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ORIGINAL RESEARCH

Neutrophils in Rheumatoid Arthritis Synovium: Implications on Disease Activity and Inflammation State

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Background: Rheumatoid arthritis (RA) is characterized by chronic synovial inflammation driven by immune cell infiltration. While neutrophils have traditionally been associated with acute inflammation, emerging evidence suggests their significant role in chronic RA synovitis. Synovial pathology reports from our center reveal lymphocyte-predominant infiltration in most RA cases, with synovial neutrophils (SNs) observed in only 30% of patients. This finding suggests that neutrophil involvement in RA pathogenesis is not universal but subtype-specific, potentially linked to distinct clinical phenotypes.

Methods: We performed a retrospective analysis of synovial pathology and clinical data from 55 RA patients collected during 2023. Using both Hematoxylin-Eosin (H&E) staining and single-cell RNA sequencing, we analyzed the synovial tissue samples. Based on neutrophil counts, patients were classified into two groups: neutrophil-absent (<10 neutrophils) and neutrophil-present (≥10 neutrophils).

Results: In this cohort of 55 RA patients, the synovial neutrophil (SN) group demonstrated significantly elevated disease activity markers, including Disease Activity Score in 28 joints based on C-reactive protein (DAS28-CRP), swollen joint count (SJC28), Visual Analog Scale (VAS) pain scores, and tender joint count (TJC28) ($p < 0.05$ for all parameters). Synovial inflammatory infiltration and neovascularization were markedly increased in the SNs group ($P < 0.05$). Patients with SNs maintained higher disease activity and showed poorer therapeutic responses despite treatment with methotrexate and targeted biologics (TNF inhibitors, IL-6 inhibitors, or JAK inhibitors). Analysis revealed a positive correlation between lymphocyte and neutrophil counts, while multivariate analysis identified DAS28-CRP, synovial inflammation, and CD3+/CD68+ cell counts as predictors of SN infiltration. Single-cell RNA sequencing confirmed their significant presence in synovial tissue, supporting neutrophils' role in refractory disease.

Conclusion: Elevated neutrophil presence in RA synovium correlates with heightened clinical disease activity and an exacerbated inflammatory state. These findings underscore the potential significance of SNs in the pathology of RA.

Keywords: rheumatoid arthritis, synovial neutrophils, synovial biopsy, disease activity, immune cell infiltration

Introduction

Rheumatoid arthritis (RA) is a chronic, debilitating inflammatory disorder characterized by persistent synovial inflammation that progressively destroys cartilage and bone, ultimately resulting in significant disability.[1] The core pathophysiological process in RA is synovitis, where immune cells infiltrate the synovial membrane and produce pro-inflammatory cytokines and mediators. Through sustained production of these inflammatory substances, chronic inflammation and joint damage ensue, leading to joint erosions, cartilage deterioration, and progressive functional decline in RA patients.[2]

The inflammatory process in RA synovitis is orchestrated by a dynamic interplay among immune cells, including T cells, B cells, macrophages, and dendritic cells.[3] These cells are recruited to the synovium through chemokine and cytokine signaling, where they produce additional pro-inflammatory mediators that perpetuate inflammation and joint destruction.

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Lymphocytes dominate the synovial infiltrate in RA patients. These cells drive and sustain the chronic inflammatory environment. Inside the inflamed synovium, macrophages act as crucial mediators. They release inflammatory cytokines and actively destroy tissue. Yet another key player, plasma cells, abundantly populate RA synovial tissue. These specialized cells produce disease-specific autoantibodies - notably RF and ACPA. Indeed, the presence of synovial lymphoplasmacytic cells serves as a vital diagnostic indicator. Their detection helps track disease progression from undifferentiated arthritis to RA, highlighting their clinical importance.[4]

In rheumatoid arthritis, synoviocyte detachment exhibits compelling correlations with multifaceted disease manifestations: markedly heightened disease activity, pronounced neovascular proliferation, and - in a striking paradox - diminished inflammatory cellular infiltration. Such revelations illuminate novel dimensions of RA's clinical presentation while offering profound insights into therapeutic response variability.[5]

In contemporary rheumatoid arthritis (RA) research, investigations into neutrophil behavior have predominantly centered on cells isolated from synovial fluid specimens. While these granulocytes were historically relegated to the role of acute inflammatory responders during pathogenic invasion, mounting evidence has revolutionized our understanding of their pivotal contribution to RA synovial pathophysiology. These remarkably versatile cells orchestrate multiple pathogenic mechanisms throughout the disease continuum, most notably through their sophisticated inflammatory mediator networks and the intricate architecture of neutrophil extracellular traps (NETs). This cellular machinery generates sustained oxidative stress through the coordinated release of reactive oxygen species (ROS) and proteolytic enzymes, culminating in irreversible tissue deterioration and progressive articular erosion.[6] Moreover, neutrophils demonstrate remarkable immunomodulatory capabilities through their sophisticated crosstalk with diverse immune cell populations, fundamentally shaping the trajectory and intensity of RA pathogenesis.[7]

Despite high RF and anti-CCP antibody titers typically supporting RA diagnosis under the 2010 ACR/EULAR classification criteria, synovial biopsy remains essential in several specific clinical scenarios. These important scenarios include cases where patients present with atypical clinical symptoms that do not align with serological findings, instances where patients demonstrate refractory disease despite standard and advanced therapies, or situations where unusual pathological features are observed during disease progression. In these cases, synovial biopsy provides direct and valuable insight into the inflammatory status of the synovium, which is crucial for both confirming the diagnosis and optimizing treatment strategies. This diagnostic tool maintains its significant value even in patients with high antibody titers, enabling a more comprehensive assessment of disease pathology and facilitating more precisely targeted therapeutic approaches.

Though neutrophils play a vital role in RA pathogenesis, their synovial presence remains understudied. Our observations uncovered a remarkable pattern: Synovial neutrophils (SNs) appear in only 30% of RA synovium cases, starkly contrasting with lymphoplasmacytic cell infiltration found in 95% of cases. This cellular distribution disparity drove our investigation into the unique features and clinical significance of RA patients showing SN infiltration. We performed a thorough retrospective analysis of RA synovial biopsies from 2023, comparing clinical and pathological characteristics between groups with and without SN presence.

