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Rheumatoid arthritis

Clinical application of synovial biopsy in noninflammatory and persistent inflammatory refractory rheumatoid arthritis

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ABSTRACT

Objectives: We aimed to characterise the synovial pathology of refractory rheumatoid arthritis (RA) by comparing two hypothesised clinical phenotypes—persistent inflammatory refractory RA (PIRRA) and noninflammatory refractory RA (NIRRA)—against nonrefractory RA (NORRA).

Methods: We conducted a prospective, observational cohort study at two academic rheumatology centres. Adult patients with established RA and active disease (Clinical Disease Activity Index >10) underwent ultrasound-guided synovial tissue biopsies in accordance with European Alliance of Associations for Rheumatology–Outcome Measures in Rheumatology guidelines. Based on the Physician Global Assessment (>2/10) and C-reactive protein (>5 mg/L) thresholds, biological and targeted synthetic disease-modifying antirheumatic drugs (b/tsDMARDs)-inadequate responders patients were classified as PIRRA or NIRRA, whereas b/tsDMARD-naïve were designated NORRA. Histopathological assessments included the Krenn Synovitis Score (KSS) and immunohistochemistry pathotype evaluation (lympho-myeloid, diffuse-myeloid, pauci-immune/fibroid).

Results: Of the 93 biopsied patients, 43 were PIRRA, 21 NIRRA, and 29 NORRA. NIRRA had lower KSS ($P = .012$), lymphoid aggregates ($P < .001$) and predominantly pauci-immune/fibroid pathotype (n = 21/43, 47.6%), whereas PIRRA displayed more lympho- and diffuse-myeloid pathotypes, coupled with higher inflammatory markers and ultrasound-power Doppler scores. The relative risk of having pauci-immune fibroid synovitis for NIRRA was 1.6 (95% CI 1.2 to 2.9, $P = .006$), indicating a statistically significant increase in risk compared to PIRRA. Despite having similar overall disease activity scores, NIRRA patients reported significantly greater pain ($P = .005$) and higher opioid use ($P = .015$) than PIRRA, and worse health-related quality of life than PIRRA or NORRA, underscoring noninflammatory mechanisms.

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Conclusions: Our findings demonstrate distinct synovial and clinical phenotypes in refractory RA. Although PIRRA appears driven by active synovial inflammation and immune cell infiltration, NIRRA involves predominantly pauci-immune/fibroid histology with significant noninflammatory contributors to disease burden.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Refractory rheumatoid arthritis (RA) encompasses a heterogeneous group of patients who have persistent disease activity despite multiple treatments.
- Within refractory RA, noninflammatory drivers (eg, chronic pain syndromes, comorbidities) can mimic or overlap with persistent inflammatory disease, complicating treatment decisions.
- Synovial tissue biopsy studies in RA have identified 3 main pathotypes—lymphomyeloid, diffuse myeloid, and fibroid/pauci-immune—but their specific role in clinically refractory RA phenotypes has remained unclear.

WHAT THIS STUDY ADDS

- Noninflammatory refractory RA (NIRRA) and persistent inflammatory refractory RA (PIRRA) exhibit distinct histopathological signatures in synovial tissue. NIRRA is predominantly associated with the fibroid/pauci-immune pathotype and low synovitis scores, whereas PIRRA often has higher-grade synovitis with lympho-myeloid/diffuse-myeloid features.
- Despite comparable clinical disease activity scores, NIRRA patients display more pain, higher opioid usage, and worse health-related quality of life indices (eg, Short Form-36) in the setting of limited immune-inflammatory changes.
- Patients with PIRRA demonstrate prominent inflammatory markers (elevated C-reactive protein, erythrocyte sedimentation rate) and heavier immune cells infiltration, highlighting a fundamentally different disease mechanism compared to NIRRA.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Clinicians may need to integrate both inflammatory and noninflammatory factors (including fibromyalgia, depression, pain sensitisation) when classifying 'refractory' RA.
- Incorporating synovial biopsy into routine practice could help distinguish between inflammatory and noninflammatory drivers of disease, guiding more targeted therapies (eg, advanced immunosuppression for PIRRA vs a focus on pain-modulating treatments and other nonimmunosuppressive strategies for NIRRA).
- In research and policy, these findings highlight the necessity of phenotyping refractory RA more precisely, which may improve trial design and resource allocation by sparing patients with predominantly noninflammatory mechanisms from additional, potentially unnecessary immunosuppressive drugs.

INTRODUCTION

The introduction of advanced treatments, such as biological and targeted synthetic disease-modifying antirheumatic drugs (b/tsDMARDs), has ushered in a novel approach to treating the 'refractory' subset of rheumatoid arthritis (RA) [1]. Refractory RA describes an ill-defined paradox in which patients have immunological mechanisms that confer failure to multiple disease-modifying antirheumatic drugs (DMARDs). Persistent synovial hyperplasia, substantial immune cell infiltration, and increased vascularity are histological hallmarks of refractory RA [2]. With the increasing use of ultrasound-guided synovial tissue biopsy (UGSTB) in clinics [3], the molecular landscape underlying refractory RA has been gradually uncovered, allowing for the possibility of aiming for a tailored approach based on the molecular pathways involved in the disease's pathogenesis. However, a connection between molecular pathways within the synovium and clinical clues of refractoriness has not been proved. To date, in fact, very little is known about the association between the main synovial pathotypes (lymphomyeloid, diffuse myeloid, and pauci-immune fibroid) and clinical phenotypes [4].

Several noninflammatory factors contribute to refractory RA, namely comorbidities, pain syndromes, and psychosocial factors [5–8]. In theory, there are 2 primary clinical subsets of refractory RA: PIRRA, which stands for persistent inflammatory refractory RA, and NIRRA, which stands for noninflammatory refractory RA [9]. Many clinicians generally agree that PIRRA is defined by persistent inflammation even after treatment, whereas NIRRA is characterised by the absence of inflammation with paradoxical persistence of disease activity. It is worth noting that the concepts of absence of inflammation discriminating between NIRRA and PIRRA also depend on the subjectivity of the physician because clinicians inevitably integrate multiple clinical clues when judging whether residual disease activity reflects persistent synovitis or noninflammatory pain. Therefore, the 2 groups could be classified using a physician-attributed measure of disease activity, like the Physician Global Assessment (PhGA) scale, and an objective indicator of inflammation, like C-reactive protein (CRP). To date, though, the distinction between NIRRA and PIRRA is theoretical only. Moreover, the association between PIRRA and NIRRA with underlying synovial pathology remains unclear.

We hypothesised that clinically defined PIRRA and NIRRA represent biologically distinct entities. Specifically, we anticipated that NIRRA would correlate with lower Krenn Synovitis Scores (KSS) and enrichment of the pauci-immune/fibroid pathotype, whereas PIRRA would display higher-grade synovitis with lympho- or diffuse-myeloid architecture. To test this, we prospectively compared synovial histology across PIRRA, NIRRA, and nonrefractory RA in a biopsy-based cohort.

