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Clinical and biological effects of the umbilical cord-derived mesenchymal stromal cell (UC-MSC) secretome in rheumatoid arthritis: A randomised double-blind placebo-controlled trial

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Abstract

Background

Although the management of rheumatoid arthritis (RA) with disease-modifying antirheumatic drugs (DMARDs) has advanced, a considerable proportion of patients never achieve durable remission, highlighting the need for adjunctive therapies that restore immune homeostasis rather than simply inhibit a individual cytokine. The secretome derived from umbilical cord-derived mesenchymal stromal cell (UC-MSC) is a promising cell-free regenerative candidate, however, randomised trials linking validated clinical outcomes with mechanistic immune biomarkers remain scarce.

Methods

In this investigator-initiated, single-centre, randomised, double-blind, placebo-controlled trial, 40 patients with moderate RA receiving stable background conventional synthetic DMARD (csDMARD) therapy were randomly assigned (1:1) to receive intramuscular UC-MSC secretome (1.5 mL weekly for six weeks) or matched placebo. The primary endpoint was ACR20 response at week 7. Secondary outcomes included ACR50/70 responses, DAS28, pain intensity, functional disability, safety, and serum TNF-α, IL-6, and TGF-β1 levels to characterise treatment-associated immunomodulation.

Results

Compared with placebo, the adjunctive UC-MSC secretome produced a significantly greater ACR20 response (75.0% vs 15.0%; risk ratio 5.0, absolute risk reduction 60%, number needed to treat 2) and major clinical response (ACR50/70: 65.0% vs 15.0%). DAS28-CRP, pain intensity, and functional disability also improved significantly. On log-scale reanalysis, TNF-α did not differ significantly between groups after adjustment for age, sex, and baseline level (geometric mean ratio [GMR] 1.07, 95% CI 0.82–1.40), with equivalence testing confirming that the difference lay within a prespecified 1.5-fold margin. IL-6 showed a nominal unadjusted increase (GMR 2.05; P = 0.034) that lost significance after adjustment (GMR 1.51; P = 0.216); TGF-β1 did not differ significantly between groups. These findings indicate the UC-MSC secretome therapy may provide a promising cell-free adjunctive treatment for RA, although its underlying

biological mechanism remains to be elucidated.

Conclusions

Adjunctive intramuscular UC-MSC secretome therapy significantly improved ACR20/50 response, DAS28 score, and pain intensity in patients with moderate RA receiving background csDMARD therapy. Larger multicentre trials with longer follow-up and pathway-specific biomarkers are warranted to confirm long-term efficacy and clarify the underlying biological mechanism.

Trial registration
ClinicalTrials.gov, NCT07715045

Keywords: Rheumatoid arthritis; Mesenchymal stromal cell secretome; Cell-free therapy; Immunomodulation; Regenerative medicine

1. Introduction

Over the past two decades, the management of rheumatoid arthritis (RA) has advanced considerably through the adoption of treat-to-target strategies and the development of conventional synthetic, biologic, and targeted synthetic disease modifying antirheumatic drugs (DMARDs). These advances have markedly improved disease activity control and established remission or low disease activity as a realistic therapeutic target. Nevertheless, a proportion of patients continue to experience persistent disease activity or suboptimal responses to available therapies, highlighting an unmet need for novel therapeutic approaches capable of more broadly modulating immune dysregulation. [1-3] However, therapeutic success in clinical trials has not been consistently translated into routine clinical practice.

Current international recommendations acknowledge that a considerable proportion of patients require sequential DMARD switching because of an inadequate response, secondary loss of efficacy, intolerance, or safety concerns, whereas others continue to experience a persistent inflammatory disease despite receiving multiple therapeutic interventions. [1-3] Data from the Global Burden of Disease (GBD) Study 2021 indicate that rheumatoid arthritis (RA) remains a significant global health concern,

with an estimated 17.9 million people living with the disease worldwide and more than 3 million disability-adjusted life years (DALYs) lost as a consequence. This burden underscores RA as one of the leading contributors to chronic musculoskeletal disability globally. [4] The coexistence of unprecedented therapeutic advances with persistent unmet clinical needs raises a fundamental question: does the remaining therapeutic gap simply reflect the incomplete suppression of inflammatory pathways, or does it also indicate that current therapies have not fully restored the immune regulatory networks required for durable disease control?

The discrepancy between therapeutic expectations and real-world clinical outcomes becomes even more apparent in health care systems with limited access to advanced therapies. Although current international guidelines recommend timely escalation to biologic or targeted synthetic DMARDs for patients with persistent disease activity, the implementation of these recommendations remains challenging in many low- and middle-income countries because of limited drug availability, health care resource constraints, and reimbursement policies. [1-2] Indonesia illustrates this therapeutic gap. In routine clinical practice, most patients continue to rely on conventional synthetic DMARDs despite persistent disease activity, limiting the likelihood of achieving sustained remission. This unmet clinical need is reflected in the most recent nationwide Indonesian rheumatoid arthritis registry, which reported that only 24.5% of patients achieved clinical remission, whereas the majority continued to present with moderate or high disease activity despite ongoing treatment. [5] Together, these observations suggest that further improvements in RA outcomes may require adjunctive therapeutic strategies capable of targeting biological processes that are not fully addressed by current DMARD-based treatment approaches, thereby providing a strong rationale for regenerative immunomodulatory therapies.

The persistent gap in rheumatoid arthritis therapies has shifted current thinking beyond the suppression of individual inflammatory mediators towards the coordinated modulation of the immune regulatory networks that sustain long-term disease control. Immune dysregulation in RA is a complex process involving the interplay of multiple cytokines rather than the activity of a single inflammatory mediator. Tumour necrosis

factor-α (TNF-α), interleukin-6 (IL-6), and transforming growth factor-β (TGF-β) form part of this network, each contributing a distinct function in regulating inflammation, immune responses, and tissue homeostasis and repair. Because these cytokines operate within an interconnected network, a change in any single cytokine does not necessarily reflect the overall immune response. Simultaneous assessment of TNF-α, IL-6, and TGF-β may therefore provide a more comprehensive picture of the immune milieu following a therapeutic intervention. [6-9]

Importantly, the modulation of immune biomarkers has limited translational value unless it is accompanied by a meaningful clinical improvement. This concept has stimulated increasing interest in regenerative immunomodulatory therapies capable of coordinately influencing multiple immune pathways rather than targeting a single cytokine. The UC-MSC secretome represents a promising cell-free approach, as it contains a diverse array of bioactive mediators capable of simultaneously modulating inflammatory and immune-regulatory pathways. This compositional complexity provides a biological rationale for its potential to influence the immune microenvironment more broadly. Accordingly, the UC-MSC secretome provides a biologically plausible platform for investigating whether the coordinated modulation of the TNF-α–IL-6–TGF-β cytokine network translates into meaningful clinical benefit in patients with rheumatoid arthritis. [10-12]

Whether the coordinated modulation of the TNF-α–IL-6–TGF-β cytokine network is genuinely translated into clinically meaningful therapeutic responses in patients with rheumatoid arthritis remains an unresolved question. Although experimental studies have established the biological plausibility of regenerative immunomodulation and early clinical investigations have suggested therapeutic potential, these two domains have largely evolved independently rather than within an integrated translational framework. Consequently, the mechanistic relationship between immune biomarker modulation and a validated clinical response has not been adequately characterised. In particular, whether changes in TNF-α, IL-6, and TGF-β levels are merely indicators of biological activity or represent mechanistic correlates of a clinical improvement as assessed by the American College of Rheumatology (ACR20,

ACR50, and ACR70) response criteria remains uncertain. Addressing this clinical–biological translational gap is essential for elucidating the mechanism of action of regenerative immunomodulatory therapies and for establishing biologically informed therapeutic strategies for rheumatoid arthritis. [13-15]

To address this translational gap, we conducted a randomised, double-blind, placebo-controlled trial to evaluate whether adding the UC-MSC secretome to background csDMARD therapy would confer clinical benefit and influence the immune response in patients with moderate rheumatoid arthritis (RA) activity. Clinical efficacy was evaluated using the American College of Rheumatology (ACR20, ACR50, and ACR70) response criteria, whereas the levels of mechanistically relevant immune biomarkers, including TNF-α, IL-6, and TGF-β, were simultaneously assessed to explore the biological correlates of the treatment response. We hypothesised that adjunctive UC-MSC secretome therapy would induce the coordinated modulation of the TNF-α–IL-6–TGF-β cytokine network and that these biological changes would be associated with superior clinical responses compared with the placebo, thereby providing translational evidence linking immune modulation to therapeutic efficacy in patients with rheumatoid arthritis.

2. Methods

2.1 Study Design

This randomised, double-blind, placebo-controlled trial was designed to test the superiority of adjunctive UC-MSC secretome therapy over placebo, although the sample size was determined pragmatically rather than from a formal a priori power calculation; it was a single-centre, investigator-initiated study conducted at the Rheumatology Outpatient Clinic, Department of Internal Medicine, Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia. This study was conducted between October 2025 and June 2026 and included study preparation, ethical approval, participant screening, recruitment, randomisation, intervention, follow-up, and outcome assessment. The last participant completed the six-week (seven-week, counting from baseline) follow-up assessment in February 2026; the interval to the

formal study completion date of 4 June 2026 reflects the time required for centralised laboratory batch analysis of stored biomarker samples, database cleaning and lock, and finalisation of the statistical analysis. This trial investigated the clinical efficacy and immunomodulatory effects of adjunctive therapy with the human umbilical cord-derived mesenchymal stromal cell (UC-MSC) secretome in patients with rheumatoid arthritis receiving stable conventional synthetic disease-modifying antirheumatic drugs (csDMARDs).

The study protocol (title: "Effectiveness of Umbilical Cord Mesenchymal Stem Cell Secretome Addition on Clinical Conditions and Biomarkers in Rheumatoid Arthritis Patients") was approved on 5 February 2026 by the Health Research Ethics Committee of Dr. Mohammad Hoesin General Hospital (Approval No. DP.04.03/D.XVIII.06.08/ETIK/038/2026). Written informed consent was obtained from all participants before enrolment. This study was registered at ClinicalTrials.gov (NCT07715045). Registration occurred retrospectively, after participant recruitment had already commenced and, in fact, after the study had been completed (study completion: 4 June 2026), with initial submission on 15 July 2026; the trial record was first publicly posted on ClinicalTrials.gov on 20 July 2026 and has remained publicly searchable since that date. This deviation from prospective registration is reported transparently in the Limitations section, including the reasons for the retrospective registration and the steps taken to mitigate its impact on the interpretation of the findings.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki (2013 revision) and is reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) 2025 Statement. No amendments were made to the trial protocol, eligibility criteria, randomisation procedure, or the prespecified primary and secondary clinical efficacy outcomes and their planned analyses after the trial commenced. [16-17] The analytical approach applied to one exploratory biomarker (TNF-α) was subsequently revised from an arithmetic-scale to a log-scale, equivalence-based framework for the methodological reasons detailed in Section 2.8; this revision is reported transparently alongside, rather

than in place of, the original arithmetic-scale result. A total of 40 eligible patients were enrolled and randomly assigned at a 1:1 ratio to receive adjunctive treatment with the UC-MSC secretome (n=20) or a placebo (n=20) in addition to stable csDMARD therapy. Detailed descriptions of participant eligibility, recruitment, randomisation, intervention, laboratory procedures, outcome assessment, safety monitoring, and statistical analysis are presented in the subsequent sections. Patient and public involvement. No patients or members of the public were directly involved in the design, conduct, or reporting of this study.

2.2 Participants

Patients were recruited consecutively from the Rheumatology Outpatient Clinic, Department of Internal Medicine, Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia. Recruitment employed a consecutive sampling method, whereby all patients fulfilling the eligibility criteria during the study period were considered for inclusion. This approach was adopted to minimise potential selection bias during recruitment.

Adult patients with rheumatoid arthritis who fulfilled the 2010 ACR/EULAR classification criteria [18], tested positive for RF at screening, and demonstrated moderate disease activity based on both DAS28-ESR and DAS28-CRP at baseline were included in the study. Participants were required to have received stable conventional synthetic disease-modifying antirheumatic drug (csDMARD) therapy for at least 3 months without achieving clinical remission according to the treating rheumatologist during the preceding 3 months, and to provide written informed consent before enrolment.

Patients were excluded if they had previously achieved complete remission or had concomitant systemic diseases, including mixed connective tissue disease (MCTD), active malignancy, diabetes mellitus, chronic kidney disease, chronic hepatitis B or C infection, active tuberculosis, human immunodeficiency virus infection, pregnancy, or previous exposure to biological disease-modifying antirheumatic drugs (bDMARDs). All participants remained on an unchanged background csDMARD regimen consisting

of methotrexate (≤12.5 mg/week), methylprednisolone (≤ 4 mg/day), and hydroxychloroquine (200 mg/day) throughout the study period to minimise treatment-related confounding factors. The sample size of 40 participants (20 per group) was determined pragmatically, on the basis of feasibility and the anticipated recruitment rate at this single centre over the planned study period, rather than a formal a priori power calculation.