Materials and Methods

PATIENTS and Study Design

We retrospectively analyzed clinical data from 55 RA patients who underwent synovial biopsy at the Rheumatology and Immunology Department of the First Affiliated Hospital of Nanchang University between January and December 2023. All patients fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA.[8] To ensure reliable histological assessment, only patients with biopsy samples larger than 3mm² and intact synovial tissue structure were included in the study.

Even in patients with high RF and anti-CCP antibody titers meeting the 2010 ACR/EULAR classification criteria for RA, synovial biopsy played a crucial diagnostic role in specific clinical scenarios. Primarily, biopsies were performed in patients presenting with atypical features, including: (1) predominant large joint involvement instead of the characteristic small joint pattern; (2) asymmetric joint involvement; (3) acute onset with systemic manifestations (significant weight loss, fever, or constitutional symptoms) rather than typical gradual onset; (4) unusual extra-articular manifestations; and

(5) clinical symptoms inconsistent with serological findings, despite positive RF and anti-CCP antibodies.[9] Additionally, synovial biopsy served as an essential diagnostic tool for refractory RA patients who demonstrated inadequate response to both conventional and biologic therapies.

During synovial biopsy, we gathered data across three key areas: patient demographics (gender, age, disease duration, and medication history), clinical evaluations, and lab tests. The laboratory analysis focused on RF, ACPA, ESR, and CRP markers. We documented these measurements thoroughly for every RA patient.

Synovial Tissue Acquisition and Histological Assessment

Synovial tissue specimens were harvested from inflamed joints utilizing a novel biopsy needle puncture technique (Figure 1). The physical components of the synovial biopsy device including the outer cannula, biopsy needle, puncture needle, and stylet are displayed in Figure 1A. The step-by-step procedure of the biopsy technique, including joint insertion, needle positioning, and tissue sampling under negative pressure, is illustrated in Figure 1B. The harvested synovial tissue was fixed in 4% paraformaldehyde buffered with PBS for 48 hours, followed by dehydration and embedding in paraffin. The paraffin tissue blocks were then sectioned into 3–4 μm thick slices, stained with Hematoxylin-Eosin (H&E), and observed under a light microscope to evaluate synovial structure and perform semi-quantitative scoring under a 20x high-power microscope.

The histopathological assessment encompassed four cardinal parameters of synovial pathophysiology: neovascularization, inflammatory cell infiltration, synovial lining hyperplasia, and stromal matrix activation. Evaluation was conducted utilizing a validated semi-quantitative scoring system with a gradation from 0 to 3. Specifically, neovascularization was quantified through enumeration of vascular structures, including capillaries, venules, and arterioles (0 = 0–3 vessels, 1 = 4–9 vessels, 2 = 10–15 vessels, 3 = ≥16 vessels). Stromal activation was evaluated through assessment of synovial stromal cellularity (0 = normocellular matrix, 1 = mild hypercellularity, 2 = moderate hypercellularity with occasional multinucleated cells, 3 = marked hypercellularity characterized by multinucleated giant cells, pannus formation, and potential rheumatoid granulomatous lesions). Synovial hyperplasia was characterized by quantification of lining cell stratification (0 = monostratified layer, 1 = 2–3 stratified layers, 2 = 4–5 stratified layers with potential multinucleated cellular elements, 3 = >5 stratified layers with potential ulcerative changes and multinucleated cellular components).[10] The inflammatory infiltration was determined through assessment of lymphocytic, macrophage, and plasma cell density (0 = 1–9 cells [absent infiltration], 1 = 10–99 cells [minimal infiltration], 2 = 100–999 cells [moderate infiltration], and 3 = exceeding 1000 cells [extensive infiltration]).[11]

Independent histopathological evaluation was performed by two expert reviewers, with any inter-observer scoring discrepancies resolved through collaborative re-examination to establish consensus. Rheumatoid arthritis (RA) subjects

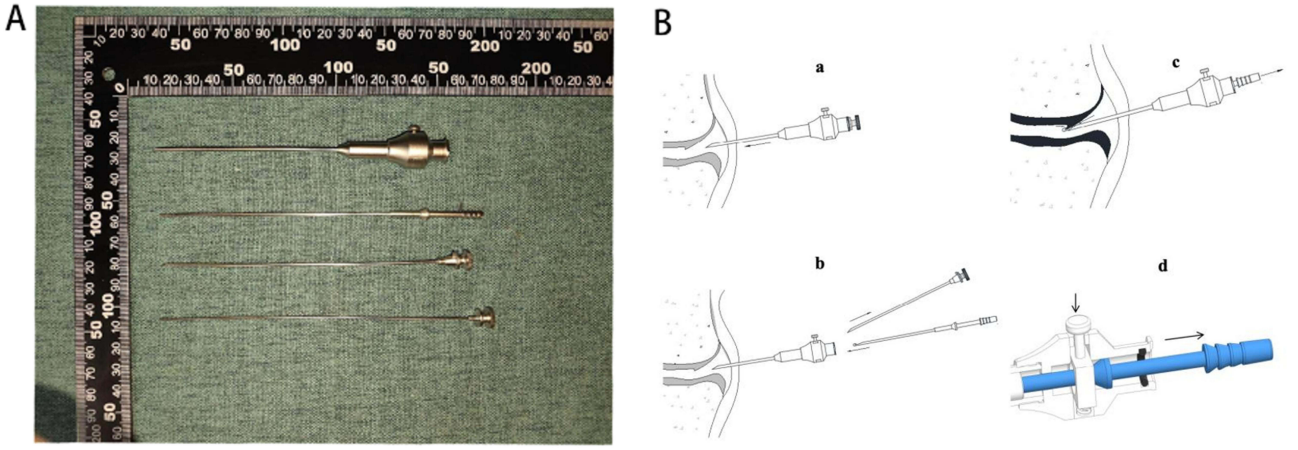

Figure 1 Synovial biopsy device and procedure. (**A**) Key components: outer cannula (1.7mm diameter, 50mm length), sampling needle, puncture needle, and stylet. (**B**) Procedural steps: insertion of combined cannula-puncture needle into joint cavity; removal of puncture needle followed by sampling needle insertion; activation of firing mechanism; connection of sampling needle to negative pressure system facilitating synovial tissue acquisition.