METHODS

Study design and setting

MATRIX-D2T is an observational, prospective, longitudinal cohort study conducted at 2 academic rheumatology units in Italy (University Hospital of Padova and University Hospital of Ferrara). The study was approved by 2 territorial ethics committees (CET-ACEV 6029/AO/24; 698/2020/Sper/AOUFe) in compliance with the principles of the Declaration of Helsinki as revised in 2000. The study enrolled patients with established RA who underwent synovial biopsy as part of routine clinical practice between January 1, 2020, and November 15, 2024. At this stage, only baseline data are reported. The reporting of the study follows the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.

Inclusion and exclusion criteria

The following inclusion criteria applied: age ≥18 years old, diagnosis of RA according to 2010 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) classification criteria, moderate or high disease activity (Clinical Disease Activity Index [CDAI] > 10), inadequate response to at least 1 conventional synthetic DMARD (csDMARD). To guard against recruiting patients with true remission plus nociplastic pain, we also mandated clinical and ultrasound (grade ≥1) synovitis of at least 1 joint for study entry. The following exclusion criteria applied: unwillingness to undergo UGSTB, overlap connective tissue diseases except for secondary Sjogren's syndrome (ie, systemic lupus erythematosus, systemic sclerosis, and dermatomyositis), suspected or confirmed infectious or crystal arthritis, current glucocorticoid dose exceeding 10 mg/day of prednisone-equivalent, intra-articular corticosteroid injection within the previous 6 weeks, and use of warfarin. The study flow chart is illustrated in Figure 1.

Study definitions

In light of the absence of universally accepted definitions for NIRRA and PIRRA, the definitions of NIRRA and PIRRA were formulated through a consensus between 2 principal investigators (AG and ES). When MATRIX-D2T was conceived, the formal EULAR definition of D2T-RA had not yet been issued and there was no consensus cut-off for multijoint power Doppler (PD) activity in refractory RA. We therefore adopted the most widely accepted operational marker of refractoriness at that time—failure of at least 1 b/tsDMARD—as used in several earlier biopsy-driven studies, including the stratification of biological therapies for rheumatoid arthritis by pathobiology (STRAP) trials and the Pathobiology of early/established (PEAC/PEsAC) cohort studies. Drug discontinuation solely due to intolerance, in the absence of objective or clinical evidence of persistent disease activity, did not meet the failure criterion. Therefore, the definition was based on 3 criteria: failure of at least 1 b/tsDMARD, a standard threshold for CRP (>5 mg/L), due to stronger associations with synovitis and radiographic progression than DAS28 [10], and the PhGA. Our pragmatic definition of refractoriness required failure of ≥1 b/tsDMARD in the presence of persistent inflammation or pain after csDMARD optimisation. While the EULAR criteria for DT2-RA recommend ≥2 failed b/tsDMARDs with different modes of action, we deliberately chose a lower threshold because, in real-world practice, uncontrolled disease after the first b/tsDMARD already represents a clinically relevant therapeutic impasse. Nonetheless, we acknowledge that some experts might label such cases as nonresponse rather than true refractoriness; our findings should therefore be interpreted within this spectrum. The threshold for PhGA was derived from latent class analysis of 255 patients fulfilling the EULAR definition of D2T-RA in the study by Novella-Navarro et al. [11] The upper quartile for PhGA at 24 months for class 2, which was most prominent in non-DT2-RA patients, was selected as the cut-off deemed to discriminate between NIRRA and PIRRA as a provisional, literature-based threshold, under the assumption that NIRRA would compare to non-D2T RA more than PIRRA. Eventually, we defined NIRRA as PhGA ≤20/100 mm or CRP ≤5 mg/L and PIRRA as PhGA >20/100 mm and CRP >5 mg/L; patients who were b/tsDMARD-naïve, irrespective of CRP or PhGA, were designated as nonrefractory RA (NORRA; Fig 2) and served as a low-inflammation benchmark.

In this study, the term "sex" refers to biological attributes (male/female) as recorded in patients' medical records. The term "gender" is not used, as gender identity was not assessed.

Study procedures

All study procedures and collected data are described thoroughly in the Supplementary Material. Briefly, clinical examination for tender (Tender Joint Count (28 joints)) and swollen (Swollen Joint Count (28 joints)) joint count in 28 joints was performed by independent joint assessors (FF, KK, and MC) to ensure consistency and minimise observer bias. Musculoskeletal ultrasound (MSUS) of clinically swollen joints was performed by 2 rheumatologists trained in MSUS (MS and CG). The examination was graded for synovial hypertrophy (SH) (0-3) and PD (0-3) according to Outcome Measures in Rheumatology consensus [12]. Each patient underwent a UGSTB by 2 trained rheumatologists (AG and ES) following the EULAR and European Synovitis Study Group consensus [10,13]. The procedure is proven to be efficacious and well-tolerated [14,15]. The UGSTB was performed on the most active joint identified based on the highest SH and PD scores; as recommended, larger joints were preferentially sampled to optimise tissue yield and quality whenever feasible [16], and a minimum of 6 to 8 tissue fragments were obtained from each joint [17].

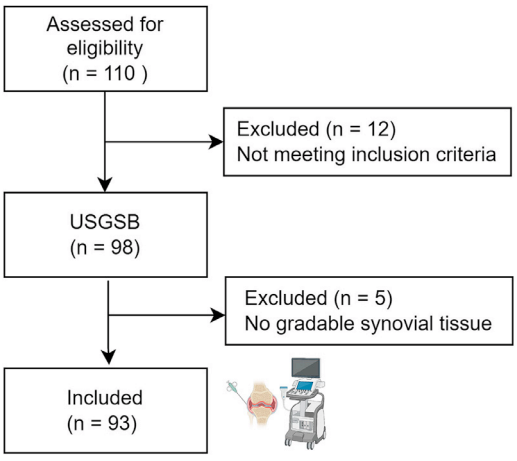

Figure 1. Study flow chart. USGSB, ultrasound-guided synovial biopsy.

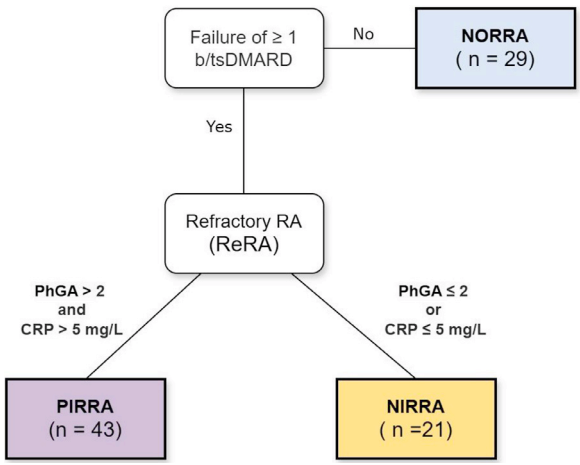

Figure 2. Proposed stratification in PIRRA and NIRRA used to classify patients in the present study. b/tsDMARD, biological and targeted synthetic disease-modifying antirheumatic drug; CRP, C-reactive protein; NIRRA, noninflammatory refractory rheumatoid arthritis; NORRA, nonrefractory rheumatoid arthritis; PhGA, Physician Global Assessment; PIRRA, persistent inflammatory refractory rheumatoid arthritis.