2.3 Randomisation and Blinding

Participants were randomly allocated at a 1:1 ratio to receive either adjunctive UC-MSC secretome or placebo, based on a computer-generated randomisation sequence. Randomisation was performed using a simple, unrestricted procedure, without stratification or blocking. The allocation sequence was prepared by an independent statistician who was not involved in participant recruitment, intervention delivery, outcome assessment, laboratory procedures, or data analysis.

Allocation concealment and maintenance of blinding were ensured by the Department of Pharmacy, Dr. Mohammad Hoesin General Hospital. The investigational products were prepared and dispensed exclusively by designated pharmacy personnel according to the randomisation sequence. The placebo was manufactured and supplied by PT Bifarma Adiluhung (Regenic by Kalbe, Jakarta, Indonesia), the same pharmaceutical company that produces the UC-MSC secretome, to ensure identical pharmaceutical characteristics. Both investigational products were pharmaceutically matched and provided in identical prefilled syringes containing 1.5 mL of solution with the same syringe type, external appearance, labelling, packaging, and visual characteristics, making them visually indistinguishable. The syringes were labelled only with allocation codes (A or B). Both participants and the physician administering the injections were blinded to treatment allocation. The allocation codes were held and accessible only to designated pharmacy staff until the completion of the study. The rheumatologist responsible for recruitment and intervention delivery, the participants, and personnel involved in outcome assessment had no access to the allocation sequence throughout the study.

A clinical response was evaluated according to the ACR20, ACR50, and ACR70 response criteria by an independent rheumatologist who was blinded to the treatment allocation. The DAS28-ESR and DAS28-CRP scores required for determining the clinical response were assessed using standardised procedures. The laboratory measurements of TNF-α, IL-6, and TGF-β levels were independently performed by two laboratory analysts who were likewise blinded to the treatment allocation. Statistical analyses were performed only after the completion of data collection and formal unblinding of the treatment allocation.

2.4 Study Intervention

The investigational product was Regenic Secretome, a clinical-grade human umbilical cord-derived mesenchymal stromal cell (UC-MSC) secretome manufactured by PT Bifarma Adiluhung (Regenic by Kalbe, Jakarta, Indonesia) under Good Manufacturing Practice (GMP)-certified conditions (Certificate No. PW-S.01.04.1.333.09.21-0082) and produced under authorisation from the Ministry of Health of the Republic of Indonesia (Licence No. 81202131002260001). UC-MSCs were isolated from Wharton's jelly obtained from human umbilical cords donated after written informed consent was obtained. An assessment of donor eligibility, infectious disease screening, and a cytogenetic evaluation were completed before cells were isolated.

Cell identity and biological characteristics were confirmed according to the minimal criteria of the International Society for Cell & Gene Therapy (ISCT). The cells expressed CD73, CD90, and CD105 (>95%) and lacked expression of CD45, CD34, CD14, CD19, and HLA-DR (<2%). Multipotency was confirmed by assessing adipogenic, osteogenic, and chondrogenic differentiation. The secretome was produced using a standardised xeno-free GMP-compliant manufacturing process. Each production batch met predefined release criteria, including sterility, endotoxin levels, a lack of mycoplasma contamination, batch consistency, and the absence of residual viable cells before clinical use.

Participants assigned to the intervention group received 1.5 mL of the UC-

MSC secretome by intramuscular injection into the deltoid muscle once weekly for six consecutive weeks. The dosing regimen was based on preliminary investigational studies conducted during product development. All injections were administered by the same rheumatologist using a standardised procedure. As this was a single-centre trial with a single treating rheumatologist administering all injections, no additional site- or provider-level eligibility criteria beyond routine clinical qualification were applied.

The placebo group received 1.5 mL of 0.9% normal saline supplied by the same manufacturer. The placebo and UC-MSC secretome were packaged in identical prefilled syringes with the same appearance, labelling, and volume to maintain blinding. Participants, treating physicians, and outcome assessors remained unaware of the treatment allocation throughout this study. All participants continued stable background conventional synthetic disease-modifying antirheumatic drug (csDMARD) therapy, which consisted of methotrexate (≤12.5 mg/week), methylprednisolone (≤4 mg/day), and hydroxychloroquine (200 mg/day), throughout this study.

Table 1. Characteristics of the investigational UC-MSC secretome product

Category	Parameter	Specification

Product	Investigational product	Regenic® Secretome
	Manufacturer	PT Bifarma Adiluhung (Regenic by Kalbe), Jakarta, Indonesia
	Manufacturing standard	Good Manufacturing Practice (GMP)-certified facility
Cell source	Tissue source	Human umbilical cord-derived Wharton's jelly mesenchymal stromal cells (UC-MSCs)
	Donor eligibility	Written informed consent, donor eligibility assessment, and infectious disease screening
Manufacturing	Isolation and culture	Xeno-free, enzyme-free isolation with controlled hypoxic conditioning
	Final formulation	Sterile, preservative-free, cell-free biological solution
	Excipients	No antibiotics, phenol red, synthetic serum, or exogenous growth factors
Product characterisation	Secretome content	bFGF, TGFβ-1, KGF, HGF, Pro collagen 1 α-1
	ISCT immunophenotype	CD73+, CD90+, and CD105 (>95%); CD34−, CD45−, CD14−, CD19−, and HLA-DR (<2%)
	Differentiation potential	Adipogenic, osteogenic, and chondrogenic differentiation confirmed
Quality control	Potency assay	Keratinocyte growth factor (KGF)
	Safety testing	Sterility, endotoxin levels, a lack of mycoplasma contamination, and absence of residual viable cells
	Batch release	Visual inspection and batch-to-batch consistency were confirmed before clinical use
Clinical administration	Storage	−10 °C to −20 °C
	Dose	1.5 mL was administered

	intramuscularly once weekly for 6 weeks
Injection site	Deltoid muscle

2.5 Laboratory Assessment of Immunological Biomarkers

Peripheral venous blood samples were collected from all participants at baseline (before the first intervention) and week 7 (one week after the sixth injection) to evaluate treatment-induced changes in the circulating levels of immunological biomarkers. At each time point, 6 mL of venous blood was collected in Vacutainer Serum Separator Tubes (SSTs) without anticoagulant. Following clot formation, the samples were centrifuged at 1,000 × g for 20 minutes, and the separated serum was aliquoted and stored at −80 °C until analysis.

Serum concentrations of tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and transforming growth factor-beta 1 (TGF-β1) were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. These biomarkers were selected to represent complementary biological pathways involved in rheumatoid arthritis pathogenesis, including proinflammatory signalling (TNF-α and IL-6) and immune regulation (TGF-β1), thereby enabling a mechanistic interpretation of clinical responses to UC-MSC secretome therapy. TNF-α levels were measured using the Elabscience® Human TNF-α ELISA Kit (Catalogue No. E-EL-H0109), IL-6 levels were measured using the Elabscience® Human IL-6 ELISA Kit (Catalogue No. E-EL-H6156), and TGF-β1 levels were measured using the Elabscience® Human TGF-β1 ELISA Kit (Catalogue No. E-EL-0162). All the assays were performed in duplicate, and the absorbance was measured at 450 ± 2 nm using a Bio-Rad iMark™ Microplate Absorbance Reader. The detailed characteristics of the assay are summarised in Table 2.

Table 2. Analytical performance of the ELISAs used to quantify immunological biomarkers

Biomarker	ELISA Kit	Catalogue No.	Sample	Detection Range	Analytical Sensitivity	Intra-assay CV	Interassay CV	Assay Format
TNF-α	Elabscience® Human TNF-α ELISA Kit	E-EL-H0109	Serum	7.81 –500 pg/mL	4.69 pg/mL	5.25–6.10%	6.03–8.18%	Duplicate
IL-6	Elabscience® Human IL-6 ELISA Kit	E-EL-H6156	Serum	1.56–100 pg/mL	0.94 pg/mL	5.06–5.96%	6.06–8.49%	Duplicate
TGF-β1	Elabscience® Human TGF-β1 ELISA Kit	E-EL-0162	Serum	0.16–10 ng/mL	0.09 ng/mL	5.07–6.75%	6.15–8.90%	Duplicate

CV, coefficient of variation; TNF-α, tumour necrosis factor-alpha; IL-6, interleukin-6; TGF-β1, transforming growth factor-beta 1. All assays were performed in duplicate; absorbance was measured at 450 ± 2 nm using a Bio-Rad iMark™ Microplate Absorbance Reader.

All laboratory analyses were independently performed by two laboratory analysts who were blinded to the treatment allocation to ensure analytical reliability. Baseline and week-7 serum samples from each participant were analysed within the same analytical batch to minimise interassay variability and batch-to-batch variation. The internal quality control procedures, instrument calibration, and assay validation were completed according to the manufacturers' recommendations before the samples were analysed. In accordance with the manufacturers' validation data, the ELISA results demonstrated acceptable analytical precision. The intra-assay/interassay coefficients of variation were 5.25–6.10%/6.03–8.18% for TNF-α, 5.06–5.96%/6.06–8.49% for IL-6, and 5.07–6.75%/6.15–8.90% for TGF-β1, respective

2.6 Clinical and Immunological Outcome Measures

The primary endpoint was clinical efficacy at week 7, which was defined as the achievement of the American College of Rheumatology 20% improvement (ACR20) response criteria following adjunctive UC-MSC secretome therapy. A clinical response was assessed by an independent rheumatologist who was blinded to the treatment allocation using the standardised ACR response criteria.

The key clinical efficacy endpoints included the proportions of participants who achieved ACR50 and ACR70 responses at week 7, together with changes in the disease activity score (DAS28-ESR), DAS28-CRP, C-reactive protein (CRP) level, and erythrocyte sedimentation rate (ESR) between baseline and week 7, in 28 joints. Prespecified secondary clinical endpoints also included the change from baseline to week 7 in the visual analogue scale (VAS) pain score, the Health Assessment Questionnaire Disability Index (HAQ-DI), and the Patient Global Assessment (PGA).

Mechanistic secondary outcomes included changes in the serum concentrations of tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and transforming growth factor-beta 1 (TGF-β1) between the baseline and week 7. These biomarkers were evaluated to characterise the immunomodulatory effects associated with UC-MSC secretome therapy and to provide mechanistic insights into the biological processes underlying the clinical improvement.

An exploratory analysis was prespecified to investigate the associations between changes in the circulating levels of immunological biomarkers (TNF-α, IL-6, and TGF-β1) and the achievement of a clinical response (ACR20, ACR50, and ACR70), with the aim of identifying potential immunological correlates of therapeutic efficacy. Safety outcomes included the incidence, severity, and relationship of adverse events to the investigational product throughout the study period, with particular attention given to injection-site reactions and systemic adverse events.

2.7 Safety Monitoring

Safety was monitored throughout this study by scheduled clinical evaluations performed at weekly follow-up visits after each intervention. At every visit, participants underwent a symptom assessment and physical examination to identify local or systemic adverse events. The participants were also instructed to immediately report any new symptoms occurring between scheduled visits to the study investigators. Safety evaluations were performed by an independent rheumatologist who was not involved in treatment administration or the clinical outcome assessment, thereby minimising observer bias.

Adverse events were classified and graded according to the Common Terminology Criteria for Adverse Events (CTCAE). The causal relationship between each adverse event and the investigational product was determined by the clinical judgement of the independent rheumatologist after a consideration of the temporal relationship, clinical presentation, and alternative aetiologies. Participants were withdrawn from this study if they developed severe allergic reactions, anaphylaxis, or severe infections; voluntarily withdrew informed consent; were lost to follow-up; or experienced major protocol violations. Any serious adverse events (SAEs) were reported to the Institutional Ethics Committee within 24 hours of recognition and were managed according to institutional clinical protocols.

Because this investigator-initiated, single-centre randomised clinical trial involved a relatively small number of participants, a formal Data Safety Monitoring Board (DSMB) was not established. Instead, participant safety was overseen

continuously by the principal investigator and the independent rheumatologist throughout this study. All adverse events were documented, managed appropriately, and included in the final safety analysis. No interim safety or efficacy analyses, and no formal rule for early stopping, were planned or performed, because of the relatively short intervention period, modest sample size, and anticipated low-risk safety profile of the investigational product.

2.8 Statistical Analysis

All the statistical analyses were performed using Python version 3.12 with SciPy, statsmodels, and pingouin libraries, whereas Microsoft Excel was used exclusively for data entry and preliminary data management. Continuous variables were assessed for normality using the Shapiro–Wilk test. Normally distributed variables are presented as the means ± standard deviations (SDs), whereas nonnormally distributed variables are reported as medians (interquartile ranges [IQRs]). Categorical variables are presented as frequencies and percentages.