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were subsequently stratified into two distinct cohorts predicated on neutrophilic infiltration parameters: Non-infiltrated (0–9 neutrophils) versus Infiltrated (≥10 neutrophils).

The innovative synovial biopsy instrumentation comprises four integral components: an external biopsy cannula, a biopsy needle, a puncture needle, and a stylet. The sampling external cannula exhibits dimensions of 1.7 mm in diameter and 50 mm in length, with a sampling aperture extending 4 mm (patent registration: 202310572174.4). As depicted in Figure 1B, the biopsy procedural algorithm progresses sequentially: (a) the external biopsy cannula, integrated with the puncture needle, is advanced into the articular cavity; (b) following puncture needle extraction, the biopsy sampling needle is introduced; (c) the external biopsy cannula's triggering mechanism is primed for activation; (d) the proximal terminus of the biopsy sampling needle is connected to a negative pressure apparatus via conduit, thereby establishing intraluminal suction that facilitates synovial tissue acquisition through the fenestrated sampling port.

Clinical Activity Assessment

RA patients rated their pain using the Visual Analog Scale (VAS).[12] Pain intensity was scored from 0 to 100, with higher scores indicating greater pain. TJC28 represented the number of tender joints out of 28, while SJC28 represented the number of swollen joints out of 28. Experienced clinicians counted these based on the patient's clinical symptoms. Disease activity was assessed using the Disease Activity Score in 28 joints based on C-reactive protein (DAS28-CRP).[13] The formula for DAS28-CRP was as follows: DAS28-CRP = [0.56 * sqr(TJC)] + [0.28 * sqr(SJC)] + [0.36 * ln(CRP+1)] + (0.014 * VAS). Based on the DAS28-CRP scores, disease activity was categorized into four levels: clinical remission (DAS28-CRP < 2.6), mild disease activity (2.6 ≤ DAS28-CRP < 3.2), moderate disease activity (3.2 ≤ DAS28-CRP ≤ 5.1), and severe disease activity (DAS28-CRP > 5.1).

Single Cell RNA-Seq Library Construction and Sequencing

Synovial tissue underwent a systematic isolation and processing procedure. Initially, the tissue was washed with RPMI-1640 medium and minced into fragments (<1mm³). These fragments were enzymatically digested in a solution containing collagenase type I, collagenase type IV, and DNase I (RPMI-1640 supplemented with 5% FBS) at 37°C for 40–60 minutes with gentle agitation at 10-minute intervals.

Following digestion, the cell suspension was diluted with an equal volume of RPMI-1640 containing 5% FBS, filtered through a 40μm cell strainer, and centrifuged at 400g for 6 minutes. The pellet was treated with red blood cell lysis buffer on ice for 5 minutes, followed by two washing cycles in RPMI-1640 with 5% FBS. Isolated cells were resuspended in RPMI-1640 medium for viability assessment (fluorescence-based methods) and cell counting.

For single-cell RNA sequencing, individual synovial cells were manually isolated using a mouth pipette and lysed. Reverse transcription was performed with a 2.5 nt oligo(dT) primer containing an 8 nt cell-specific barcode and 8 nt unique molecular identifiers. First-strand and second-strand cDNA synthesis was followed by 16 cycles of amplification. Amplified cDNAs from single cells were pooled and further processed with biotinylated preindexed primers (4-cycle PCR) to tag the 3′ ends. Approximately 300 ng of cDNA was sheared to 300 bp using a Covaris S2 system (Covaris), and 3′ terminals were purified with Dynabeads MyOne Streptavidin C1 beads (Thermo Fisher Scientific).

Statistical Analysis

Statistical analyses were performed using SPSS 26.0 (IBM). Data normality was assessed using the Kolmogorov–Smirnov test. Normally distributed quantitative variables were expressed as mean ± standard deviation and analyzed with one-way ANOVA, while non-normally distributed quantitative variables were presented as median (quartiles) and evaluated using the Kruskal–Wallis test. Categorical variables, such as medication usage, were expressed as frequencies (percentages) and compared using chi-square or Fisher's exact test as appropriate. Bivariate Spearman correlation coefficients were calculated to assess relationships between clinical and synovial features, with results visualized in a correlation heatmap. The nonlinear association between lymphocytes and SNs was modeled using polynomial regression. To identify factors independently associated with SN presence in RA patients, both univariate and multivariate logistic regression analyses were conducted. The multivariate model included significant indicators from univariate analysis along with age and disease duration. Results were expressed as odds ratios (OR) with 95% confidence intervals (95% CI). Statistical significance was set at $p<0.05$.

Results

Demographic and Disease Characteristics of RA Patients in Two Groups

This study enrolled 55 RA patients with a mean age of 53.44 years. Analysis of synovial tissue revealed neutrophil infiltration in the synovial sublining layer in 17 patients (30.09%). Baseline characteristics of all patients are summarized in Table 1. Clinical assessment showed that patients with SNs demonstrated significantly higher VAS scores and DAS28-CRP values compared to those without SNs, indicating more severe disease activity.

Synovial Histopathology in RA with SNs

The pathological manifestations of synovial tissue in RA patients are illustrated in Figure 2, with a notable difference in inflammatory infiltration between the two groups. The x-axis represents the number of patients in each grade category. Specifically, RA patients in the group with SNs demonstrated significantly higher inflammatory infiltration scores compared to those without SNs.

The infiltration patterns of immune cells in the synovial tissue of RA patients are depicted in Figure 3. The y-axis represents the number of immunocompetent cells per 100 cells in synovial tissue. Analysis revealed that the group with SNs exhibited significantly higher levels of both lymphocyte and macrophage infiltration compared to the group without SNs.

Correlation Analysis of SNs with Clinical and Synovial Features

The correlations between clinical and synovial variables are visualized through a heatmap in Figure 4, where color intensity reflects both the strength and direction of relationships. Positive correlations are depicted in red, while negative correlations appear in blue. Analysis revealed significant positive correlations between SNs and multiple factors, including VAS, DAS28-CRP, synovial inflammatory infiltration, neovascularization, sublining lymphocytes, and macrophages. The synovial inflammatory infiltration demonstrated strong positive associations with disease activity indicators (VAS and DAS28-CRP), neovascularization and inflammatory cell populations (lymphocytes and macrophages), indicating that elevated synovial inflammation corresponds with heightened disease activity and inflammatory response.