Synovitis score and synovial pathotypes

Analysis of synovial tissue (ST) is described in detail in the Supplementary Material. Briefly, all ST specimens were examined by 3 trained and expert pathologists (AA, GL, and MF) who were blinded to the patients' clinical and immunologic characteristics. Synovitis severity was assessed using the KSS [18], which evaluates 3 key features of the synovial tissue—namely, the synovial lining cell layer, stromal cell density, and inflammatory infiltrates—each graded on a scale from 0 (none) to 3 (strong). The values of the parameters were summed and interpreted as follows: a score of 0 to 1, no synovitis; 2 to 4, low−grade synovitis; and 5 to 9, high−grade synovitis. ST samples obtained from patients were processed for pathotype assessment using immunohistochemistry (IHC) analysis. All IHC markers were used to stratify lympho-myeloid, diffuse-myeloid, and pauci-immune fibroid histological patterns (pathotype) according to previously published histological features [19]: lympho-myeloid is characterised by the presence of grades 2 to 3 CD20 + aggregates, CD20 ≥2, and/or CD138 ≥2; diffuse-myeloid is characterised by CD68 SL ≥2, CD20 ≤1 and/or CD3 ≥1, and CD138 ≤2; pauci-immune-fibroid is characterised by the CD68 SL <2 and CD3, CD20, and CD138 <1.

Statistical analysis

Data were first tested for their normality graphically and with the Kolmogorov−Smirnov test. Continuous variables were presented as mean ± SD for parametric data and median with interquartile range for nonparametric data. Parametric data were compared using the independent samples Student's t-test and the Mann−Whitney U test used for nonparametric data. Categorical variables were presented using numbers and percentages. Associations between categorical variables were tested using the chi-square test, and Fisher's exact test was used when cells count less than 5 was expected. Comparisons between NIRRA, PIRRA, and NORRA were assessed through the Brown-Forsythe and Welch analysis of variance, with corrections made using Dunnett's T3 multiple comparisons tests. Statistical significance was set at a P value of .05. All statistical analyses were conducted using IBM SPSS version 26.0.

Missing data

All variables analysed had less than 10% of missing data and thus were considered with no need for imputation, and listwise deletion was used.

Patients and public involvement

Patients were not involved in the design, conduct, reporting, or dissemination of this study. The study was investigator-led and developed based on scientific and clinical considerations without direct patient input. However, patient-reported outcome measures (PROMs) were systematically collected to assess the patient's perspective on disease impact and treatment burden.

RESULTS

Clinical characteristics of the study population

A total of 98 patients (Padova n = 72, Ferrara n = 26) met the inclusion criteria and underwent UGSTB. The UGSTB procedure was safe and well-tolerated, with only 6% of participants experiencing adverse events (mild bleeding (n = 3), mild swelling (n = 2), and flushing), all of which resolved without intervention. The most frequently biopsied joints were the knees (80%), wrists (20%), and metacarpophalangeal joints (<10%). After excluding 5 patients due to inadequate sampling (ie, lack of gradable synovial tissue), 93 patients were ultimately included in the study (Fig 2). Of these, we classified 43 as PIRRA, 21 as NIRRA, and the remaining (n = 29) as nonrefractory RA (NORRA). In total 29 of the 64 refractory participants (45 %) fulfilled the full EULAR D2T definition.

Patients enrolled in Ferrara had a significantly longer disease duration than those in Padova (mean (SD) 15.8 (9.8) vs 7.9 (7.4) years, P < .001). After adjusting for the study centre, Patient Global Assessment (PtGA), and PhGA, disease duration did not predict KSS or the pauci-immune fibroid pathotype (Supplementary Table S1). Therefore, both centres enrolled patients with long-standing RA, and the disparity in disease duration did not impact the results. Otherwise, there were no significant differences in demographic characteristics, MSUS findings, or the distribution of PIRRA, NIRRA, and NORRA between the 2 enrolling centres; therefore, data were pooled for analysis.

Comparative analysis showed that NIRRA, PIRRA, and NORRA patients did not differ significantly in terms of sex distribution, age, and extra-articular manifestations (Table 1). As expected, NORRA patients exhibited significantly shorter disease duration and fewer radiographic erosions. The PIRRA group had a higher representation of seropositive patients. With regard to medications, current use and dose of oral glucocorticoids (GCs), current use of csDMARDs, and nonsteroidal anti-inflammatory drugs were similar between groups; however, NIRRA patients significantly used opioids more often than PIRRA and NIRRA. The number of failed b/tsDMARD classes was similar between NIRRA and PIRRA, with similar and substantial proportions of patients fulfilling the EULAR D2T-RA definition. By protocol, NORRA were all b/tsDMARD-naïve. One half of the NORRA group (58%) had received only a single csDMARD—usually methotrexate or hydroxychloroquine—whereas a further one-third had tried 2 agents (21%) and the remaining (21%) 3 or more. Two patients out of 29 were administered methotrexate and hydroxychloroquine as a combination of csDMARDs (Supplementary Table S2).

NIRRA and PIRRA differentiate into distinct pathotypes

The most common pathotype of NIRRA was pauci-immune fibroid, which contributed to 47.6% of all cases (n = 10/21). It was followed by diffuse myeloid (n = 6/21, 28.6%) and lymphomyeloid (n = 5/21, 23.8%). In PIRRA, diffuse myeloid (n = 14/43, 33%) and lymphomyeloid (n = 23/43, 53%) were the most prevalent pathotypes, with only a minority of patients presenting with the pauci-immune fibroid pathotype (n = 6/43, 13.9%) (Fig 3A). The relative risk (RR) of having pauci-immune fibroid synovitis for NIRRA was 1.6 (95% CI 1.2 to 2.7, P = .006; Fig 3B), indicating a statistically significant increase in risk compared to PIRRA. We have repeated the main analysis in D2T-RA patients only (PIRRA, n = 19/43; NIRRA, n = 10/21; Fig 3B), finding an even stronger association (RR = 2.0, 95% CI 1.1 to 4.9, P = .045).

NIRRA also had low-grade synovitis and scarce plasmacellular infiltrate. The mean (SD) synovial inflammatory score in NIRRA was 1.7 (1.0), comparable to NORRA (1.3 (0.6); P = .235), whereas it was significantly higher in PIRRA (2 (1.1); P = .015), indicating a 33% relative increase. Scores for SH and stromal density were comparable between groups.

Table 1
Clinical characteristics of the study population (n = 93)

n (%) or median (25th, 95th percentile)	PIRRA N = 43	NIRRA N = 21	NORRA N = 29	PIRRA vs NIRRA	PIRRA vs NORRA	NIRRA vs NORRA
Female sex	77.1	77.3	63	0.396		
Age, y	61 (44.8, 68)	59 (54, 68)	60 (46, 65)	0.848		
Disease duration, y	10.5 (5, 16.5)	10 (5.5, 16)	3 (1, 5)	0.985	0.002	0.024
Erosions	66.7	47.8	12		<0.001	
RF or ACPA positive	80.6	65.0	60.0		0.007	
Extra-articular manifestations	20.8	26.1	22.2		0.949	
Current GCs	52.1	52.4	26.9		0.100	
PDN, mg daily	5 (5, 8.8)	5 (4.4, 5)	5 (3.8, 6.3)		0.158	
NSAID	37.5	52.4	38.5		0.517	
Opioid	8.3	23.8	0		0.014	
csDMARDs, n	2 (1, 3)	2 (1, 3)	1 (1, 1)	0.999	0.002	0.141
Current csDMARD	52.1	57.1	34.6		0.229	
b/tsDMARDs	1 (1, 3)	1 (1, 3)	0	0.944	<0.001	<0.01
- TNFi	82.5	77.8				
- Abatacept	22.9	21.7				
- Anti-IL6	22.9	21.7				
- Rituximab	8.3	17.4				
- JAKis	31.3	39.1				
D2T RA (≥2 classes)	41.7	43.5	0		<0.001	