Baseline characteristics were compared between treatment groups using the independent-samples t test or Mann–Whitney U test for continuous variables, according to the data distribution, and the chi-square test or Fisher's exact test for categorical variables. Within-group changes from baseline to week 7 were evaluated using the paired t test or Wilcoxon signed-rank test, whereas between-group treatment effects were assessed by comparing change scores (Δ values) using the independent-samples t test, Welch's t test, or Mann–Whitney U test, as appropriate.

Clinical response rates according to the American College of Rheumatology 20%, 50%, and 70% improvement criteria (ACR20, ACR50, and ACR70) were compared using the chi-square test or Fisher's exact test, depending on the expected cell frequencies. Effect estimates for binary outcomes are presented as odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs). Correlations between changes in clinical outcomes and immunological biomarkers were evaluated using Spearman's rank correlation coefficient.

Given the observed baseline imbalances in age and ESR, additional covariate-

adjusted analyses were performed to assess whether the treatment effect estimates were robust to adjustment for these baseline differences. Multivariable linear regression models adjusted for age, sex, and baseline values were used to identify independent treatment effects on continuous outcomes. A multivariate logistic regression analysis was performed to identify independent predictors of an ACR50 clinical response. In addition, a multivariate analysis of variance (MANOVA) was conducted to evaluate the overall treatment effect across multiple correlated clinical outcomes, whereas longitudinal changes in weekly pain scores were analysed using a linear mixed-effects model incorporating participant-specific random effects and fixed effects for the treatment group, time, and their interaction.

All randomised participants were analysed according to the intention-to-treat (ITT) principle. Because no participants withdrew, crossed over, or were lost to follow-up, the ITT population was identical to the per-protocol population. Only one posttreatment DAS28-ESR value was unavailable because of a data entry error. Given the minimal proportion of missing data (<3%) and the absence of participant attrition, data imputation was not performed. All the statistical tests were two-sided, and a P value <0.05 was considered to indicate statistical significance.

Because a substantial proportion of the TNF-α, IL-6, and TGF-β1 measurements approached or fell below the lower limit of quantification of the respective ELISA assays, and the raw concentrations were markedly right-skewed, a subsequent reanalysis of the cytokine data was performed using log-transformed values. Within-group and between-group changes are expressed as geometric mean ratios (GMRs) with 95% confidence intervals; between-group GMRs were additionally adjusted for age, sex, and the log-transformed baseline concentration using analysis of covariance (ANCOVA) on log-transformed week-7 values. Two one-sided tests (TOST) were used to assess statistical equivalence between groups at prespecified margins of 1.5-fold and 2.0-fold. Associations between the ratio of change in each biomarker and changes in clinical outcomes were assessed using Spearman's rank correlation with 95% confidence intervals derived from 5,000 bootstrap resamples, and the resulting P values across the prespecified set of comparisons were adjusted for multiple testing using the

Benjamini–Hochberg false discovery rate (FDR) procedure. A post hoc power and minimum-detectable-effect analysis was also performed for each biomarker given the observed variance and the sample size of 20 participants per group. This log-scale, equivalence- and power-aware approach was adopted because the arithmetic-scale summary statistics reported for these biomarkers in earlier analyses of this dataset were disproportionately influenced by measurements clustered near the assay detection limit and by a small number of outlying values; the results of this reanalysis, rather than the earlier arithmetic-scale comparisons, are reported in Section 3.5 and interpreted in the Discussion.

3. Results

3.1 Participant Enrolment and Follow-up

Between November 2025 and January 2026, 60 patients with rheumatoid arthritis underwent screening to determine eligibility for the study. Twenty patients were excluded: 8 due to comorbidities meeting the exclusion criteria, 7 for not meeting the criterion for moderate disease activity based on DAS28-ESR and DAS28-CRP, and 5 for being unable to comply with the scheduled follow-up visits. The remaining 40 eligible participants then underwent 1:1 randomisation, with 20 allocated to the UC-MSC secretome group and 20 to the placebo group.

All randomised participants received their allocated intervention and completed the six-week treatment protocol as scheduled. The trial proceeded to its planned completion as designed, with no early termination and no stopping for efficacy, futility, or safety. No participants discontinued treatment, withdrew consent, or were lost to follow-up. Consequently, all 40 randomised participants (100%) completed the study and were included in the final analyses of clinical efficacy, immunological biomarkers, and safety outcomes. The flow diagram of participants throughout this study is illustrated in Figure 1.

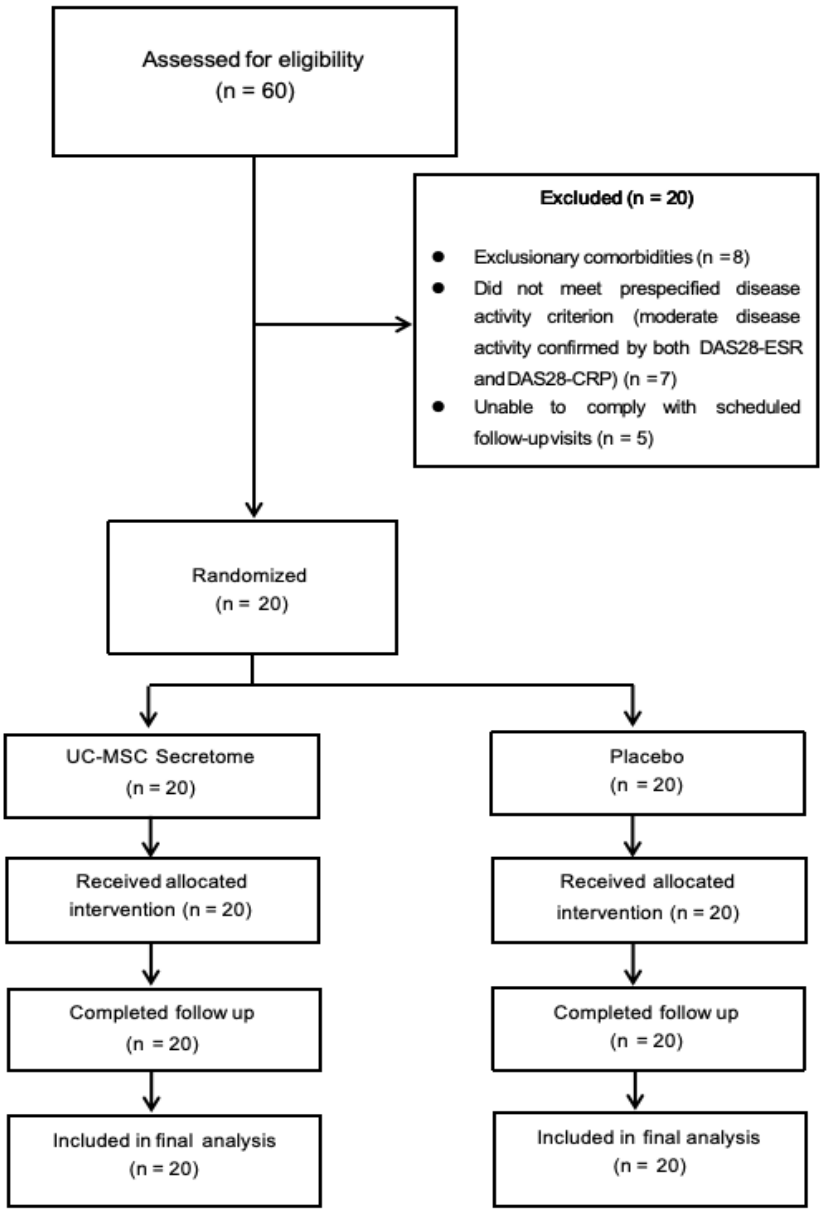


Figure 1. CONSORT flow diagram of participant enrolment and study allocation. The diagram summarises participant screening, eligibility assessment, randomisation, treatment allocation, follow-up, and inclusion in the final analysis. Among the 60 participants assessed for eligibility, 40 fulfilled the predefined eligibility criteria and were randomised at a 1:1 ratio to receive an adjunctive UC-MSC secretome treatment or the placebo. All randomised participants received the allocated intervention, completed the 6-week follow-up, and were included in both the efficacy and safety analyses. CONSORT, Consolidated Standards of Reporting Trials.

3.2 Baseline Demographic and Clinical Characteristics

The baseline demographic, clinical, and laboratory characteristics of the study participants are summarised in Table 3. Overall, randomisation produced two groups with generally comparable baseline characteristics, particularly regarding the sex distribution, disease activity, inflammatory biomarkers, and background laboratory findings. Consistent with the seropositivity eligibility criterion, all 40 participants (100%) tested positive for rheumatoid factor at baseline in both treatment groups. Although differences in age, baseline erythrocyte sedimentation rate (ESR), and the haemoglobin concentration were observed between the treatment groups, no clinically relevant differences were identified in the baseline disease activity score in 28 joints using the erythrocyte sedimentation rate (DAS28-ESR), disease activity score in 28 joints using C-reactive protein (DAS28-CRP), visual analogue scale (VAS) pain score, or C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), or transforming growth factor-beta 1 (TGF-β) levels. Considering the modest sample size, these baseline differences were considered compatible with random variation following allocation and were addressed during multivariable statistical modelling through an adjustment for age.

Table 3. Characteristics of patients receiving the investigational UC-MSC secretome product and the placebo

Variable	UC-MSC Secretome (n = 20)	Placebo (n = 20)	P value
Demographic characteristics			
Age, years	39.50 (34.00–48.50)	48.00 (43.00–53.75)	**0.012**
Female sex, n (%)	17 (85.0)	19 (95.0)	0.605
Disease activity			
VAS score	6.00 (5.00–6.00)	6.00 (6.00–6.00)	0.297
Patient Global Assessment (PGA)	6.00 (5.00–6.00)	6.00 (6.00–6.00)	0.297
DAS28-ESR	4.25 (3.88–5.30)	4.60 (3.90–4.98)	0.472
DAS28-CRP	3.58 (3.15–4.11)	3.33 (3.07–4.23)	0.617
ESR, mm/hour	28.00 (17.75–42.50)	47.50 (39.00–55.00)	**0.004**
hsCRP, mg/L	1.85 (1.00–11.23)	4.35 (3.50–5.70)	0.135
Rheumatoid factor positivity, n (%)	20 (100.0)	20 (100.0)	NA
Laboratory parameters			
Haemoglobin, g/dL	13.10 (12.65–13.62)	12.30 (11.22–12.90)	**0.025**
Leucocyte count, /μL	8150 (6845–9443)	7855 (6708–8958)	0.646
Platelet count, /μL	333000 (287750–359000)	331500 (289750–362750)	0.860
AST, U/L	19.00 (17.00–22.00)	20.50 (18.75–25.00)	0.446
ALT, U/L	18.50 (12.50–22.25)	16.50 (12.25–23.25)	0.626
Blood urea, mg/dL	19.00 (17.50–24.50)	19.00 (15.75–25.00)	0.796
Serum creatinine, mg/dL	0.67 (0.63–0.76)	0.66 (0.61–0.80)	0.694

Data are presented as median (interquartile range) unless otherwise indicated.

Categorical variables are presented as number (%). Continuous variables were compared using the Mann–Whitney U test, and categorical variables were compared using Fisher's exact test.

Statistically significant differences (P < 0.05) are shown in bold. Although the Visual Analogue Scale (VAS) pain score and Patient Global Assessment (PGA) are numerically identical at baseline in this

cohort, they are distinct patient-reported instruments (pain intensity versus overall disease impact, respectively); their post-treatment values, reported in Section 3.4, diverge. Baseline serum concentrations of TNF-α, IL-6, and TGF-β1 are presented together with the corresponding week-7 values in Table 7, rather than being duplicated here.

3.3 Primary Outcome: Clinical Efficacy

At week 7, the proportion of patients achieving a clinical response based on ACR criteria was higher in the UC-MSC secretome group than in the placebo group (Table 4 and Figure 2). Fifteen of 20 patients (75.0%) in the secretome group met the ACR response criteria, compared with 3 of 20 patients (15.0%) in the placebo group. Conversely, 85.0% of patients in the placebo group remained nonresponders, compared with only 25.0% in the UC-MSC secretome group, indicating a substantially greater probability of achieving a clinically meaningful improvement following adjunctive UC-MSC secretome therapy.

The magnitude of the clinical response was also greater among patients treated with the UC-MSC secretome. Within the secretome group, 2 patients (10.0%) achieved ACR20, 9 patients (45.0%) achieved ACR50, and 4 patients (20.0%) achieved ACR70. Thus, 65.0% of all patients receiving the UC-MSC secretome achieved a major clinical response (ACR50 or ACR70). In contrast, only 3 patients (15.0%) in the placebo group achieved any ACR response, including 2 ACR50 responders (10.0%) and 1 ACR70 responder (5.0%), whereas the remaining 17 patients (85.0%) experienced no clinically meaningful improvement.

The corresponding treatment effect estimates, including the relative risk (RR), odds ratio (OR), absolute risk reduction (ARR), number needed to treat (NNT), 95% confidence intervals (95% CI), and P values, are presented in Table 4, providing quantitative measures of the magnitude and clinical relevance of the observed treatment effect. Adjunctive UC-MSC secretome therapy was associated with a greater clinical response than placebo, in terms of both the proportion of patients achieving a response and the magnitude of clinical change. These findings support an additional benefit of the UC-MSC secretome when combined with background csDMARD therapy in patients with RA.