Table 1 Clinical Characteristics of the RA Patients in Two Groups

Clinical Characteristics	Group with SNs (n=17)	Group without SNs (n=38)	*p*
Age, years	50.24±13.59	54.87±10.77	0.225
Duration, months	76.94±59.10	97.11±90.40	0.331
RF, IU/mL	172.46±239.28	243.47±408.27	0.434
ACPA, U/mL	249.67±287.88	137.24±244.89	0.144
ESR, mm/h	42.47±25.83	47.82±31.75	0.514
CRP, mg/L	36.82±32.74	38.13±37.49	0.896
DAS28-CRP	5.28±0.67	4.71±0.94	**0.029**
VAS	81.47±6.06	58.16±7.92	**<0.001**
Medications			
GC, mg	5.11±3.13	5.21±1.29	0.334
MTX, n (%)	12 (70.6%)	17 (44.7%)	0.061
JAKi, n (%)	3 (17.6%)	3 (7.9%)	0.494
TNFi, n (%)	6 (35.3%)	5 (13.2%)	0.670
IL-6i, n (%)	1 (5.9%)	3 (7.9%)	0.506

Notes: Bold type indicates that the p-value is less than 0.05, which is statistically significant.
Abbreviations: RA, rheumatoid arthritis; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; SJC28, Swollen joint count from 28 joints; TJC28, Tender joint count from 28 joints; VAS, Visual Analogue Scale for pain assessment; DAS28-CRP, Disease Activity Score in 28 joints based on C-reactive protein; MTX, methotrexate; JAKi, Janus kinase inhibitor; TNFi, tumor necrosis factor inhibitors; IL-6i, interleukin-6 inhibitor.

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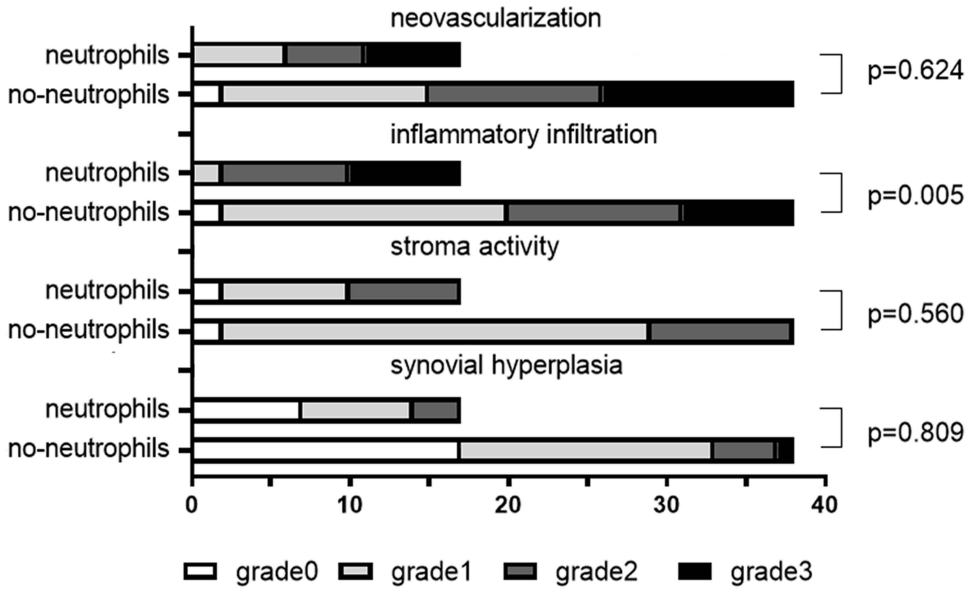

Figure 2 Synovial pathology scoring in RA patients. X-axis represents patient distribution across scoring grades. Four panels display neovascularization, inflammatory infiltration, stromal activity, and synovial hyperplasia (scored 0–3).

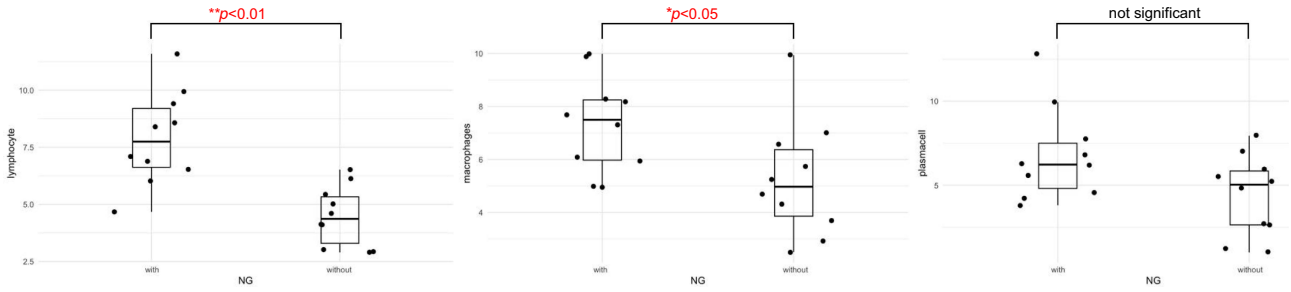

Figure 3 Comparison of synovial immune cell infiltration in RA. Box plots illustrate lymphocyte (p=0.007), macrophage (p=0.010), and plasma cell (p=0.219) counts between neutrophil-negative and neutrophil-positive groups.

Nonlinear Relationship Between Lymphocyte Count and Odds Ratio (OR) for Synovial Neutrophil Infiltration

Figure 5 demonstrates a significant nonlinear relationship between lymphocyte count and the odds of synovial neutrophil infiltration. Anchored at a reference lymphocyte count of 200, the analysis reveals that elevated lymphocyte counts are associated with progressively higher odds ratios. This trend is further illustrated by deviations from the reference point, which track how the odds of neutrophil infiltration increase with rising lymphocyte counts.

Logistic Regression Analysis on Significant Predictors for SNs

Indicators that demonstrated statistical significance in the univariate logistic regression analysis were subsequently incorporated into a multivariate logistic regression model, with adjustments for gender, age, and medications. As presented in Table 2, the analysis revealed that VAS, DAS28-CRP, inflammatory infiltration, and the counts of both lymphocytes and macrophages emerged as independent significant factors associated with the presence of SNs.