ACPA, Anti-citrullinated protein antibodies; ANOVA, analysis of variance; b/tsDMARDs, biologic or targeted synthetic disease-modifying antirheumatic drugs; D2T RA, difficult-to-treat rheumatoid arthritis; GC, glucocorticoid; IL6, interleukin-6; JAKis, Janus kinase inhibitors; NIRRA, noninflammatory refractory RA; NORRA, nonrefractory rheumatoid arthritis; NSAID, nonsteroidal anti-inflammatory drug; PDN, prednisone; PIRRA, persistent inflammatory refractory rheumatoid arthritis; RF, rheumatoid factor; TNFi, tumour necrosis factor inhibitor.
P values refer to chi-square or Fisher test for categorical variables and ANOVA with Dunnett's T3 multiple comparisons tests for continuous variables.

The median (IQR) KSS of NIRRA (4; IQR: 2, 6) was significantly lower than that of PIRRA (6; IQR: 3, 8), $P = .012$; Fig 4A). Compared to PIRRA, NIRRA exhibited significantly fewer lymphoid aggregates ($P < .001$; Fig 4B) and fewer plasma-cellular ($CD138^+$) infiltrates (Fig 4C). In addition, macrophages ($CD68^+$), lymphocytes ($CD3^+$), and B cells ($CD20^+$) markers showed numerically lower values for NIRRA (Fig 4C), reflecting lower inflammatory scores. The key histopathological contrasts between PIRRA and NIRRA—higher KSS, number of aggregates—were reproduced almost verbatim within the EULAR-defined difficult-to-treat subset (Supplementary Table S3).

Physical function and comorbidities across the refractory RA continuum

NIRRA patients had the worst physical function among all patients' subgroups. In the majority of the Short Form (SF)-36 domains (5/8), NIRRA patients scored the lowest, and their physical functioning scores were significantly worse than those of NORRA ($P = .041$; Fig 5).

Although there was no significant difference in Health Assessment Questionnaire Disability Index (HAQ-DI) scores between NIRRA and PIRRA, both were significantly higher than NORRA ($P = .019$ and $P = .002$, respectively).

Fibromyalgia (17.4 % in NIRRA vs 6.3 % in PIRRA) and depression (17.4 % vs 8.3 %) were numerically more frequent in NIRRA; however, neither difference reached statistical significance (both $P > .05$). Body mass index, obesity, and comorbidities of NIRRA patients were the same as those of PIRRA patients.

Differences in disease activity across PIRRA, NIRRA, and NORRA

In comparison to PIRRA, NIRRA exhibited a significantly reduced number of swollen joints, increased pain, and morning stiffness scores while maintaining comparable overall disease activity scores (Table 2). CRP and erythrocyte sedimentation rate (ESR) were also significantly elevated in PIRRA compared to NIRRA or NORRA, whereas there were no significant differences in the levels of inflammatory markers between NIRRA and NORRA.

MSUS differences across PIRRA, NIRRA, and NORRA

The gradings of PD, grey scale (GS) and the combined scores of the target joint and global joint assessment in the 3 groups are illustrated in Figure 6. To confirm whether the chosen joint

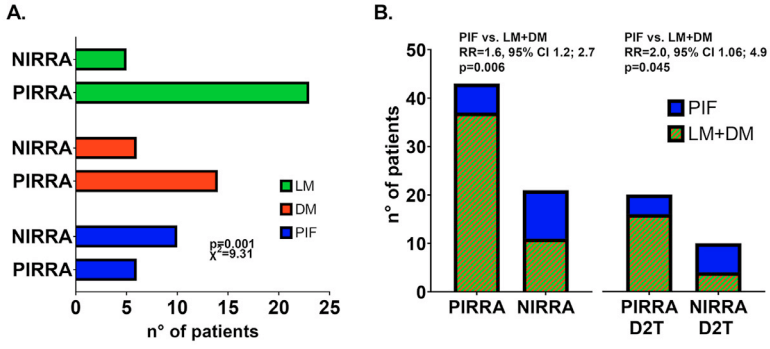

Figure 3. The pauci-immune fibroid pathotype is more abundant in NIRRA patients. (A, B) Pauci-immune was the most prevalent pathotype in NIRRA, accounting for 47.6% of all cases. In PIRRA, diffuse myeloid (33%) and lympho-myeloid (53%) were the most prevalent pathotypes. (C) Pauci-immune fibroid was the most prevalent pathotype in NIRRA-D2T. D2T, difficult-to-treat; DM, diffuse-myeloid; LM, lympho-myeloid; NIRRA, noninflammatory refractory rheumatoid arthritis; NORRA, nonrefractory rheumatoid arthritis; PIF, pauci-immune fibroid; PIRRA, persistent inflammatory refractory rheumatoid arthritis; RR, relative risk.

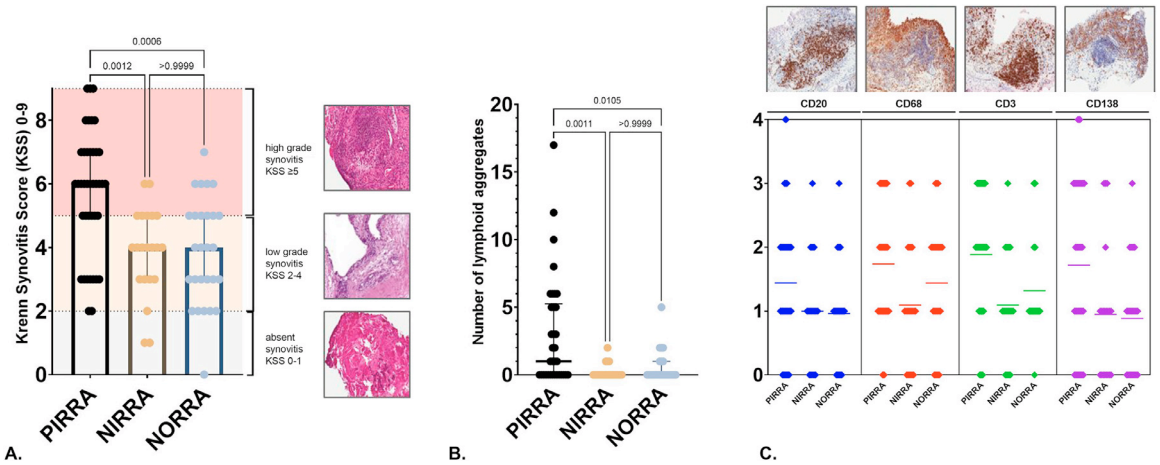

Figure 4. NIRRA is associated with low-grade, pauci-immune synovitis. (A) The KSS of NIRRA was significantly lower than that of PIRRA. (B) Compared to PIRRA, NIRRA exhibited significantly fewer lymphoid aggregates and fewer CD138 infiltrates. (C) All IHC markers showed lower values for NIRRA. IHC, immunohistochemistry; KSS, Krenn Synovitis Score; NIRRA, noninflammatory refractory rheumatoid arthritis; NORRA, nonrefractory rheumatoid arthritis; PIRRA, persistent inflammatory refractory rheumatoid arthritis.

was representative of each patient's overall inflammatory burden, we correlated its target-joint Global OMERACT-EULAR Synovitis Score (GLOESS) with the patient-level sum of GS scores, PD scores and combined scores (GLOESS) from all examined joints in a post hoc sensitivity analysis. The Spearman correlation coefficient was 0.548 ($P < .001$), 0.548 ($P < .001$) and 0.699 ($P < .001$), respectively, indicating that the index joint signal tracked well with the global ultrasound burden.