Table 4. Clinical Response According to the American College of Rheumatology (ACR) Response Criteria at Week 7

Clinical Response	UC-MSC Secretome (n = 20)	Placebo (n = 20)
Clinical responders (ACR20 or higher)	15 (75.0%)	3 (15.0%)
Non-responders (ACR0)	5 (25.0%)	17 (85.0%)
Distribution of responders		
ACR20	2 (10.0%)	0 (0.0%)
ACR50	9 (45.0%)	2 (10.0%)
ACR70	4 (20.0%)	1 (5.0%)

Data are presented as number (%). Clinical responders were defined as participants achieving ACR20, ACR50, or ACR70 at week 7 according to the American College of Rheumatology response criteria. In the 'Distribution of responders' rows, ACR20, ACR50, and ACR70 denote mutually exclusive categories corresponding to each participant's single highest level of response achieved and therefore do not sum cumulatively; this differs from the cumulative 'ACR20 or higher' convention used to define 'Clinical responders' above, in which a participant achieving ACR70 is also counted within ACR50 and ACR20.

Table 5. Treatment Effect Estimates for ACR Response at Week 7

Response threshold	RR (95% CI)	OR (95% CI)*	ARR	NNT	P value†
ACR20 or higher (overall response)	5.00 (1.71–14.63)	17.00 (3.46–83.44)	60.0%	2	<0.001
ACR50 or higher (major response)	4.33 (1.45–12.91)	10.52 (2.27–48.76)	50.0%	2	0.003
ACR70 (complete response)‡	4.00 (0.49–32.72)	4.75 (0.48–46.91)	15.0%	7	0.342

*RR, relative risk; OR, odds ratio; ARR, absolute risk reduction; NNT, number needed to treat; CI, confidence interval. *Unadjusted OR calculated from the 2×2 contingency table using the Haldane–Anscombe logit method. The multivariable-adjusted OR for an ACR50-or-higher response (12.18, 95% CI 2.11–70.39, adjusted for age, sex, and baseline DAS28-CRP) is reported in Section 3.6.3 and Figure 6B. †P values from Fisher's exact test. ‡ACR70 is the highest response tier and is therefore not nested within a higher category; the comparison is included for completeness as a prespecified secondary endpoint but was underpowered given the small number of responders in this tier and did not reach statistical significance.*

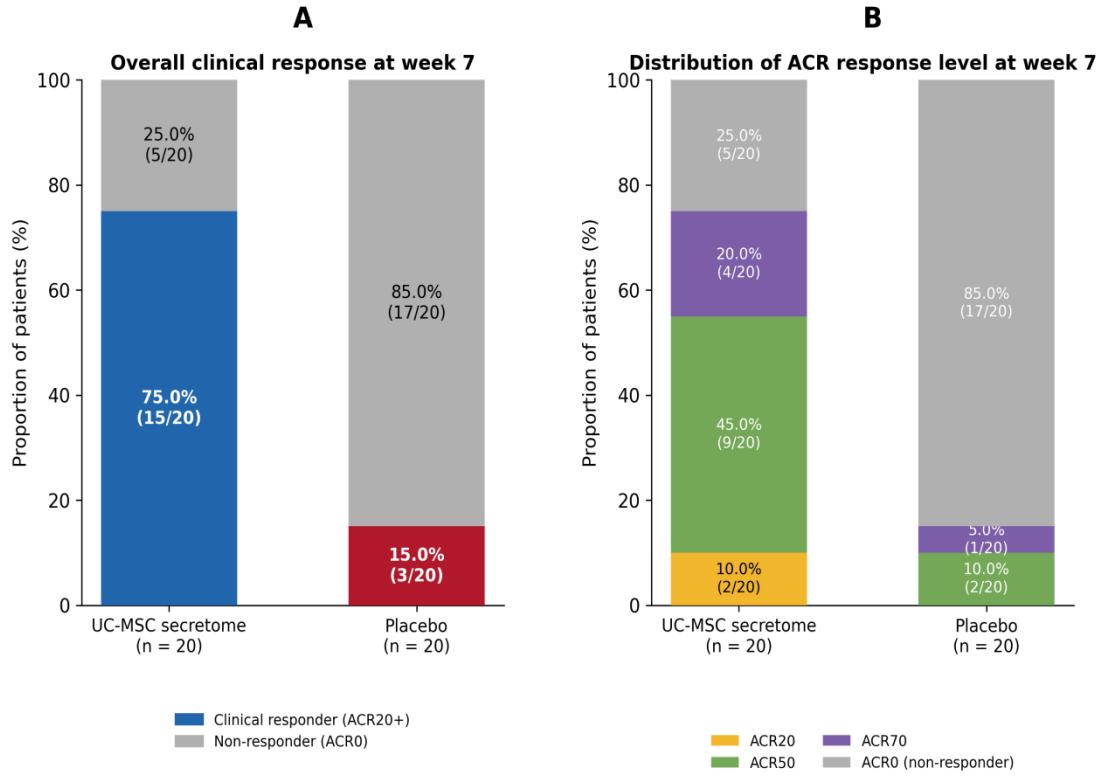


Figure 2. Overall clinical response and distribution of ACR response levels at week 7. (A) Overall proportions of responders (ACR20/50/70) and nonresponders (< ACR20) at week 7 in the UC-MSC secretome and placebo groups. (B) The distribution of the level of the clinical response achieved among responders according to the American College of Rheumatology (ACR) response criteria (ACR20, ACR50, and ACR70). Stacked bars represent the percentage of participants within each treatment group, with the corresponding number of patients shown in parentheses. Participants who did not achieve an ACR20 response were classified as nonresponders. Compared with the placebo group, the UC-MSC secretome group displayed a higher overall responder rate and a higher proportion of patients who achieved higher-level clinical responses (ACR50 and ACR70) at week 7.

3.4 Secondary Clinical Outcomes

Compared with placebo, adjunctive UC-MSC secretome therapy resulted in greater improvements in disease activity and patient-reported outcomes. Both groups showed change from baseline, but the magnitude of change over time was greater in the secretome group, indicating a differential treatment effect between groups.

Compared with participants receiving the placebo, participants receiving the UC-MSC secretome presented significantly greater reductions in visual analogue scale (VAS) pain scores (median Δ −3.00 vs. −1.00; Hodges–Lehmann median difference −2.00, 95% CI −3.00 to 0.00; P = 0.010), significantly greater improvements in the

Health Assessment Questionnaire Disability Index (HAQ-DI) (Hodges–Lehmann median difference −0.50, 95% CI −0.88 to −0.13; P = 0.004), and significantly greater reductions in the disease activity score in 28 joints using C-reactive protein (DAS28-CRP) (median Δ −1.17 vs. −0.40; Hodges–Lehmann median difference −0.68, 95% CI −1.19 to −0.09; P = 0.040). Although the improvement in the disease activity score in 28 joints determined using the erythrocyte sedimentation rate (DAS28-ESR) was greater in the UC-MSC secretome group (median Δ −0.95 vs. −0.40; Hodges–Lehmann median difference −0.60, 95% CI −1.20 to 0.00), the between-group difference did not reach statistical significance (P = 0.055). Adjunctive UC-MSC secretome therapy was also associated with a significantly greater improvement in the Patient Global Assessment (PGA) than placebo (median Δ −4.00 vs. −1.00; Hodges–Lehmann median difference −2.00, 95% CI −3.00 to −1.00; P = 0.006), consistent with the pattern observed for VAS pain scores. Adjunctive UC-MSC secretome therapy showed a consistent pattern of improvement in pain scores, patient-reported global disease impact, functional disability, and composite disease activity measures.

A prespecified multivariate analysis of variance (MANOVA) was performed on the four correlated clinical outcome change scores (ΔVAS, ΔHAQ-DI, ΔDAS28-CRP, and ΔDAS28-ESR; n = 39, one participant missing a week-7 DAS28-ESR value). Treatment allocation had a statistically significant overall multivariate effect on this combined set of clinical outcomes (Wilks' $\lambda = 0.730$, $F(4,34) = 3.14$, $P = 0.027$), corroborating the univariate between-group comparisons reported above.

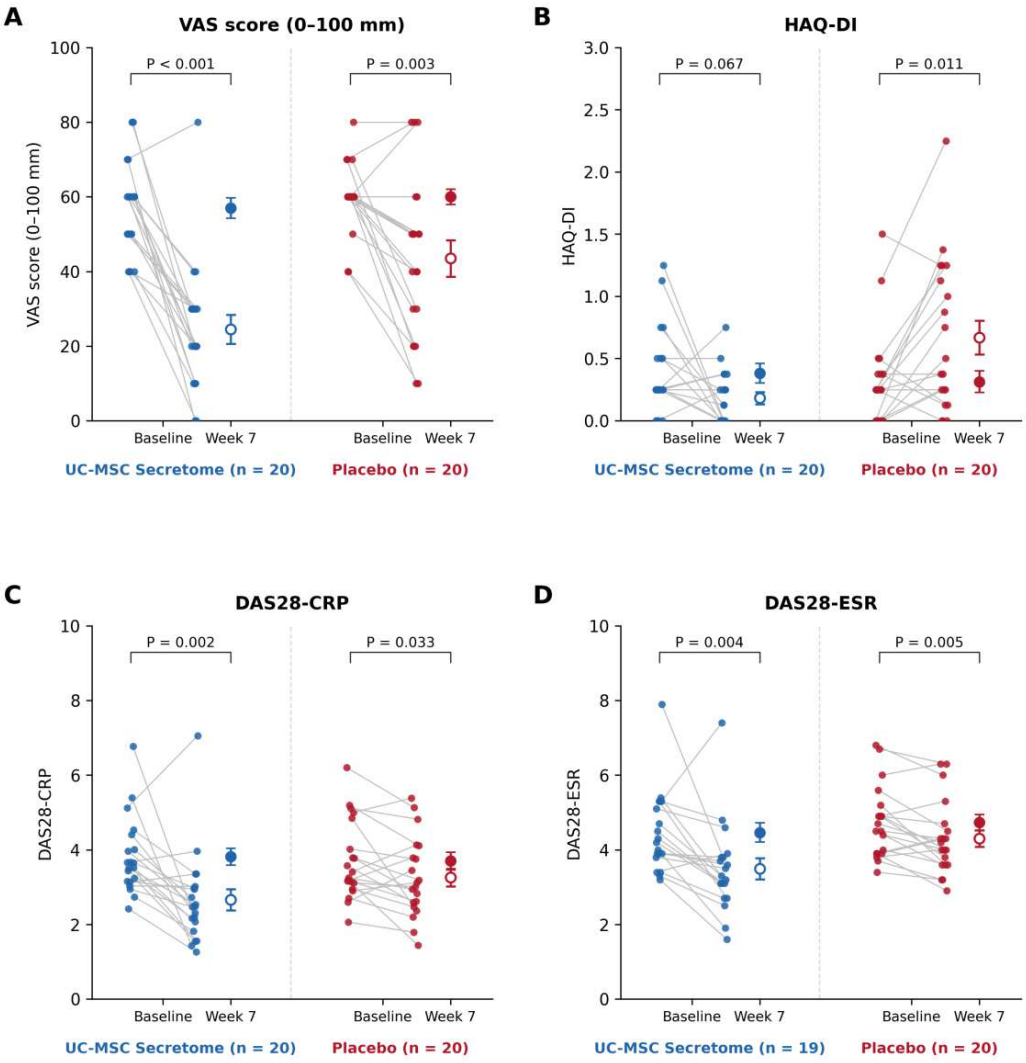


Figure 3. Changes in secondary clinical outcomes following adjunctive UC-MSC secretome therapy. (A) Visual analogue scale (VAS) pain score, (B) Health Assessment Questionnaire–Disability Index (HAQ-DI), (C) disease activity score in 28 joints using C-reactive protein (DAS28-CRP), and (D) disease activity score in 28 joints using the erythrocyte sedimentation rate (DAS28-ESR), measured at baseline and week 7. Individual participant values are presented as coloured dots connected by grey lines. The filled coloured circles indicate the group mean at baseline, the open coloured circles indicate the group mean at week 7, and the error bars represent the standard errors of the means (SEMs). Within-group comparisons between baseline and week 7 were performed using the Wilcoxon signed-rank test; between-group comparisons of change scores were analysed using the Mann–Whitney U test. Exact P values for within-group comparisons are shown above each panel. One participant in the UC-MSC secretome group had no recorded week-7 DAS28-ESR value (n = 19 for panel D; n = 20 for all other groups and panels)

Additional longitudinal analysis of weekly pain assessments further demonstrated the temporal pattern of the treatment response. Patients receiving the

adjunctive UC-MSC secretome treatment exhibited a progressive reduction in VAS scores beginning early during treatment, whereas placebo-treated patients showed only modest improvement throughout the 6-week follow-up period (Figure 4). The linear mixed-effects analysis revealed a significant treatment-by-time interaction, indicating that the trajectory of pain reduction differed significantly between treatment groups. These findings suggest that the adjunctive UC-MSC secretome treatment not only resulted in greater overall pain reduction at week 7 but also accelerated the onset and magnitude of analgesic improvement during treatment.