Synovial Tissue Single Cell RNA Sequencing

Single-cell RNA sequencing analysis revealed a predominant myeloid cell population in the synovial tissue of patients, characterized by substantial neutrophil and granulocyte presence, while T cells and B cells comprised smaller proportions

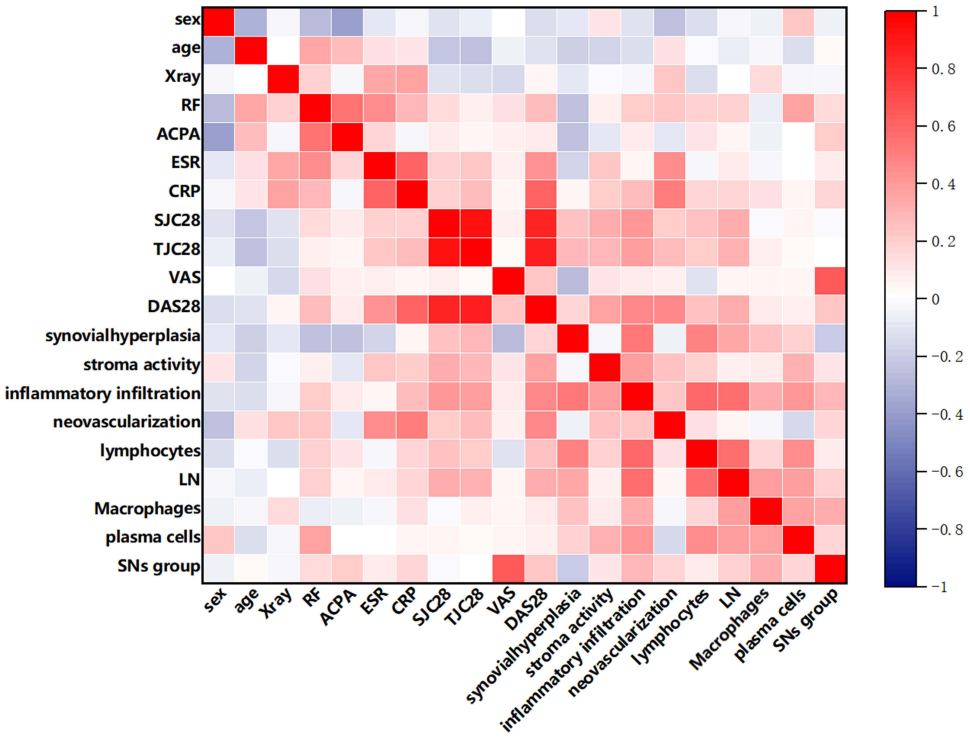

Figure 4 Correlation heatmap of clinical and synovial features in rheumatoid arthritis. Color intensity reflects both the strength and direction of relationships, with positive correlations depicted in red and negative correlations in blue. Analysis revealed significant positive correlations between synovial neutrophils (SNs) and multiple factors, including VAS pain scores, DAS28-CRP disease activity, synovial inflammatory infiltration, neovascularization, sublining lymphocytes, and macrophages. Synovial inflammatory infiltration demonstrated strong positive associations with disease activity indicators (VAS and DAS28-CRP), neovascularization, and inflammatory cell populations (lymphocytes and macrophages), indicating that elevated synovial inflammation corresponds with heightened disease activity and inflammatory response.

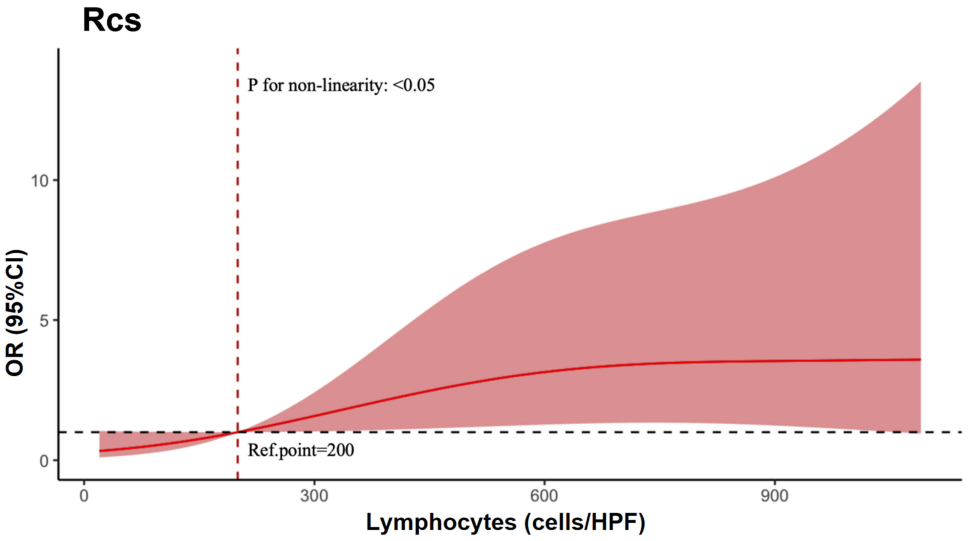

Figure 5 Relationship between lymphocyte count and neutrophil infiltration probability. Graph demonstrates nonlinear increase in neutrophil infiltration odds with rising lymphocyte counts (nonlinearity test p<0.05). Using 200 lymphocytes as reference point, counts exceeding 900 significantly increase neutrophil infiltration likelihood. Red line indicates mean odds ratio with pink area showing 95% confidence interval.

(Figure 6A). The identification of distinct synovial cell types was supported by characteristic marker gene expression patterns (Figure 6B), with their respective proportions quantified and presented in Figure 6C.