At the target joint level (illustrated in Fig 6A), the chi-squared test revealed a significant association between MSUS score and refractoriness ($\chi^2(4) = 18.78$, $P < .001$; Cramér's V = 0.32). Post hoc analysis of standardised residuals indicated

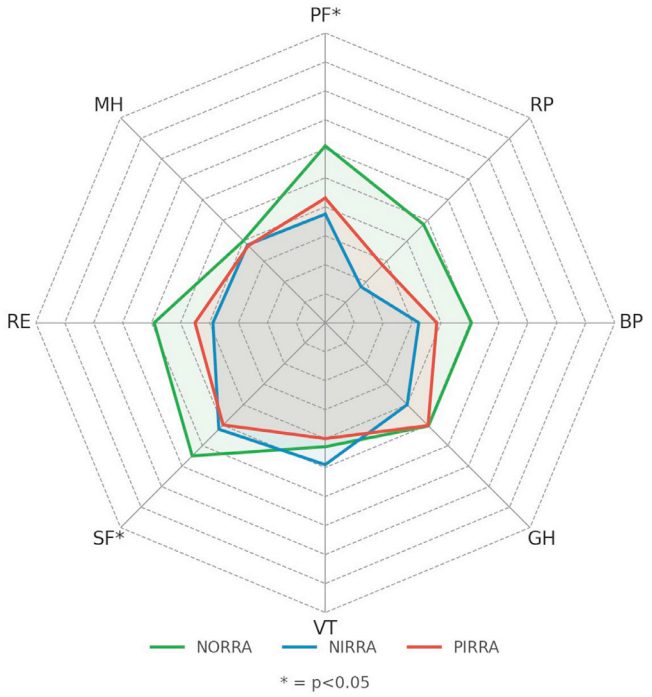

Figure 5. Physical and social function of NIRRA, PIRRA and NORRA patients, according to SF-36 in 60 out of 93 patients. * $P<0.05$ for between groups comparisons. BP, Bodily pain; GH, general health; MH, mental health; NIRRA, noninflammatory refractory rheumatoid arthritis; NORRA, nonrefractory rheumatoid arthritis; PF, physical functioning; PIRRA, persistent inflammatory refractory rheumatoid arthritis; RE, role emotional; RP, role physical; SF, social functioning; VT, vitality.

that PIRRA with grade 3 synovitis (R = 2.19, $P < .05$) and NORRA with grade 1 synovitis (R = 2.17, $P < .05$) were significantly overrepresented, whereas NORRA (R = −2.12, $P < .05$) grade 3 synovitis was significantly under-represented compared to expected frequencies. In contrast, the distribution of NIRRA MSUS scores were uniform across all subgroups, indicating that there was no clinically significant correlation between NIRRA and the MSUS grade of synovitis.

At the global joint level (Fig 6B), mean (SD) GLOESS was significantly higher in PIRRA (8.3 (4.5)) compared to NIRRA (5.2 (2.8), $P = .020$) and compared to NORRA (1.5 (0.8), $P < .001$), with statistical significance also for single components GS ($P = .017$) and PD ($P = .099$). However, NIRRA and NORRA had comparable GLOESS scores ($P = .542$), as well as PD ($P > .999$) and GS ($P = .2870$) scores.

The area under the receiver operating characteristic curve to predict a pauci-immune fibroid pathotype was 0.39 (95% CI 0.18 to 0.48) for PD alone of the target joint and 0.40 (0.21 to 0.59) for the global PD score, below the performance of the CRP plus PhGA criterion (0.66, 95% CI 0.54 to 0.78).

Sensitivity analyses

In order to verify the primary findings of this investigation, we implemented numerous sensitivity analyses.

First, we repeated the primary analyses after reclassifying patients according to having PD ≥ 1 in any swollen joint as PIRRA and PD = 0 as NIRRA. Of 64 refractory patients, 43 out of 43 PIRRA met the PD-based PIRRA definition, and 9 out of 21 NIRRA met the PD-based NIRRA definition. The synovial findings replicated those obtained with the clinical CRP/PhGA criteria: the pauci-immune/fibroid pathotype remained enriched in NIRRA (60% vs 18.5%), whereas lympho-/diffuse-myeloid patterns predominated in PIRRA (81.5% vs 40%; $P = .009$ for Fisher's exact test).

Second, all 29 NORRA participants were observed for a median (IQR) of 23.0 (16.1, 25.4) months after the index biopsy. During this time-frame, only 3 out of 29 (10.3%) initiated their first b/tsDMARD; one of those (3.4%) subsequently fulfilled the PIRRA definition as well as the DT2-RA definition. Importantly, removing these 3 individuals from the baseline analyses did not alter any of the key histopathological contrasts (Supplementary Table S4).

Table 2
Disease activity of the study population (n = 93)

n (%) or median (25th, 95th percentile)	PIRRA N = 43	NIRRA N = 21	NORRA N = 29	PIRRA vs NIRRA	PIRRA vs NORRA	NIRRA vs NORRA
SDAI	23.6 (17.3, 31.2)	20.2 (14.1, 30.4)	22.3 (10.8, 28.5)	0.244		
CDAI	22.3 (15.6, 34.4)	19.7 (14, 30.3)	21.4 (10.4, 23.8)	0.502		
DAS28	4.3 (3.8, 5.2)	4 (3.3, 4.7)	3.8 (2.8, 4.8)	0.148		
TJC28	3 (2, 10)	4 (2, 9)	2 (1, 4)	0.997	<0.001	0.022
SJC28	5 (2, 10)	2 (1, 4)	6 (4, 7)	0.029	<0.001	0.016
PhGA	60 (50, 79)	50 (30, 60)	20 (10, 30)	0.093	<0.001	0.003
PtGA	72 (50, 90)	62 (50, 81)	49 (22, 68)	0.960	0.005	0.008
VAS-Pain	62 (28)	69 (17)	38 (27)	0.492	0.005	<0.001
VAS-MS	61 (29)	73 (22)	39 (30)	0.518	0.031	0.011

ANOVA, analysis of variance; CDAI, Clinical Disease Activity Index; DAS28, Disease Activity Score using 28 joints; NIRRA, noninflammatory refractory rheumatoid arthritis; NORRA, nonrefractory rheumatoid arthritis; PIRRA, persistent inflammatory refractory rheumatoid arthritis; PhGA, Physician Global Assessment; PtGA, Patient Global Assessment; SDAI, Simplified Disease Activity Index; SJC28, Swollen Joint Count (28 joints); TJC28, Tender Joint Count (28 joints); VAS-MS, Visual Analog Scale for Morning Stiffness; VAS-Pain, Visual Analog Scale for Pain.
P values refer to ANOVA with Dunnett's T3 multiple comparisons tests for continuous variables.