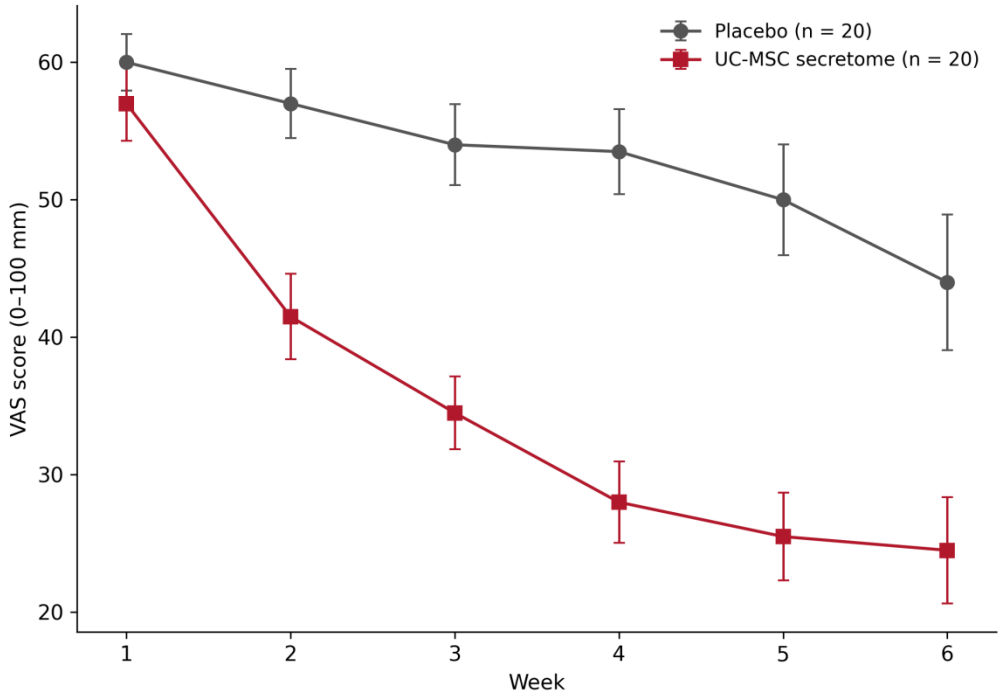


Figure 4. Weekly trajectory of pain intensity during adjunctive UC-MSC secretome therapy. The pain intensity was assessed weekly using the visual analogue scale (VAS, 0–100 mm) over the 6-week treatment period in the UC-MSC secretome (n = 20) and placebo (n = 20) groups. The data are presented as group means, with error bars representing the standard errors of the means (SEMs). The figure illustrates the temporal pattern of VAS scores in both treatment groups throughout the study period. Longitudinal changes were analysed using a linear mixed-effects model with fixed effects for the treatment group, time, and the treatment-by-time interaction. The treatment-by-time interaction was statistically significant ($P < 0.001$), indicating that the trajectories of pain reduction differed between treatment groups over time.

3.5 Mechanistic Immunological Outcomes

Because TNF-α, IL-6, and TGF-β1 are key inflammatory and immunoregulatory mediators involved in the pathogenesis and immune regulation of rheumatoid arthritis, these biomarkers were evaluated to characterise the biological response associated with adjunctive UC-MSC secretome therapy. The serum concentrations of TNF-α, IL-6, and TGF-β1 were measured at baseline and again at week 7 following treatment. The corresponding changes in the levels of these biomarkers are summarised in Table 6 and illustrated in Figure 5, with the corresponding arithmetic-scale median (IQR) concentrations at baseline and week 7 presented separately in Table 7 for descriptive completeness.

Because a substantial proportion of the TNF-α and TGF-β1 measurements were near or below the lower limit of quantification of the respective ELISA assays and the raw concentrations were markedly right-skewed (Additional file 1), the changes in biomarker levels were reanalysed on a log scale, as described in Section 2.8. On this reanalysis, circulating TNF-α concentrations decreased numerically in both groups between baseline and week 7 and did not differ significantly between the UC-MSC secretome and placebo groups, either without adjustment or after adjustment for age, sex, and the baseline level; prespecified equivalence testing confirmed that the between-group difference was within a 1.5-fold margin. Serum IL-6 concentrations increased in the UC-MSC secretome group and decreased in the placebo group; this unadjusted between-group difference reached nominal statistical significance, but the difference was no longer significant after adjustment for baseline covariates, and equivalence testing was inconclusive at the prespecified margins. Serum TGF-β1 concentrations increased modestly in both groups, with no significant between-group difference either unadjusted or adjusted.

Table 6. Changes in Immunological Biomarkers from Baseline to Week 7

Biomarker	Within-group GMR (95% CI)	Between-group GMR (95% CI), adjusted*	P (adjusted)
TNF-α	Secretome 0.93 (0.75–1.17); Placebo 0.94 (0.82–1.07)	1.07 (0.82–1.40)	0.612
IL-6	Secretome 1.41 (0.85–2.34); Placebo 0.69 (0.41–1.15)	1.51 (0.78–2.92)	0.216
TGF-β1	Secretome 1.29 (0.79–2.10); Placebo 1.13 (0.66–1.94)	1.08 (0.61–1.88)	0.792

Values in the within-group columns are geometric mean ratios (GMR; week 7 relative to baseline) with 95% confidence intervals; between-group values are adjusted GMRs (UC-MSC secretome relative to placebo) with 95% confidence intervals from ANCOVA of log-transformed week-7 concentrations, adjusted for age, sex, and the log-transformed baseline value. A GMR of 1.0 indicates no change. Because a substantial proportion of TNF-α and TGF-β1 values were near or below the assay lower limit of quantification, arithmetic-scale median (IQR) summaries are presented separately in Table 7; unadjusted between-group GMRs and equivalence testing are provided in Additional file 1.

P values (adjusted) are from ANCOVA of log-transformed week-7 concentrations. Prespecified two one-sided tests (TOST) confirmed statistical equivalence between groups for TNF-α at both 1.5-fold and 2.0-fold margins; equivalence testing was inconclusive for IL-6 and TGF-β1 at both margins (Additional file 1).

Table 7. Arithmetic-Scale Serum Biomarker Concentrations at Baseline and Week 7

Biomarker	Group	Baseline, median (IQR)	Week 7, median (IQR)	P
IL-6	Secretome	4.42 (2.74–18.20)	10.32 (3.31–19.34)	0.648
	Placebo	8.94 (3.00–12.27)	4.04 (1.90–15.02)	0.114
TNF-α	Secretome	12.88 (9.58–15.82)	10.93 (9.76–13.03)	0.177
	Placebo	9.94 (8.70–12.12)	9.26 (8.46–9.86)	0.430
TGF-β1	Secretome	0.28 (0.20–0.33)	0.39 (0.20–0.48)	0.255
	Placebo	0.33 (0.23–0.51)	0.43 (0.28–0.57)	0.452

Data are presented as arithmetic-scale median (interquartile range, IQR); TNF-α and IL-6 values are in pg/mL, and TGF-β1 values are in ng/mL. P values are from the Wilcoxon signed-rank test for the within-group change from baseline to week 7; these values correspond to those

shown above each panel in Figure 5A–C. These arithmetic-scale summaries are provided for descriptive completeness; because a substantial proportion of TNF-α and TGF-β1 values were near or below the assay lower limit of quantification, the log-scale, covariate-adjusted between-group comparisons reported in Table 6 are considered the more reliable basis for between-group inference. UC-MSC, umbilical cord-derived mesenchymal stromal cell.

The clinical benefit observed in the UC-MSC secretome group was therefore not accompanied by a corresponding, statistically robust reduction in circulating TNF-α, IL-6, or TGF-β1 concentrations once the skewed distribution of these biomarkers and multiple baseline covariates were accounted for. This dissociation between the clinical and the biomarker findings is considered further in the Discussion.

Taken together, adjunctive UC-MSC secretome therapy was not associated with a statistically robust or equivalence-confirmed change in circulating IL-6 or TGF-β1 levels, and the apparent between-group difference in TNF-α levels reported in earlier arithmetic-scale analyses of this dataset was not reproduced once log-transformation, covariate adjustment, and equivalence testing were applied; TNF-α was instead found to be statistically equivalent between groups. A detailed biological interpretation of these immunological findings is presented in the Discussion section.

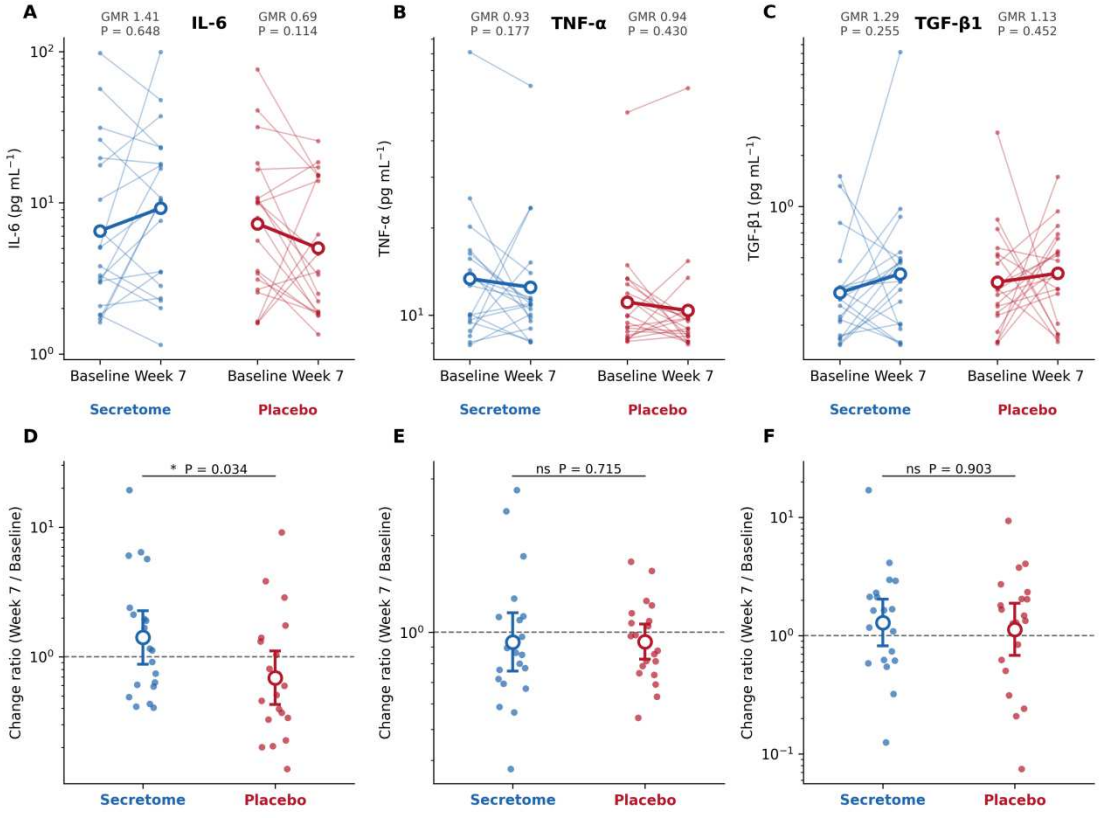


Figure 5. Serum cytokine responses to adjunctive UC-MSC secretome therapy: individual trajectories and treatment-effect ratios. (A) Interleukin-6 (IL-6), (B) tumour necrosis factor-α (TNF-α), and (C) transforming growth factor-β1 (TGF-β1) concentrations at baseline and week 7, plotted on a logarithmic scale. Individual participant values are presented as coloured dots connected by lines. The coloured circles indicate group geometric means, and the geometric mean ratio (GMR, week 7 relative to baseline) is shown above each group. Within-group comparisons between baseline and week 7 were performed using the Wilcoxon signed-rank test. (D–F) The corresponding individual change ratios (week 7 value divided by baseline value) for IL-6 (D), TNF-α (E), and TGF-β1 (F) in the UC-MSC secretome (n = 20) and placebo (n = 20) groups. The circles indicate group geometric means, and the error bars represent 95% confidence intervals (CIs); the dashed horizontal line denotes no change (ratio = 1). Between-group comparisons of change ratios were analysed using the Mann–Whitney U test on the log-transformed values. Exact P values are shown above each panel. Between-group comparisons shown here (D–F) are unadjusted, log-scale Mann–Whitney U tests; the corresponding baseline-, age-, and sex-adjusted ANCOVA estimates are reported in Table 6. UC-MSC, umbilical cord-derived mesenchymal stromal cell.