To elucidate neutrophil heterogeneity beyond basic cell enumeration, we conducted comprehensive transcriptional profiling of synovial tissue neutrophils. Uniform manifold approximation and projection (UMAP) visualization identified

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Table 2 Logistic Regression Analysis of Predictors for Non-SNs Vs SNs Groups

Characteristic	Univariable		Multivariable	
	OR (95% CI)	*p*	OR (95% CI)	*p*
VAS	1.511 (1.115–2.046)	0.008	1.542 (1.119–2.125)	0.008
DAS28-CRP	2.233 (1.055–4.724)	0.036	4.013 (1.522–10.585)	0.005
Inflammatory infiltration	2.867 (1.303–6.306)	0.009	4.007 (1.483–11.211)	0.006
Lymphocytes	3.603 (1.360–9.548)	0.010	4.273 (1.311–13.924)	0.016
Macrophages	2.170 (1.211–3.890)	0.009	2.409 (1.146–5.061)	0.020

two distinct neutrophil subpopulations: Neu_CXCR2 and Neu_IDO1 (Figure 7A). Transcriptional analysis revealed subpopulation-specific expression patterns of key marker genes, visualized through dot plots depicting both expression levels and the percentage of expressing cells (Figure 7B). Analysis of these neutrophil subsets within anti-citrullinated protein antibody-positive (Acpa-P) samples illuminated their relative distribution, providing insights into their functional states within the synovial microenvironment (Figure 7C).

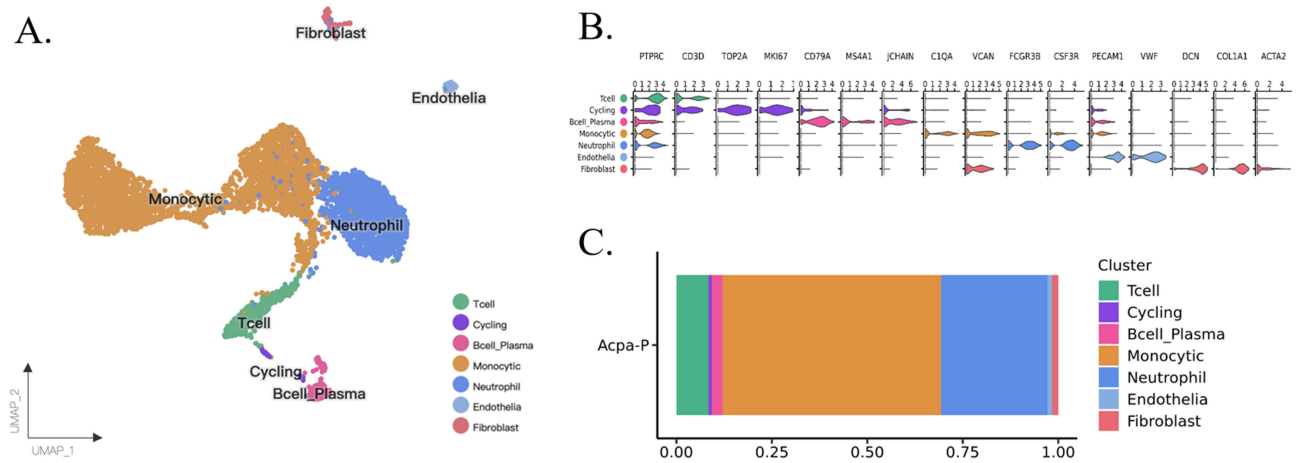

Figure 6 (**A**) The UMAP plot shows the cell types present in the tissue. (**B**) The violin plot displays the marker genes used for identifying these types. (**C**) The bar plot illustrates the proportion of cell numbers for each cell type in ACPA-positive samples.

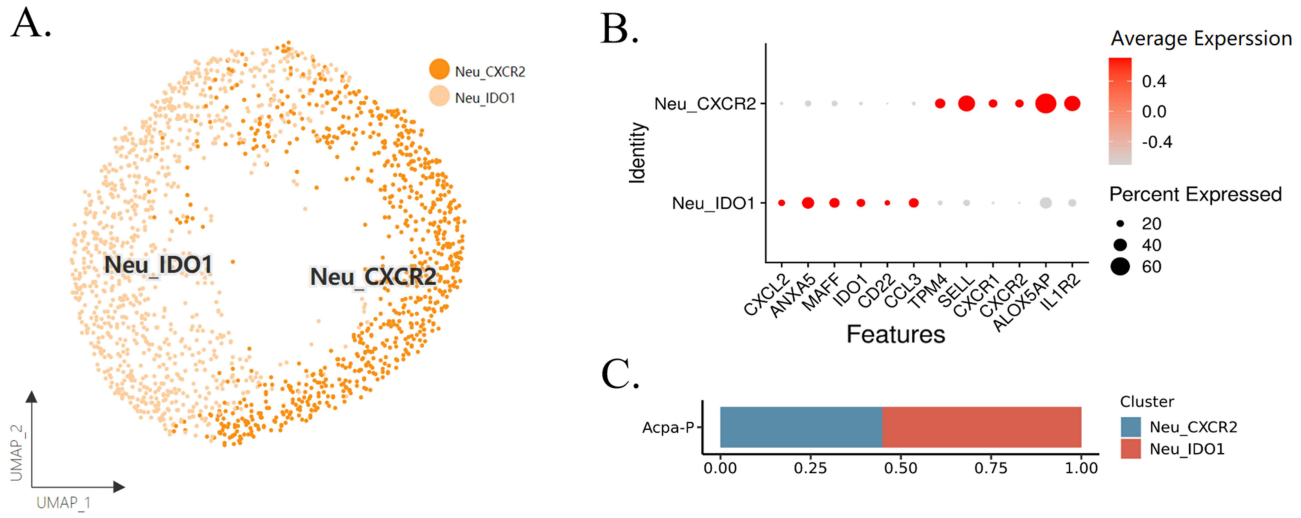

Figure 7 (**A**) The UMAP plot shows two neutrophil subpopulations (Neu_CXCR2 and Neu_IDO1) in the tissue. (**B**) The dot plot displays differential gene expression profiles characterizing these neutrophil populations. (**C**) The bar plot illustrates the proportion of Neu_CXCR2 and Neu_IDO1 in Acpa-P positive samples.

Discussion

In examining rheumatoid arthritis patients, we initially discovered neutrophils localizing within synovial sublining regions, which showed clear connections to disease progression and inflammatory cell presence. When we evaluated the patient cohort, we found that neutrophils had infiltrated the sublining tissue in roughly a quarter (27%) of the cases. Notably, these particular patients displayed markedly increased disease activity markers, coupled with substantial accumulation of lymphocytes and macrophages. To validate these clinical and pathological findings, we employed single-cell transcriptomic analysis, which confirmed our observations by identifying distinct neutrophil-specific transcriptional patterns.

