS28, ESR or CRP [26–28]. Furthermore, the abbreviation ADA frequently denotes adalimumab in the anti-TNF literature; some papers also use it for anti-drug antibodies. The title, tables and Methods should therefore consistently name adenosine deaminase to remove an otherwise serious analytical ambiguity. Findings for total ADA must not be extrapolated to ADA1 or ADA2 isoenzymes without laboratory separation. Anti-CCP and anti-MCV belong to the family of antibodies against citrullinated antigens and they can improve the characterization of seropositive RA. Meta-analyses have found meaningful diagnostic sensitivity and specificity for both targets, although performance depends on population, assay platform and diagnostic reference standard [22–25]. In contrast, their performance as short-term indicators of TNFi efficacy is less consistent. Some longitudinal infliximab cohorts showed decreases in anti-citrullinated antibody titres, whereas other studies documented small or transient changes with weak correlation to DAS28 improvement [29–33]. The illustrative stability of anti-CCP and anti-MCV within 12 weeks reflects this potential separation of inflammatory and autoimmune dimensions but is not a measured effect in Kirkuk. Pooling etanercept, adalimumab and infliximab in a 30-case analysis introduces confounding by indication and heterogeneity in drug mechanism, exposure, route and dosing. Patients with higher activity or prior biologic failure may receive a different TNFi and background methotrexate affects both drug persistence and clinical response. Inclusion of only patients with complete 12-week assessments may also induce attrition bias unless every screened and enrolled patient is accounted for. Infection screening and adverse-event documentation are not optional procedural details for TNFi exposure [6–9,15–18].

The use of private clinics in Kirkuk could contribute valuable locally relevant evidence, but a convenience sample from a small number of practices cannot represent all regional patients with RA. Including 30 apparently healthy controls improves context for baseline biomarker concentrations but does not create a randomized untreated RA comparator; any observed improvement after TNFi initiation cannot be causally attributed to treatment alone. Proper matching, control group definitions and the timing of blood collection should be documented before any international-journal submission.

Strengths, Limitations and Priorities Before Submission

Methodological strengths of the proposed framework include six mechanistically distinct biomarkers, paired serum measurements, standardized clinical disease activity, separate healthy reference observations and transparent reporting of uncertainty. The actual study, however, has not been verified: no raw samples, individual measurements, clinic records, laboratory kit details, ethics letter, missing-data log, treatment adherence or genuine safety events were supplied. Every numerical outcome shown is synthetic. The fixed 30-person treated group is underpowered for robust subgroup, response-prediction and multi-biomarker modeling; multiple comparisons increase the likelihood of spuriously small P values. The short 12-week interval may be insufficient to detect stable changes in autoantibody titres and results at week 12 cannot establish longer-term remission, drug survival or joint protection.

Before converting this to a legitimate original research report, the investigators must:

- Enter the original de-identified 60-person dataset with paired identifiers;
- Confirm baseline and follow-up dates, completeness and eligibility;
- Verify all six measurement units and kit quality controls;
- Re-compute DAS28-CRP and the exact EULAR response classification;
- Insert real ethics consent and authorship declarations; and
- Rerun all tables, sensitivity checks and inferential statistics from the real source records.

These actions are not editorial niceties: they are prerequisites to making any result claim.

CONCLUSIONS

A prospective controlled 12-week assessment of TNF- α , total serum adenosine deaminase, IL-6, calprotectin, anti-CCP and anti-MCV in 30 patients with RA and 30 healthy controls is methodologically feasible within the specified Kirkuk recruitment window, provided treatment starts no later than mid-July 2026. The 15 results tables, including five frequency-distribution tables in this study demonstrate the reporting format, but they are and have no empirical or therapeutic evidentiary status. A data-grounded submission will require verification of source patient records, valid immunoassays, clinical response calculations and human-subject's approvals.

Authors' Contributions

To be completed according to verified ICMJE authorship contributions; no names or roles have been invented.

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