3.6 Exploratory Correlation and Multivariable Analyses

3.6.1 Correlations Between Changes in the Levels of Biomarkers and Clinical Outcomes

A Spearman correlation analysis, with 95% confidence intervals derived from 5,000 bootstrap resamples, was performed between the ratio of change (week 7 relative to baseline) in each circulating biomarker and the change in each clinical outcome at week 7, with the resulting P values across the 24 prespecified biomarker–outcome comparisons adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) procedure. On this reanalysis, the previously reported inverse correlation between the change in IL-6 concentration and the change in pain intensity, as measured using the visual analogue scale (VAS), was weaker and no longer statistically significant ($\rho = -0.28$, 95% CI −0.55 to 0.02, P = 0.080, P(FDR) = 0.507).

In addition, changes in hsCRP levels were moderately positively correlated with changes in DAS28-CRP scores ($\rho = 0.481$, P = 0.002), confirming the internal consistency between a biochemical marker of inflammatory activity and a composite disease activity assessment; this correlation was not part of the cytokine reanalysis described above and is unaffected by it. After FDR correction, none of the 24 biomarker–outcome correlations evaluated for TNF-α, IL-6, and TGF-β1 remained

statistically significant (all P(FDR) ≥ 0.507).

These findings indicate that, after accounting for the skewed distribution of the biomarker data and for multiple testing, none of the evaluated changes in TNF-α, IL-6, or TGF-β1 levels were significantly correlated with the clinical outcomes assessed in the present study, whereas hsCRP, an independently measured inflammatory marker, remained closely related to disease activity as assessed using the DAS28-CRP.

3.6.2 Multivariate Linear Regression Analysis

Multivariable linear regression analyses were performed after adjustment for age, sex, and corresponding baseline values to determine whether the observed therapeutic effects were independent of the baseline characteristics. Treatment allocation (UC-MSC secretome versus the placebo) remained an independent predictor of an improvement in functional disability, as demonstrated by a significantly greater reduction in HAQ-DI scores (adjusted β = −0.508, 95% CI −0.83 to −0.19; P = 0.003; adjusted R^2 = 0.352) (Figure 6A).

An earlier arithmetic-scale linear regression analysis of this dataset had reported an apparent independent association between UC-MSC secretome treatment and a greater reduction in circulating TNF-α concentrations after adjustment for potential confounders. On independent verification against the underlying patient-level data, this specific arithmetic-scale result could not be reproduced, and its previously reported magnitude and P value should not be relied upon. The log-scale ANCOVA reanalysis described in Section 2.8, which was independently verified against the raw dataset, found no significant between-group difference in TNF-α levels after an equivalent set of covariate adjustments (adjusted GMR 1.07, 95% CI 0.82–1.40; P = 0.612), together with confirmed statistical equivalence on prespecified TOST testing. This log-scale, equivalence-tested result is therefore reported as the verified estimate of the between-group TNF-α effect for this trial. The independent association between treatment allocation and functional improvement, reflected in the reduction in HAQ-DI scores reported above, is unaffected by this reanalysis. The results of the multivariable analyses therefore support the robustness of the clinical, but not the TNF-α biomarker,

findings after adjustment for clinically relevant covariates.

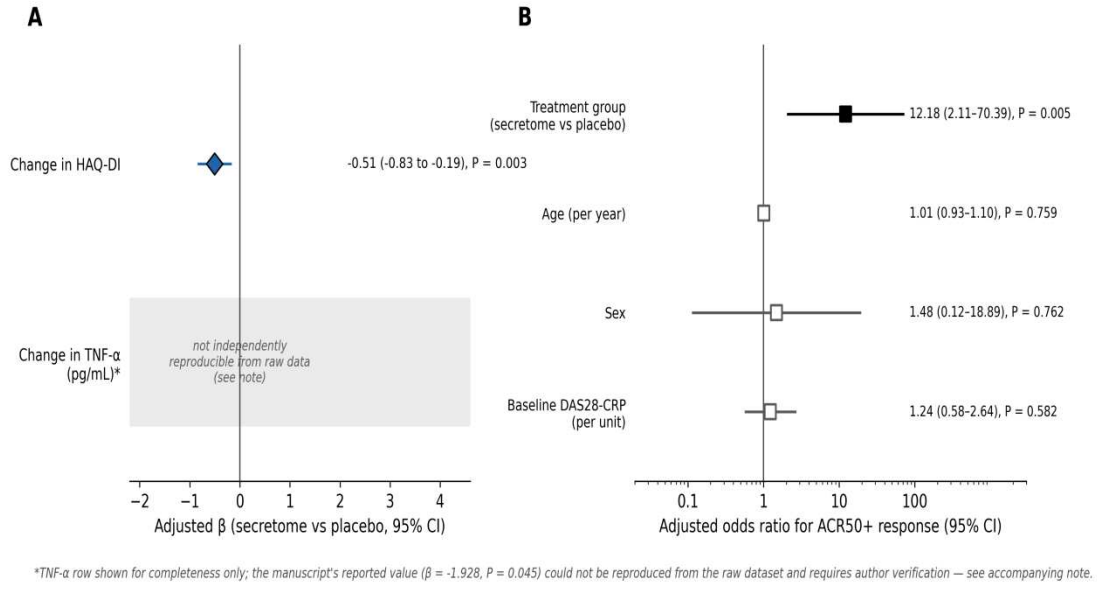


Figure 6. Multivariable regression analyses of predictors of clinical response following adjunctive UC-MSC secretome therapy. (A) Forest plot showing the adjusted regression coefficient (β) and 95% confidence interval (CI) from a multivariable linear regression model for the change in Health Assessment Questionnaire–Disability Index (HAQ-DI) score, adjusted for treatment group, age, sex, and the baseline HAQ-DI value (Figure 6A). (B) Forest plot showing adjusted odds ratios (ORs) and 95% CIs from a multivariable logistic regression model evaluating the achievement of an ACR50 response at week 7, with treatment group, age, sex, and baseline DAS28-CRP as covariates (Figure 6B). The diamond (A) and squares (B) represent point estimates, horizontal lines indicate 95% CIs, and the vertical solid line denotes the null effect (β = 0 or OR = 1). Exact P values are shown for each variable. An arithmetic-scale linear-regression estimate for the change in TNF-α reported in earlier analyses of this dataset is not plotted in panel A, as this specific result could not be independently reproduced from the underlying data (Section 3.6.2); the log-scale ANCOVA analysis (adjusted GMR 1.07, 95% CI 0.82–1.40; P = 0.612) is reported instead as the verified estimate of the treatment effect on TNF-α. UC-MSC, umbilical cord-derived mesenchymal stromal cell.

3.6.3 Predictors of a Clinical Response

A multivariable logistic regression analysis was performed using the achievement of a clinical response (ACR50 or higher) at week 7 as the dependent variable to identify independent predictors of a treatment response. The regression

model included the treatment allocation, age, sex, and baseline DAS28-CRP score as clinically relevant covariates. Treatment with the UC-MSC secretome emerged as the only independent predictor of a clinical response, increasing the likelihood of achieving an ACR50 or higher response by more than twelve-fold compared with the placebo (adjusted OR = 12.18, 95% CI 2.11–70.39; P = 0.005) (Figure 6B). In contrast, age (P = 0.759), sex (P = 0.762), and the baseline DAS28-CRP score (P = 0.582) were not significantly associated with the treatment response. After adjustment for age, sex, and baseline DAS28-CRP, treatment allocation remained significantly associated with achieving an ACR50 response or higher.

An additional effect size analysis was performed to facilitate the clinical interpretation, and the results showed that patients receiving the UC-MSC secretome were more than four times more likely to achieve an ACR50-or-higher clinical response than those receiving the placebo (risk ratio 4.33, 95% CI 1.45–12.91). The absolute risk difference was 50%, corresponding to a number needed to treat (NNT) of 2, indicating that the treatment of two patients with the UC-MSC secretome would result in one additional clinical responder compared with the placebo.

3.7 Safety and Tolerability

The safety outcomes are summarised in Table 8. Overall, 7 of 20 participants (35.0%) in the UC-MSC secretome group and 8 of 20 participants (40.0%) in the placebo group experienced at least one adverse event during the study period. All reported adverse events were mild, self-limiting, and resolved without specific medical intervention.

The most frequently reported adverse events in the UC-MSC secretome group were injection-site pain (n = 3), followed by injection-site swelling (n = 1), pruritus (n = 1), bruising/ecchymosis (n = 1), and headache (n = 1). In the placebo group, the most common adverse events included pruritus (n = 3), bruising/ecchymosis (n = 2), injection-site pain (n = 1), injection-site swelling (n = 1), and chills (n = 1).

No serious adverse events (SAEs), treatment discontinuation due to adverse events, hepatotoxicity, nephrotoxicity, opportunistic infections, hospitalisation, or

deaths were observed in either treatment group. A causality assessment was performed via clinical judgement by an independent rheumatologist, and all adverse events were considered unrelated or unlikely to be related to the investigational product. Intramuscular administration of the UC-MSC secretome displayed a favourable short-term safety profile, with adverse event frequencies comparable to those observed in the placebo group.

Table 8. Safety and Tolerability Outcomes

Outcome	UC-MSC Secretome (n = 20)	Placebo (n = 20)
Participants with ≥1 adverse event, n (%)	7 (35.0)	8 (40.0)
Serious adverse events	0	0
Discontinuations due to adverse events	0	0
Injection-site pain	3	1
Injection-site swelling	1	1
Pruritus	1	3
Bruising / ecchymosis	1	2
Chills	0	1
Headache	1	0
Hepatotoxicity	0	0
Nephrotoxicity	0	0

Data are presented as number of participants or number (%). All adverse events were classified as mild in severity and resolved without sequelae. No serious adverse events, treatment discontinuations, hospitalisations, or deaths occurred.

4. Discussion

4.1 Principal Findings

The present randomised, double-blind, placebo-controlled trial demonstrated that the intramuscular administration of the UC-MSC secretome in combination with csDMARDs resulted in a significant clinical improvement in patients with rheumatoid

arthritis at week 7. Compared with patients receiving the placebo, patients receiving the UC-MSC secretome achieved substantially higher clinical response rates, accompanied by greater reductions in pain intensity, functional disability, and disease activity. Importantly, the treatment was well tolerated, with no serious adverse events or treatment-related discontinuations observed throughout this study.

In addition to its clinical efficacy, this study evaluated the immunomodulatory effects of the UC-MSC secretome through serum TNF-α, IL-6, and TGF-β1 measurements. On reanalysis using log-transformed values with covariate adjustment (Section 3.5), none of these three biomarkers changed significantly, or differed significantly between groups, and equivalence testing confirmed that the TNF-α change was statistically equivalent between groups; the unadjusted, arithmetic-scale IL-6 comparison that had appeared significant did not survive adjustment for baseline covariates. Rather than demonstrating a mechanistically informative biomarker signal, these findings indicate that the clinical benefit observed with the UC-MSC secretome in this trial was not accompanied by a measurable, statistically robust change in the three circulating cytokines assessed. Because the secretome contains a broad repertoire of paracrine mediators, extracellular vesicles, growth factors, and immunomodulatory molecules, it remains biologically plausible that its clinical effects are mediated through pathways not captured by these three serum biomarkers, or through local synovial rather than systemic immune effects, but the present data neither confirm nor refute any specific mechanism. [19,20]

This study integrates randomised clinical evidence with immunological biomarker assessments and clinical effect size analyses, providing a comprehensive translational evaluation of intramuscular UC-MSC secretome therapy in patients with rheumatoid arthritis. Collectively, these findings support the clinical potential of the UC-MSC secretome as a safe adjunctive therapeutic strategy, while the absence of a robust systemic TNF-α, IL-6, or TGF-β1 signal indicates that the biological mechanism underlying this clinical benefit remains unresolved. Future studies incorporating pathway-specific biomarkers, including membrane-bound and soluble IL-6 receptor (sIL-6R), gp130 signalling, downstream intracellular mediators, and, ideally, synovial

tissue or local biomarker assessments, are needed to clarify the biological basis of these observations. [19,20]

4.2 Clinical Significance of UC-MSC Secretome Therapy

In this study, adjunctive UC-MSC secretome therapy was associated with consistent improvement across multiple clinical outcomes, including ACR response, pain, physical function, and disease activity. This pattern across several distinct parameters suggests that the clinical effect of the secretome is unlikely to be confined to a single dimension of RA, but may instead extend to both patient reported and physician-assessed aspects of the disease. These findings support the potential of the UC-MSC secretome as an immunomodulatory approach capable of delivering multidimensional clinical benefit when used as an adjunct in RA. Importantly, these clinical improvements were accompanied by an excellent short-term safety profile without serious adverse events or treatment discontinuation, supporting the feasibility of this cell-free therapeutic approach.