Third, we revisited the NORRA cohort to distinguish an inflammatory phenotype from a noninflammatory one within the NORRA group. We applied the same a priori thresholds that were used to define PIRRA and NIRRA, with only 2 out of 29 NORRA patients having PhGA>2 and CRP>5 mg/L; hence, analysis of this post hoc reclassification was not feasible.

Finally, to test whether the 20 mm cut-off for PhGA exerts undue influence on our results, we re-analysed the data using an additional, stricter threshold (PhGA >50 mm). For this scenario we reclassified patients with CRP ≤5 mg/L accordingly and repeated all primary histopathological comparisons (data shown in Supplementary Table S5). After applying this stricter threshold, NIRRA remained significantly enriched for the pauci-immune fibroid pathotype (39.3%), whereas the KSS gap between PIRRA and NIRRA slightly decreased. This finding is consistent with NIRRA patients with PhGA between 20 and 50 mm having slightly higher synovitis severity than NIRRA with PhGA ≤20 mm.

DISCUSSION

We showed that a pragmatic bedside tool (PhGA and CRP) stratifies refractory RA into 2 histologically divergent phenotypes. Almost half of NIRRA patients exhibited pauci-immune fibroid synovitis, whereas PIRRA was dominated by lympho- or diffuse-myeloid patterns; inflammatory pathotypes were 3-fold more common in PIRRA. The RR of pauci-immune synovitis in NIRRA versus PIRRA was 1.6 (95 % CI 1.2 to 2.9), confirming a biological separation that routine composite scores alone cannot capture.

The clinical tool was derived from physician-subjective disease activity assessment and objective inflammation without incorporating the patient's perception of disease activity. However, the optimal PhGA threshold may differ between early-stage refractory and fully EULAR-defined D2T disease, and prospective validation in independent cohorts will be required before the tool can be adopted in routine practice.

Our findings support the relevance of clinical anchors for contextualising histological heterogeneity while also highlighting their limitations as surrogates for synovial inflammation. Because composite scores alone are heavily influenced by subjective components (as shown in Supplementary Table S6), they cannot disentangle inflammatory from noninflammatory refractoriness; the identification of distinct pathotypes in patients with similar clinical profiles underscores the potential of tissue analysis as an independent and complementary metric. Because PtGA correlates more strongly with pain amplification and psychosocial factors than with objective synovitis in several [20,21] reports, we elected not to incorporate it into the algorithm, recognising that its discriminatory value for PIRRA versus NIRRA remains to be formally tested. Alternatively, PtGA thresholds—analysed alongside other patient-reported outcomes—should be assessed to determine whether PtGA provides incremental phenotypic discrimination beyond CRP and PhGA.

The association of NIRRA with poor functional outcomes despite limited synovial inflammation highlights the multidimensional impact of noninflammatory drivers in RA. Physical function, as assessed by SF-36 and HAQ-DI scores, was worst in NIRRA patients. NIRRA had significantly lower SF-36-physical functioning and SF-36-social functioning compared to NORRA, while the scores were numerically lower in 5 out of 8 domains compared to PIRRA. This finding likely reflects SF-36 capturing more refined physical functioning than visual analogue scale-pain. Notwithstanding the greater pain amplification observed in NIRRA, vitality did not differ significantly between phenotypes. The vitality scale, although widely adopted, may lack sensitivity to the multidimensional fatigue experienced in RA and is influenced by mood, anaemia, and sleep quality, factors that were distributed evenly across groups in our study. Future work should incorporate RA-specific fatigue instruments such as Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) to clarify whether central sensitisation contributes distinctly to fatigue as well as pain.

Many patients with NIRRA were codiagnosed with fibromyalgia and depression, an observation aligned with prior research identifying fibromyalgia and depression as significant contributors to disability in refractory RA populations [5,9,22,23] and from studies delineating different RA trajectories according to function and mental states [24]. The elevated pain and morning stiffness in NIRRA, despite low inflammatory markers, reflect noninflammatory mechanisms, potentially linked to central sensitisation or altered pain perception, as suggested by recent studies exploring the neuro-immune axis in RA [25]. Opioid use was markedly higher in NIRRA than in PIRRA (39% vs 11%), mirroring both a significantly worse SF-36 Bodily Pain score and greater fibromyalgia-screen positivity. When such nociplastic pain is misinterpreted as persistent synovitis, treatment is frequently intensified with additional biologic DMARDs or Janus-kinase inhibitors, exposing patients to unnecessary immunosuppression and increasing healthcare costs. Recognition of a pauci-immune fibroid synovial pattern—and the concomitant absence of systemic inflammation—should instead redirect