The synovial membrane, a distinct mesenchymal tissue, consists of a superficial lining layer overlying a deeper sublining region. Within the superficial layer, MLSCs (macrophage-like synovial cells) and FLSCs (fibroblast-like synovial cells) form tightly organized structures spanning one to three cell layers. Through their active synthesis and secretion of synovial fluid components, these specialized cells play an essential role in preserving joint homeostasis and ensuring proper lubrication.[14,15]

The sublining layer, nestled beneath the superficial stratum, demonstrates remarkable complexity and cellular heterogeneity. This profound region contains diverse cellular components, encompassing resident fibroblasts, adipocytes, vascular endothelial cells, and a dynamic immune cell population including lymphocytes, macrophages, and dendritic cells. During rheumatoid arthritis (RA), the architectural framework and cellular makeup of the sublining layer experience substantial modifications. Immune cell infiltration into this region propels synovitis progression and bone erosion via pro-inflammatory cytokines and proteolytic enzymes.[14,16,17] Notably, the intensity of RA-associated synovitis correlates directly with both the concentration and variability of these infiltrating immune cells within the synovial environment.

Neutrophils dominate the synovial fluid composition. Yet the synovium presents a distinct cellular profile. Here, lymphoplasmacytic cells prevail. Our analysis confirms this pattern. Over 95% of RA synovial tissues show extensive lymphoplasmacytic infiltration. In contrast, neutrophil infiltration appears in merely 27% of cases. This cellular distribution pattern sets RA synovitis apart from SpA. In SpA, both neutrophils and macrophages show heightened presence.[18] The lymphoplasmacytic cell dominance points to adaptive immunity's central role. This aligns with RA's autoimmune character. These cells guide immune responses through autoantigen interaction. They also produce crucial autoantibodies. Both processes underpin RA pathophysiology. Neutrophils maintain significance despite comprising only 31% of infiltrating cells. This limited presence merits attention. Typically, neutrophils lead acute inflammatory responses. They rapidly deploy to infection or injury sites. Yet RA's chronic autoimmune setting alters this dynamic. Adaptive immune cells - T cells, B cells, and macrophages - modulate neutrophil function. The precise mechanics of this immune cell interplay demands further study.

Neutrophil recruitment represents a pivotal phase in inflammatory progression.[19] Within the spectrum of circulating leukocytes, neutrophils constitute the initial cellular population to infiltrate the synovial microenvironment. Their mobilization from hematopoietic reservoirs is orchestrated via the granulocyte colony-stimulating factor (G-CSF),[20] which facilitates neutrophil egress through modulation of the homeostatic balance between CXCR4 and CXCR2 chemokine ligand interactions.[21] The complex interplay of chemotactic factors and adhesion molecules mediates neutrophil activation and subsequent transendothelial migration into inflamed synovial tissue.[22] The transmigration process across the endothelial barrier may proceed via either intercellular junctional pathways (paracellular route) or directly through endothelial cellular bodies (transcellular route).

Within the inflammatory synovial milieu, immune complexes engage Fcγ receptors expressed on neutrophil membranes, thereby initiating degranulation cascades and promoting reactive oxygen species (ROS) generation.[23] These highly reactive oxygen intermediates possess the capacity to induce genomic instability through DNA damage, while simultaneously promoting oxidative modifications of cellular components including lipids, proteins, and lipoproteins, culminating in articular cartilage destruction and periarticular tissue deterioration. Historically, the predominant contribution of neutrophils to rheumatoid arthritis (RA) pathophysiology was attributed to their cytotoxic potential,[24,25] whereby they amplify inflammatory processes, facilitate cartilaginous matrix degradation, and promote osteoclast-mediated bone resorption through the elaboration of multiple effector molecules including ROS, reactive nitrogen species (RNS), collagenases, gelatinases, neutrophil myeloperoxidase (MPO), elastase, and cathepsin G.[26]

Our investigations demonstrate that RA patients exhibiting synovial neutrophil (SN) infiltration manifest significantly elevated DAS28-CRP scores (p=0.005) and demonstrate more pronounced synovial inflammatory cellular infiltration (p=0.006) compared with cohorts lacking synovial neutrophilic presence, thereby implicating neutrophils as central mediators in perpetuating inflammatory activity in RA pathogenesis. Of particular significance, we have further substantiated the presence of neutrophilic infiltration within the synovial microenvironment of RA patients through comprehensive single-cell RNA sequencing analysis, thus elucidating potential molecular therapeutic targets for subsequent precision-based therapeutic interventions in RA management.

Medication's impact on synovial pathology necessitates careful interpretation of findings. Patient treatment status during biopsy included MTX or biologics administration, potentially influencing synovial inflammatory cell distribution. Analysis demonstrated MTX utilization prevalence in the SNs group (70.6%) surpassing the non-SNs group (44.7%), approaching significance (p=0.061). This elevation in MTX administration correlates with heightened disease manifestation (DAS28-CRP 5.28±0.67 vs 4.71±0.94, p=0.029) and symptom severity (VAS 81.47±6.063 vs 58.16±7.920, p<0.001) within the SNs group. The increased prescription frequency aligns with established therapeutic protocols, advocating conventional synthetic disease-modifying antirheumatic drugs as primary intervention.

The conspicuous persistence of elevated disease activity in the SNs cohort, notwithstanding their exposure to intensified MTX intervention, indicates the potential identification of a therapeutically recalcitrant patient subpopulation. The emergence of this treatment-resistant phenomenon necessitates critical examination of current therapeutic paradigms and the prospective implementation of alternative interventional strategies. The heterogeneous therapeutic responsiveness across distinct patient populations potentially elucidates the observed variability in synovial neutrophilic infiltration, conceivably attributable to treatment-refractory inflammatory milieus or differential pharmacological tissue penetration and localized effects. The elucidation of these intricate pathophysiological dynamics mandates future investigations with systematic evaluation of medication chronologies, encompassing precise treatment-to-biopsy temporal relationships, therapeutic agent combinations, and intervention durations. Such methodologically rigorous analyses would yield invaluable insights into the interplay between therapeutic modalities and patterns of synovial neutrophilic infiltration.