Our findings are consistent with previous clinical evidence pointing to the potential of MSC-based therapy in RA, notwithstanding differences in therapeutic platform, route of administration, and outcome parameters used. In one of the earlier studies in this area, Wang et al reported that intravenous UC-MSC administration, given alongside conventional DMARD therapy, reduced DAS28 and HAQ scores and increased the proportion of patients achieving ACR20, ACR50, and ACR70 responses compared with DMARD therapy alone. Furthermore, the reductions in CRP and rheumatoid factor levels together with the expansion of peripheral regulatory T cells suggested that MSC therapy induced sustained immunomodulatory effects beyond symptom improvement. [21]

Similarly, Álvaro-Gracia et al. reported that repeated intravenous administration of allogeneic adipose-derived MSCs (Cx611) was safe and showed encouraging clinical activity, with improvements in the DAS28-ESR score, EULAR response, and ACR20 response during the first three months after treatment. However, the clinical benefit gradually diminished during longer follow-up, particularly in patients with highly refractory disease, suggesting that repeated dosing strategies or

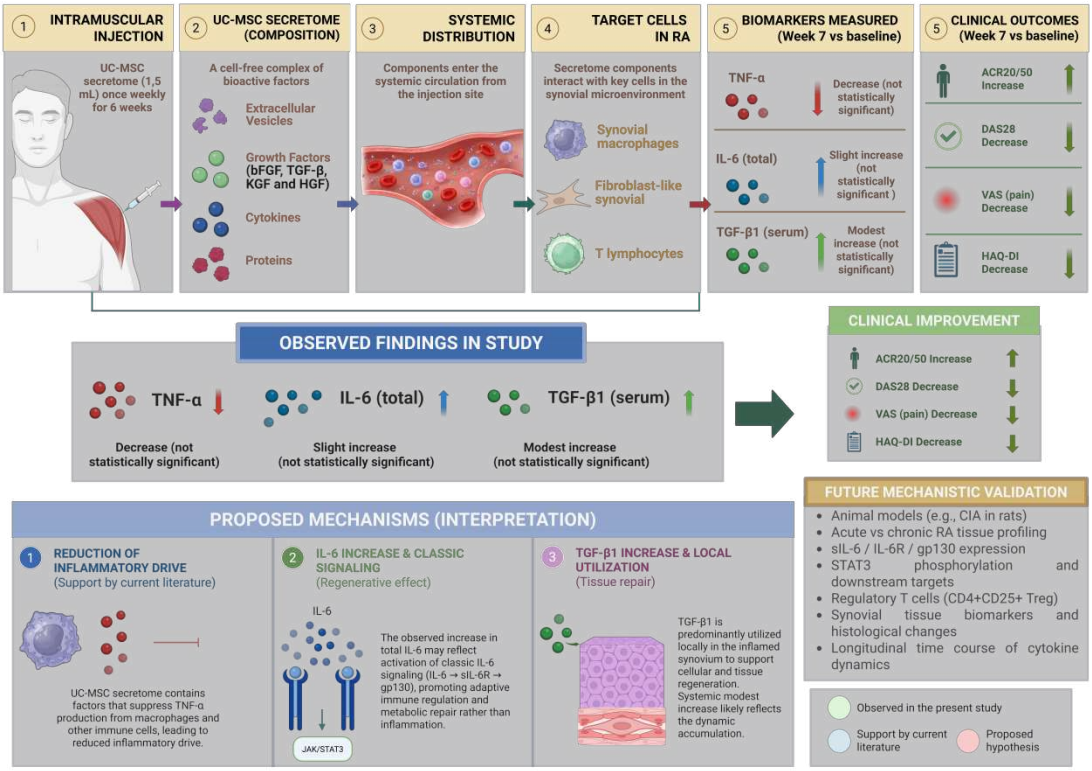
optimised patient selection may be required to maintain therapeutic efficacy. [22]

Another important study by Yang et al. revealed that approximately half of patients receiving intravenous UC-MSCs achieved a good or moderate EULAR response after treatment, accompanied by reductions in the DAS28 score, ESR, and CRP level in responders, although the changes in autoantibody levels were less pronounced. [31] These findings further support the concept that MSC-based therapy primarily modulates inflammatory disease activity rather than directly altering the levels of established autoimmune serological markers.

Comparisons with previous studies should account for differences in MSC source, dosing regimen, route of administration, and outcome definitions across trials.[23] Nonetheless, our results broadly mirror earlier clinical findings; consistent with Wang et al, we observed improvements in clinical response and disease activity despite the absence of biologic DMARD therapy. This study differs from previous MSC-based trials in two key respects. First, we used a cell-free secretome rather than viable MSCs, potentially avoiding limitations inherent to cell-based therapy such as uncertain engraftment, post-administration persistence, and unintended differentiation. Second, the secretome was administered intramuscularly, a simpler and less invasive route than the intravenous infusion used in most prior MSC trials. Third, in addition to conventional clinical outcomes, our study incorporated immunological biomarker assessments together with multivariable analyses and clinical effect size measures, including the risk ratio, absolute risk difference, and number needed to treat, providing a more comprehensive translational evaluation of treatment efficacy.

One of the most clinically relevant findings of the present study is the magnitude of the treatment benefit. Patients receiving the UC-MSC secretome were more than four times more likely to achieve an ACR50-or-higher clinical response than those receiving the placebo, with an absolute risk difference of 50% and a number needed to treat of only two patients. These effect size estimates indicate that the observed improvements were not only statistically significant but also clinically meaningful. Although the relatively small sample size and short follow-up period warrant a cautious interpretation, these findings compare favourably with those of

previously published MSC clinical trials and support further evaluation of the UC-MSC secretome in larger multicentre randomised studies.



Graphical abstract. Proposed mechanism of action and observed clinical and biomarker effects of adjunctive UC-MSC secretome therapy in rheumatoid arthritis. The UC-MSC secretome, a cell-free complex of bioactive factors (extracellular vesicles, growth factors including basic fibroblast growth factor, transforming growth factor-β, keratinocyte growth factor and hepatocyte growth factor, cytokines, and proteins), was administered by weekly intramuscular injection for 6 weeks. Following systemic distribution from the injection site, secretome components are proposed to interact with synovial macrophages, fibroblast-like synoviocytes, and T lymphocytes within the rheumatoid synovial microenvironment. At week 7 (one week after the sixth and final dose), a decrease in serum TNF-α (not statistically significant), a slight increase in serum interleukin-6 (IL-6; not statistically significant after adjustment), and a modest increase in serum transforming growth factor-β1 (TGF-β1; not statistically significant) were observed relative to baseline. These biomarker changes accompanied clinically meaningful improvement, including an increased proportion of participants achieving an ACR20/50 response and reductions in the disease activity score in 28 joints (DAS28), visual analogue scale (VAS) pain score, and Health Assessment Questionnaire–Disability Index (HAQ-DI) score. Three tentative, literature-based mechanisms are considered to explore these observations, none of which is established by the present biomarker data once log-transformation, covariate adjustment, and equivalence testing were applied (see Discussion, Reassessment of Cytokine Findings): (1) a possible contribution of TNF-α suppression, although the reanalysed between-group difference in TNF-α was not statistically significant and was confirmed to be statistically equivalent between groups; (2) a possible shift towards

classical IL-6 signalling (IL-6 → membrane-bound IL-6 receptor → gp130), as opposed to soluble-receptor-mediated trans-signalling, which may promote adaptive immune regulation and tissue repair rather than inflammation; and (3) local synovial utilisation of TGF-β1 supporting tissue regeneration, reflected in only a modest, non-significant rise within the systemic (serum) compartment. Elements labelled "observed in the present study" reflect findings reported in the Results; those labelled "support by current literature" and "proposed hypothesis" represent literature-based and speculative interpretive elements, respectively, none of which is confirmed by the present biomarker findings, and all of which require confirmation in dedicated mechanistic studies, including animal models, synovial tissue profiling, STAT3 phosphorylation assays, and regulatory T-cell quantification. UC-MSC, umbilical cord-derived mesenchymal stromal cell; RA, rheumatoid arthritis. Created in BioRender. Darwin, B. (2026) https://BioRender.com/3lmfwd3

4.3 Reassessment of Cytokine Findings and Biological Interpretation

Interpretation of the biomarker results requires consideration of the statistical approach applied. Following log-transformation and adjustment for age, sex, and baseline biomarker concentration, no significant between-group difference was observed for TNF-α, IL-6, or TGF-β1. For TNF-α, further equivalence testing confirmed that the between-group difference fell within the prespecified 1.5-fold equivalence margin. These findings indicate that the observed biomarker changes provide no evidence of a meaningful systemic difference between the two groups once confounding factors and baseline values were accounted for. This reanalysis supersedes the arithmetic-scale comparisons reported in earlier analyses of this dataset, in which TNF-α had appeared to be significantly suppressed; that specific arithmetic-scale result could not be independently reproduced from the underlying dataset and should not be relied upon, and the log-scale, equivalence-tested reanalysis is reported as the verified finding for this biomarker. General statistical considerations further support reliance on the log-scale approach.

Arithmetic-scale summary statistics are sensitive to distributional skew and to the influence of a small number of extreme values, both of which characterised the raw TNF-α and TGF-β1 measurements in this study; a substantial proportion of these measurements also clustered near the lower limit of quantification of the respective ELISA assays (Table 2). Under these conditions, means and mean differences on the raw concentration scale can be disproportionately driven by a few high-leverage

observations, whereas geometric means and log-scale contrasts are markedly less sensitive to this pattern and are the more appropriate and more conservative summary for this type of biomarker data. We therefore regard the log-scale, covariate-adjusted, equivalence-tested results as the more reliable characterisation of the biological effect of adjunctive UC-MSC secretome therapy on these three cytokines in this trial.

For TNF-α specifically, the reanalysis found no significant between-group difference either without adjustment (GMR 1.00, 95% CI 0.77–1.28) or after adjustment for baseline covariates (GMR 1.07, 95% CI 0.82–1.40), and formal equivalence testing confirmed that the two groups were statistically equivalent at both a 1.5-fold and a 2.0-fold margin. This finding does not support a TNF-α-suppression-centred mechanism of action for the UC-MSC secretome in this study, and the mechanistic discussion of macrophage polarisation and NF-κB-dependent signalling that had previously been proposed on the basis of the apparent TNF-α reduction should accordingly be regarded as unsupported by the present data.

For IL-6, an unadjusted between-group difference reached nominal statistical significance (GMR 2.05, 95% CI 1.02–4.10; P = 0.034), with a numerical increase in the UC-MSC secretome group; however, this difference was no longer significant after adjustment for age, sex, and baseline concentration (GMR 1.51, 95% CI 0.78–2.92; P = 0.216), equivalence testing was inconclusive at both prespecified margins, and none of the correlations between the change in IL-6 and the evaluated clinical outcomes remained significant after correction for multiple testing. Taken together, these results are compatible with, at most, a modest and imprecisely estimated treatment-associated change in circulating IL-6 that this trial was not adequately powered to characterise; a post hoc analysis indicated that a sample of approximately 37 participants per group would have been required to detect the observed effect with 80% power. Several non-mutually exclusive explanations could account for the direction of this signal. One biologically plausible possibility relates to the distinction between classical and trans-IL-6 signalling. In classical signalling, IL-6 binds to the membrane-bound IL-6 receptor (mIL-6R) expressed mainly on hepatocytes and selected leucocyte subsets, and the resulting IL-6/mIL-6R complex associates with gp130 to activate JAK/STAT3

signalling; this pathway has been associated with physiological immune regulation, tissue repair, and the restoration of homeostasis. In contrast, trans-signalling occurs when IL-6 binds to soluble IL-6 receptor (sIL-6R), enabling activation of gp130-expressing cells that do not otherwise respond to IL-6, a pathway more consistently linked to chronic synovial inflammation and joint destruction in rheumatoid arthritis [19,20]. It is therefore conceivable that the modest increase in circulating IL-6 observed after adjunctive UC-MSC secretome therapy could, in principle, reflect a shift favouring classical rather than trans-signalling. However, this trial measured only total circulating IL-6 and did not quantify membrane-bound or soluble IL-6 receptor, gp130, or downstream STAT3 phosphorylation, so the present data cannot distinguish between classical and trans-signalling, nor can they exclude alternative explanations such as pre-analytical or assay variability, or a chance nominal finding given the number of comparisons performed. We therefore consider the biological interpretation of this signal, including any hypothesis regarding classical versus trans-signalling, to be premature given the present evidence, and future studies with larger samples and pathway-specific biomarkers (membrane-bound and soluble IL-6 receptor, gp130, STAT3 phosphorylation) would be needed to evaluate it.

For TGF-β1, no significant between-group difference was observed either unadjusted (GMR 1.14, 95% CI 0.56–2.31) or adjusted (GMR 1.08, 95% CI 0.61–1.88), and equivalence testing was inconclusive at both margins. A post hoc power analysis indicated that more than 1,000 participants per group would be required to detect the observed effect size with 80% power, indicating that this trial was substantially underpowered to characterise a TGF-β1 effect of the magnitude observed; the present data are accordingly uninformative as to whether a true TGF-β1 effect exists.