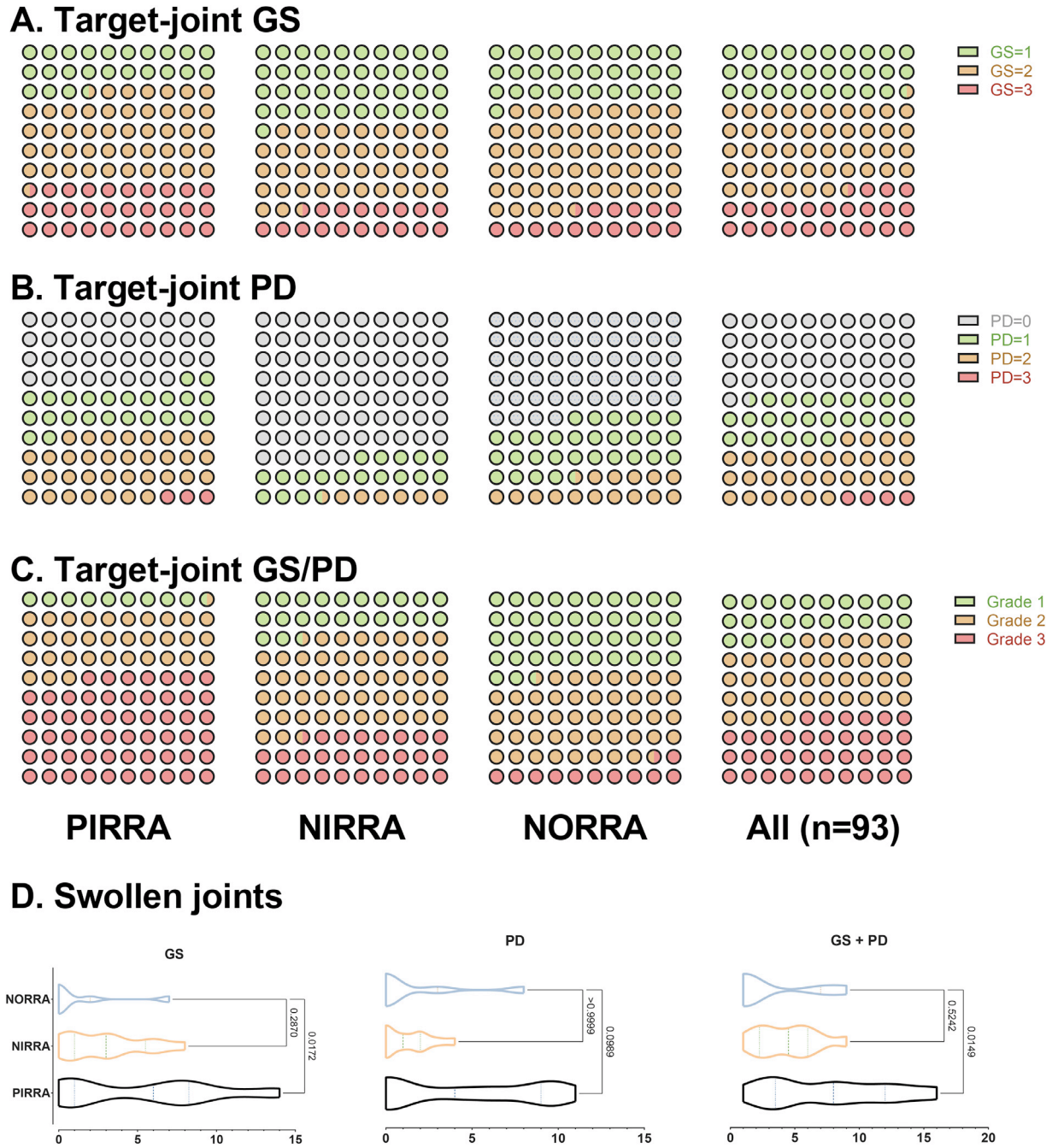

Figure 6. Musculoskeletal ultrasound findings in the target joint (A-C) and in all swollen joints scanned (D), according to the clinical phenotype group (PIRRA, NIRRA, NORRA). GS, grey scale; NIRRA, noninflammatory refractory rheumatoid arthritis; NORRA, nonrefractory rheumatoid arthritis; PD, power Doppler; PIRRA, persistent inflammatory refractory rheumatoid arthritis.

management towards non-immunological strategies, including opioid-sparing analgesics (eg, serotonin-noradrenaline reuptake inhibitors), structured exercise programmes, cognitive–behavioural therapy, and referral to multidisciplinary pain services.

Our findings align with the NIRRA phenotype proposed in literature, which associates persistent symptoms with mechanisms beyond overt inflammation, such as central sensitisation and fibromyalgia [26,27]. Compared to NIRRA, PIRRA exhibited a significantly higher number of swollen joints and inflammatory markers. PIRRA is predominantly associated with lympho-myeloid or diffuse-myeloid pathotypes, characterised by higher levels of CRP, ESR, and higher-grade synovial inflammation with a greater number of plasma cells and lymphoid aggregates, corroborating prior studies emphasising the inflammatory burden in D2T RA [28,29].

Refractoriness resulting from inadequate treatment response

A major issue with understanding refractoriness is that most research on synovial tissue has focused on treatment-naive patients rather than those with multi-failure to b/tsDMARDs. While synovial pathology in treatment-naive RA can change due to persistent inflammation [30], medications may affect refractoriness, for instance, prolonged use of GCs [31,32], or poor optimisation of methotrexate [31,32]. Although some studies have reported an overall shift towards lymphocyte-poor synovitis after prolonged exposure to biologic DMARDs [33], our data provide evidence that this trend is not universal: patients classified here as PIRRA, despite extensive prior immunomodulation, still exhibited a predominantly lympho- or diffuse-myeloid pathotype. This finding underscores that treatment history interacts

with, but does not fully determine, the underlying tissue phenotype.

All NIRRA patients with the pauci-immune fibroid pathotype in our cohort were inadequate responders to tumour necrosis factor inhibitors (TNFis), which is consistent with a previous observation from the PEAC/PEsAC cohorts where a significantly higher number of patients with a lympho-myeloid and diffuse-myeloid pathotype achieved a clinical response to certolizumab-pegol in comparison with the pauci-immune pathotype [34]. Cell signatures that decreased following TNFi treatment were predominantly associated with lymphocytes, and fewer were associated with myeloid and fibroblast populations [35]. Another interesting finding is that 17.4% of NIRRA patients who were given rituximab did not achieve a response. This is in line with the STRAP trials, which found that none of the pauci-immune fibroid patients had an ACR50 or ACR70 response. [36]. Sustained cytokine blockade can attenuate lymphocyte recruitment and thereby contribute to the pauci-immune histology observed in a subset of refractory patients. Whether such treatment-conditioned states persist after drug withdrawal, or merely mask an inflammatory potential that re-emerges once the inhibitory pressure is lifted, remains to be determined.

In this regard, Zhang et al. [37] investigated whether cell-type abundance phenotypes (CTAPs) changed over time in a cohort of 45 TNFi-inadequate responder RA patients from the R4RA trial who had synovial tissue biopsies before and 16 weeks after starting treatment (tocilizumab and rituximab). Although CTAPs partially explained variance in Krenn's synovial pathotypes (notably, the inflammation and stromal density components), it is relevant that CTAPs were dynamic, with 30/45 (67%) patients changing to a different CTAP after treatment, irrespective of treatment response. Among patients who changed CTAPs, CTAP with enriched fibroblasts was the most common CTAP at week 16 (n = 16/30 (53%)), consistent with rituximab and tocilizumab targeting inflammatory cells and pathways. Our NIRRA- and PIRRA-stratified synovial tissue analysis could be applied to the multi-drug-experienced patients described by Zhang and colleagues. It is crucial to note that, in accordance with our findings, they did not observe any correlation between CTAP assignment and disease activity measures, yet indisputably important data like SF-36, PROMs, and, remarkably, PhGA, were not provided. While this limitation prevents fully comparing the 2 cohorts, this result corroborates the hypothesis that CTAPs are indicative of distinct inflammatory phenotypes that are responsible for synovitis, rather than variations in clinical disease activity. Finally, almost all NORRA patients could not be classified as 'inflammatory' post hoc, further supporting the evidence that RA refractory to b/tsDMARDs is biologically distinct from RA refractory to csDMARDs, as it indicates a reduced inflammatory burden that is comparable to a nonrefractory status.

MSUS may not adequately characterise NIRRA

A clinically active joint with synovial thickening on MSUS does not necessarily indicate the presence of immune cells in the corresponding biopsy. On the other hand, a definition of NIRRA that hinges on the absence of PD may miss patients with low-grade but biologically relevant inflammation.

Despite the lack of high-quality direct evidence, MSUS may have an additional value in assessing the presence of inflammatory activity in EULAR-defined D2T-RA patients, including those with concomitant obesity or fibromyalgia [38]. Ultrasound assessment of synovial tissue faithfully reflects synovial vascularity [39]. In early RA, both greyscale and PD synovitis are associated with a proinflammatory cellular and cytokine profile, providing considerable validity in their use as an objective assessment of synovial inflammation in clinical practice [39]. We observed reduced GS and PD scores in NIRRA relative to PIRRA; however, it is important to note that MSUS lacked adequate discriminatory capability in distinguishing between PIRRA and NIRRA. This finding implies that NIRRA and PIRRA cannot be distinguished solely based on MSUS. One single-centre study defined PIRRA if MSUS synovitis (GS $\geq$1 and PD $\geq$1) was detectable in one or more swollen joints and NIRRA if absent, and classified 57% of EULAR-defined D2T-RA patients as PIRRA and 43% as NIRRA [27]. Our results show that defining NIRRA by only a nonclinical criterion of complete absence of MSUS synovitis may risk missing the nuances of the NIRRA phenotype and forgo a consistent subset of patients with noninflammatory symptoms but true low-grade active inflammation. For this reason, all patients in our study had to demonstrate MSUS synovitis (GS $\geq$1) for being included. Although in our study PD scores were lower in NIRRA compared to PIRRA, GS was consistently increased across NIRRA and PIRRA joints, a finding that is corroborated by a recent systematic literature review [40]. Future iterations of the algorithm may well integrate quantitative multi-joint PD scores once robust, externally validated cut-offs become available; for the present, the CRP plus PhGA tool offers a pragmatic approximation that aligns closely with objective synovial histology in our cohort.