Contemporary investigations have increasingly illuminated the intricate modulatory influence of synovial neutrophils (SNs) on immune cell functionality in rheumatoid arthritis (RA)-associated synovial inflammation. Our empirical observations reveal a robust positive association between SN prevalence and the quantification of both synovial lymphocytic and macrophage populations. The implementation of nonlinear modeling demonstrates a particularly striking phenomenon: lymphocyte population expansion correlates with an exponential amplification of SN impact. The scientific literature extensively documents neutrophilic orchestration of T-cell proliferation and macrophage activation in RA pathogenesis. This immunological interplay manifests through neutrophils' direct T-lymphocyte engagement via MHC class II molecule expression and co-stimulatory factor presentation, culminating in CD4+ T cell activation.[27] Within the RA synovial microenvironment, neutrophil-mediated presentation of citrullinated autoantigens through neutrophil extracellular trap (NET) formation potentially triggers direct T cell autoantigen recognition.[28] The neutrophilic secretion of inflammatory mediators - including IL-1β, TNF-α, and diverse chemokine populations - orchestrates macrophage recruitment and activation.[29] These molecular signals enhance both macrophage mobility and their pro-inflammatory capacity, subsequently triggering amplified cytokine and chemokine production in a self-perpetuating inflammatory cascade. Flow cytometric analysis of synovial tissue demonstrates that neutrophil abundance positively correlates with T lymphocyte and macrophage marker expression.[6] Furthermore, in vitro investigations reveal that neutrophil-T cell co-cultivation significantly augments both T cell proliferation and IFN-γ secretory capacity.[30] The integration of these research findings with our observations underscores the fundamental significance of SNs in lymphocyte and macrophage activation and proliferation, thereby advancing our understanding of RA pathogenesis.

Single-cell RNA sequencing analysis revealed significant heterogeneity within synovial neutrophils, identifying two functionally distinct subpopulations: Neu_CXCR2 and Neu_IDO1. The Neu_CXCR2 subpopulation characteristically expresses high levels of CXCR2, ALAS1, and RPF genes. CXCR2, as an IL-8 receptor, plays a crucial role in neutrophil migration to inflammatory sites, and its elevated expression may promote sustained neutrophil accumulation in synovial tissue. These cells likely participate primarily in chemotaxis and inflammatory amplification processes, closely associated with local tissue damage. In contrast, the Neu_IDO1 subpopulation expresses regulatory genes including IDO1, CCL22, and

CD83. IDO1 (indoleamine 2,3-dioxygenase) exerts immunosuppressive effects through catalyzing tryptophan metabolism, suggesting this neutrophil subset may possess immunomodulatory functions. In ACPA-positive patient samples, the Neu_IDO1 subpopulation accounts for approximately 60% while Neu_CXCR2 represents 40%, potentially reflecting dynamic processes balancing pro-inflammatory and anti-inflammatory responses within the immune system. The functional divergence between these subpopulations may partially explain the dual role of neutrophils in RA pathogenesis and individual variations in treatment response. The CXCR2-high expressing subpopulation may correlate with disease activity and more severe inflammatory phenotypes, while the IDO1-expressing subpopulation may participate in inflammation regulation. These findings provide theoretical foundations for targeted therapies directed at specific neutrophil subpopulations, such as modulating CXCR2 signaling pathways to inhibit inflammatory neutrophil recruitment or enhancing IDO1-mediated immunomodulatory effects. Future research should further explore the dynamic changes of these subpopulations across different disease stages and their interaction networks with other immune cells in the synovium.

Our findings demonstrated markedly elevated joint pain VAS scores ($p<0.001$) among RA patients with synovial neutrophils (SNs), thus establishing neutrophils as key contributors to RA-associated pain. In the inflammatory cascade, neutrophils infiltrate affected joints and subsequently unleash an array of inflammatory mediators - myeloperoxidase (MPO), matrix metalloproteinases (MMPs), hypochlorous acid, superoxide anions, and prostaglandin E2 - which together orchestrate the activation of nociceptive neurons and heighten pain sensitization. Notably, MMPs systematically break down extracellular matrix components, thereby intensifying joint deterioration and nociceptive sensitivity, while MPO-derived reactive oxygen species (ROS) dramatically amplify oxidative stress and nociception.[2] Meanwhile, prostaglandin E2 acts as a direct pain signal amplifier by targeting nociceptive nerve endings. This continuous release of mediators perpetuates an unrelenting cycle of tissue destruction and pain intensification, ultimately fostering chronic pain conditions in RA.[31] These compelling insights suggest an innovative therapeutic approach: intercepting neutrophil migration or mediator release could potentially mitigate RA-related pain through simultaneous suppression of inflammatory and nociceptive pathways, offering renewed hope for enhanced pain management in patients with high synovial neutrophil infiltration.

Recent studies have highlighted the promising potential of targeting neutrophil-mediated inflammation in RA treatment. Our findings demonstrating the significant correlation between SNs and disease severity provide additional support for this therapeutic direction. The positive associations between SNs and both lymphocyte and macrophage infiltration suggest that targeting neutrophils could potentially modulate both innate and adaptive immune responses in RA synovitis. Future research should focus on developing targeted therapies that specifically modulate neutrophil function in the synovial environment.

Conclusion

In summary, this investigation reveals new understanding of neutrophil migration into rheumatoid arthritis (RA) synovial tissue, establishing its correlation with increased disease severity and joint discomfort. The complex interplay between synovial neutrophils and additional immune cell populations indicates that neutrophils potentially function as central regulators of synovial inflammatory processes in RA. These observations suggest the therapeutic potential of interventions targeting neutrophil activity in RA management. Nevertheless, several constraints of our investigation, including limited participant numbers and single-institution data collection, warrant additional confirmation through expanded multicenter cohort analyses and comprehensive mechanistic studies at the molecular level. Subsequent research initiatives should additionally investigate the development of therapeutic approaches focusing on neutrophil-driven inflammatory pathways in RA.

Data Sharing Statement

The data are available from the corresponding author on reasonable request.

Ethical Statement

This study was approved by the Ethical Review Board of the First Affiliated Hospital of Nanchang University, with the ethics approval number IIT[2023] Clinical Ethics Review No. 011. It was conducted in accordance with the Declaration of Helsinki (as revised in 2013), and written informed consent was obtained from all participants.

Consent for Publication

The patient provided consent for publication of the images.

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Author Contributions

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

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Disclosure

The authors declare no competing interests.

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