The biomarker reanalysis did not provide sufficient evidence to link the observed clinical improvement to changes in the specific immunomodulatory pathways measured in this study. This does not diminish the clinical efficacy findings, as clinical outcomes were prespecified and analysed independently of the biomarker data. Rather, it suggests that the biological mechanism underlying the clinical benefit of the UC-MSC secretome is likely more complex than changes in TNF-α, IL-6, or TGF-β1 alone,

and warrants further investigation. We consider this an important, transparently reported limitation of the present study rather than a mechanistic conclusion, and recommend that future trials of the UC-MSC secretome incorporate serial biomarker sampling, assays with a quantification range matched to the expected concentrations, larger sample sizes powered specifically for the biomarker endpoints, and, where feasible, local (synovial) rather than only systemic biomarker assessment.

4.4 Clinical and Translational Implications

4.4.1 Clinical implications of intramuscular UC-MSC secretome therapy

The findings of this study have several clinical implications. Adjunctive intramuscular UC-MSC secretome therapy was associated with significant improvements in disease activity, pain, physical function, and ACR response while maintaining an acceptable safety profile, whereas biomarker analysis showed no significant differences between groups. These findings indicate improvements in several patient-centred clinical outcomes; however, the biological pathways underlying these clinical responses remain to be elucidated.

Current international recommendations emphasise a treat-to-target strategy, in which remission or low disease activity is achieved through timely adjustment of DMARDs. [25] Although conventional synthetic DMARDs remain the cornerstone of treatment, escalation to biologic or targeted synthetic DMARDs is not always accessible because of cost, infrastructure, and reimbursement constraints, underscoring the need for adjunctive strategies that improve outcomes without substantially increasing treatment-related toxicity. [1,27] Within this context, the present findings support the UC-MSC secretome as an adjunctive immunomodulatory therapy rather than a replacement for established DMARD treatment: all participants continued stable background csDMARD therapy throughout the study, and the observed improvements were achieved without any modification to standard care. [1,20]

As a cell-free product, the UC-MSC secretome offers several practical advantages over live-cell MSC therapies, including simpler manufacturing, storage, and product standardisation, and avoidance of biological risks associated with living cells,

such as uncertain engraftment or unintended differentiation. [8,19,20,26] Its administration by intramuscular injection is technically straightforward, minimally invasive, and readily integrated into routine outpatient rheumatology practice without the specialised infusion facilities required for intravenous cellular products, an advantage that may be particularly relevant in low- and middle-income countries, where access to advanced biologic therapies remains constrained by cost and infrastructure. [1,8,13,28] The product evaluated in this study was manufactured under standardised procedures with comprehensive quality control, including sterility, endotoxin, mycoplasma, and batch-consistency testing, directly addressing product heterogeneity as a recognised barrier to the clinical translation of MSC-derived therapeutics. [12,30]

Realising this potential will require further work on dose optimisation, treatment intervals, pharmacokinetic and pharmacodynamic characterisation, and harmonised regulatory and manufacturing standards, alongside the biomarker and mechanistic studies discussed above. [19,29,30] Larger, adequately powered multicentre trials with longer follow-up will ultimately be needed to confirm the durability of the clinical response, establish long-term safety, and define the place of the UC-MSC secretome within contemporary rheumatoid arthritis treatment algorithms, including its potential combination with biologic or targeted synthetic DMARDs. [30]

4.5 Strengths and Limitations of This Study

The present study has several important strengths that enhance both the scientific validity and translational relevance of its findings. To our knowledge, this study is among the first randomised, double-blind, placebo-controlled clinical trials to investigate the efficacy and safety of intramuscular UC-MSC secretome therapy as an adjunctive treatment for rheumatoid arthritis. Unlike many previous studies that focused primarily on either clinical outcomes or laboratory parameters, the present investigation integrated comprehensive clinical efficacy assessments, immunological biomarker evaluations, effect size analyses, multivariable regression modelling, and systematic safety monitoring within a single-study framework. This multidimensional approach provides a more comprehensive evaluation of treatment efficacy along with

biological insights that may facilitate future mechanistic investigations.

Another major strength lies in the translational design of this study. All participants continued stable background csDMARD therapy throughout the trial, allowing the effects of the UC-MSC secretome to be evaluated as an adjunctive immunomodulatory intervention rather than a substitute for established disease-modifying therapy. This design closely reflects real-world clinical practice and therefore increases the clinical applicability of the findings. Furthermore, the use of a standardised UC-MSC secretome product manufactured under controlled conditions, together with comprehensive quality control procedures, including sterility testing, endotoxin assessment, mycoplasma detection, protein characterisation, and batch consistency, increases the reproducibility of the intervention and supports its future clinical translation. The integration of clinical outcomes with immunological biomarker analyses, even though the reanalysed biomarker findings did not themselves reach statistical significance (Section 3.5, Section 4.3), reflects a comprehensive and transparent evaluation that strengthens the translational value of the present study.

Several limitations should nevertheless be considered when these findings are interpreted. First, this single-centre randomised controlled trial included a relatively small number of patients. Although statistically significant improvements were observed across multiple clinical outcomes, confirmation in larger multicentre studies is needed to establish the reproducibility and robustness of these findings. In addition, the active treatment period comprised only six weekly injections, with efficacy and safety assessed at a single post-treatment time point seven weeks after baseline; this short overall follow-up duration precluded assessments of long-term efficacy, the durability of the treatment response, optimal retreatment intervals, and late adverse events.

Another limitation relates to the external validity of the present study. All participants were recruited from a single tertiary referral centre in Indonesia; therefore, the study population may not fully represent the broader spectrum of patients with rheumatoid arthritis encountered in different geographical regions or health care systems. Variations in genetic background, ethnicity, environmental exposure, disease

characteristics, treatment accessibility, and clinical practice patterns may influence both the therapeutic response and the generalizability of the present findings. In addition, because eligibility was restricted to patients with a positive rheumatoid factor to ensure a biologically homogeneous seropositive cohort, these findings may not be generalizable to the substantial proportion of patients with seronegative rheumatoid arthritis, who can differ in underlying immunopathology and treatment response. Although the randomised double-blind study design substantially strengthened the internal validity of this trial, confirmation in multicentre studies involving larger and more ethnically diverse populations is essential before these findings can be confidently generalised to routine international clinical practice.

Additional limitations are related to the biological characterisation of the treatment response. The present study only evaluated circulating serum biomarker levels and therefore did not directly assess immunological changes within the synovial microenvironment, where the primary pathological processes of rheumatoid arthritis occur. Furthermore, circulating total IL-6 and total TGF-β1 levels were measured without distinguishing between biologically active and inactive fractions or evaluating downstream signalling pathways, including membrane-bound and soluble IL-6 receptor activation, gp130 signalling, STAT3 phosphorylation, SMAD activation, and regulatory T-cell responses. Consequently, the mechanistic interpretations proposed in this study should be regarded as biologically plausible hypotheses supported by the current scientific evidence rather than definitive mechanistic conclusions. Future studies integrating serial biomarker measurements, flow cytometry-based immune profiling, synovial tissue analysis, and multiomics technologies will be essential to validate the proposed immunological mechanisms.

Finally, the present trial was not designed to determine the optimal therapeutic dose, treatment interval, pharmacokinetic profile, pharmacodynamic characteristics, or cost-effectiveness of UC-MSC secretome therapy. Likewise, direct comparisons with biologic or targeted synthetic DMARDs were beyond the scope of the present study. Addressing these important clinical questions through adequately powered comparative effectiveness studies and long-term multicentre randomised trials will be essential

before the UC-MSC secretome can be considered for routine clinical implementation.

An important limitation concerns the immunological biomarker findings. As detailed in Section 4.3, a log-scale, covariate-adjusted reanalysis of the TNF-α, IL-6, and TGF-β1 data found no statistically significant between-group differences, in contrast to the significant TNF-α reduction reported in earlier arithmetic-scale analyses of this dataset; the sample size of 20 participants per group provided a post hoc power of only 5.0% and 6.6% to detect the observed TNF-α and TGF-β1 effect sizes, respectively, and 53.0% for the observed IL-6 effect size. This reanalysis, together with the ELISA assay characteristics summarised in Table 2, should be taken into account when interpreting the biological claims of this study; both are reported here in full for transparency rather than omitted.

A further limitation concerns the timing of trial registration. As noted in the Methods, registration at ClinicalTrials.gov occurred after enrolment had commenced and, in fact, after the trial had already been completed, reflecting a genuine, initial oversight regarding the requirement for prospective public registration of an investigator-initiated academic trial rather than a deliberate departure from reporting standards. Several factors mitigate the risk of reporting bias arising from this delay: no amendments were made to the trial protocol, prespecified clinical outcomes, or their planned statistical analyses after the trial commenced (the post-hoc revision of the analytical approach for one exploratory biomarker, described in Section 2.8, is unrelated to trial conduct or outcome selection); ethics committee approval was obtained before participant enrolment; the trial was funded by academic and institutional sources, and the secretome manufacturer had no role in the study design, conduct, analysis, or the decision to publish; and the full dataset and analysis code are made available upon reasonable request (Availability of data and materials), allowing independent verification of the reported findings.

Despite these limitations, the present study provides preliminary clinical evidence supporting the feasibility of intramuscular UC-MSC secretome therapy as a novel cell-free regenerative immunomodulatory approach for rheumatoid arthritis. The rigorous randomised double-blind placebo-controlled design, comprehensive

evaluation of clinical outcomes and immunological biomarkers, incorporation of effect size analyses, and biologically grounded mechanistic interpretation collectively represent meaningful strengths that distinguish this study from previous investigations. Taken together, these findings provide a solid scientific foundation for future multicentre clinical trials and mechanistic studies designed to define the role of the UC-MSC secretome in precision medicine strategies for rheumatoid arthritis.

5. Conclusions

In this randomised, double-blind, placebo-controlled trial, intramuscular administration of UC-MSC secretome as an adjunct to csDMARD therapy was associated with greater short-term improvements in several clinical outcomes among patients with rheumatoid arthritis compared with placebo. The UC-MSC secretome significantly increased ACR20 and ACR50 response rates and improved disease activity, pain intensity, and physical function, without increasing the incidence of serious adverse events. These effect estimates suggest that the observed between-group differences may be clinically relevant, particularly given the higher proportion of participants achieving ACR50 or higher responses. Overall, these results provide preliminary evidence of a short-term clinical benefit of adjunctive UC-MSC secretome therapy and support its further development as a potential cell-free therapeutic approach for RA. Confirmation in larger, adequately powered, multicentre trials with longer follow-up is warranted. The immunological biomarkers (TNF-α, IL-6, and TGF-β1) showed no statistically significant between-group differences, indicating that the clinical benefit was not accompanied by a measurable systemic biological effect within this short observation period. Because biomarker levels were assessed at a single time point seven weeks after baseline, it remains possible that a longer duration of treatment and follow-up would be required for a significant change in these biomarkers to become detectable. The underlying mechanism therefore remains to be elucidated and warrants further mechanistic studies with pathway-specific biomarker assessment over an extended follow-up period.

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Authors' contributions

Radiyati Umi Partan (RUP) conceived this study, developed the study methodology, supervised the research, and critically reviewed and revised the manuscript. Rudi Erwin Kurniawan (REK) contributed to the study design, conducted the clinical investigation, collected clinical data, performed the statistical analyses, drafted the original manuscript, and revised the manuscript. MR, SD, AR and LAL contributed to patient recruitment, clinical investigation, and clinical data collection. MHR, DHH, and NP performed laboratory investigations, biomarker analyses, and data validation. All the authors reviewed the manuscript, approved the final version, and agree to be accountable for all the aspects of the work.

Availability of data and materials

The datasets generated and/or analysed during the current study, together with the statistical code (Python scripts) used for the analyses, are available from the corresponding author upon reasonable request. The full trial protocol and statistical analysis plan are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The study protocol (title: "Effectiveness of Umbilical Cord Mesenchymal Stem Cell Secretome Addition on Clinical Conditions and Biomarkers in Rheumatoid Arthritis Patients") was approved on 5 February 2026 by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sriwijaya/Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia (Approval No. DP.04.03/D.XVIII.06.08/ETIK/038/2026). Written informed consent was obtained from all participants before enrolment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests. PT Bifarma Adiluhung supplied the UC-MSC secretome used in this study at a preferential research price but had no role in the study design, conduct of the study, data collection, data analysis, interpretation of the findings, manuscript preparation, or the decision to submit the manuscript for publication.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with the initial drafting and Claude (Anthropic) during subsequent revisions to improve language clarity, readability, and the overall flow of the manuscript. The use of these tools was limited to writing and language assistance. AI tools were not used to develop the study hypothesis, determine the study design, collect data, perform statistical

analyses, interpret the results, or draw scientific conclusions. All scientific content, data, analyses, interpretations, and conclusions were developed and verified by the authors. Following the use of these tools, the authors reviewed and edited the entire manuscript and take full responsibility for its content and scientific integrity.

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