Proposed pathophysiology of NIRRA

The predominance of pauci-immune synovitis in NIRRA underscores the need for further exploration of its pathophysiological basis.

NIRRA had levels of pain comparable to PIRRA despite having more pauci-immune synovitis. It has previously been shown that concomitant noninflammatory pain was more prevalent in EULAR-defined D2T-RA patients compared to good-responders non-D2T patients [41,42]. A recent study from the AMP-RA consortium revealed that pain scores do not correlate with stromal cell density in RA patients with low inflammatory synovium; interestingly, although, imaging of solvent-cleared synovial tissue with scarce inflammation from humans with RA revealed Calcitonin Gene-Related Peptide $(CGRP)^+$ pain-sensing neurons encasing blood vessels growing into synovial hypertrophic papilla. Together, these findings support a model whereby synovial lining fibroblasts express genes associated with pain that enhance the growth of pain-sensing neurones into regions of synovial hypertrophy in RA [25].

We were unable to detect an increase in stromal density scores in NIRRA with respect to other groups. Notwithstanding that, our NIRRA samples do share the leukocyte paucity with noninflammatory synovium RA patients. This divergence indicates that pauci-immune synovitis may represent a spectrum in which fibroblast expansion, nociplastic pain, and immune quiescence can be variably coupled. Absence of a fibroblast-dominant signal in NIRRA may reflect biological heterogeneity, limitations of histological stromal quantification, or both. Future single-cell or spatial-transcriptomic profiling will be required to determine whether a subset of NIRRA indeed harbours the *NOTCH3*-dependent fibroblast programme or whether alternative pathways underlie their symptom burden.

NIRRA had the lowest levels of acute phase reactants, rheumatoid factor (RF), and anti-citrullinated protein antibody (ACPA) positivity, despite the presence of active disease,

reflecting the high prevalence of the pauci-immune pathotype in this group [19,40]. As we characterised it, this subset closely resembles that reported in the seminal study by Humby et al. [19], in which the pauci-immune pathotype was the least associated with RF positivity (50%) or ACPA positivity (52.9%), and it was characterised by significantly lower CRP and ESR in comparison with lympho-myeloid. A previous study of early RA patients from the PEAC cohort described 10 gene–gene interaction clusters in the pauci-immune pathotype, with most of them showing enrichment in pathways involved in extracellular matrix, proinflammatory activation of fibroblasts, nerve, vascular endothelial, and growth factors [43]. Hence, NIRRA with pauci-immune fibroid synovitis may be related more to stromal activation and synovial fibroblasts, although our findings also suggest that paucity of immune cells does not inevitably coincide with fibroblast hyperplasia, and nociplastic pain in NIRRA may arise through mechanisms other than the stromal programme. In treatment-naive patients, pauci-immune histology is associated with increased Axl/MerTK synovial expression, which is influenced by interleukin (IL)-6 inhibition [44]. Accordingly, many of our NIRRA patients were exposed to IL-6 inhibition. The observation of a high proportion of mast cells in the pauci-immune pathotype [45] is consistent with the high number of DMARDs that failed due to intolerance or hypersensitivity reactions in the NIRRA subgroup. Finally, the presence of diffuse-myeloid pathotype in a subset of NIRRA patients is consistent with the previous observation that a subset of patients has overlapping characteristics with both pauci-immune and lympho-myeloid pathotypes [43]. The absence of B and plasma cell aggregates in diffuse myeloid is a feature in common with the pauci-immune fibroid pathotype.

Implications for therapeutic decision-making in RA

The identification of distinct PIRRA and NIRRA synovial phenotypes has critical implications for therapeutic decision-making. For PIRRA, targeting inflammation through aggressive use of advanced targeted therapies remains paramount. This aligns with a previous study that demonstrated a positive association between high-grade KSS and clinical response to therapy in chronic inflammatory arthritis patients [46]. Inhibition of IL-6 or multiple cytokines through the JAK/STAT pathways, shown to modulate myeloid-driven pathways effectively, may offer substantial benefits in this subgroup [47,48]. Conversely, NIRRA may benefit from approaches directed to stromal cells rather than immunosuppression. Approaches targeted to synovial fibroblasts could potentially be combined with DMARDs to improve control of RA without increasing immunosuppression in NIRRA [49]. Moreover, NIRRA management may require a broader approach, incorporating non-pharmacological interventions aimed at central pain modulation, such as cognitive –behavioural therapy, exercise programs, and neuromodulation techniques [29]. Pharmacologically, adjunctive use of agents targeting pain mechanisms, such as duloxetine or pregabalin, could be considered over trialling b/tsDMARDs with several mechanisms of action [50]. Avoiding unnecessary immunosuppression in this group is essential to mitigate side effects and healthcare costs. Routine incorporation of ultrasound-guided synovial biopsy with validated histopathological scoring into the clinical work-up of refractory RA could enable the identification of inflammatory versus non-inflammatory pathotypes at the point of care, thereby facilitating a truly mechanistic, precision-based selection of immunomodulatory or nonimmunological interventions for each individual patient.

Limitations and strengths

A potential limitation of the study is the use of PhGA and CRP as criteria for subclassifying refractory RA. Although these markers are widely used, they may not capture the full spectrum of disease activity. However, this approach is consistent with current guidelines and supported by prior research highlighting the relevance of these parameters in distinguishing inflammatory versus noninflammatory disease drivers [9,51]. Because phenotype assignments depended on the PhGA threshold, we cannot exclude the possibility that physician subjectivity introduced misclassification bias. Although we standardised assessment procedures and documented high test–retest reliability, objective tissue-level read-outs such as synovial cellular composition or transcriptomic modules may ultimately supplant global ratings in phenotype algorithms. However, this was beyond the aim of this study. Additionally, stratifying refractory RA patients based on longitudinal disease patterns—persistent versus episodic—could refine treatment algorithms and improve outcome prediction [29]. Other limitations include the under-representation of patients from diverse ethnic backgrounds in this cohort. Finally, we acknowledge that some experts might label refractory RA patients as nonresponders rather than true refractory cases; our findings should therefore be interpreted within the spectrum of failure of ≥1 b/tsDMARD in the presence of persistent inflammation or pain after csDMARD optimisation.

The methodological rigour of this study is grounded in its prospective, multicentre design, which enhances generalisability. The incorporation of MSUS-guided synovial biopsy, a proven method for evaluating synovitis, guaranteed precise characterisation of synovial pathotypes. Moreover, reliance on validated scoring systems, such as the KSS, adds robustness to the findings. The stratification of patients into NIRRA and PIRRA subgroups based on consensus definitions and inflammatory markers aligns with emerging classifications of refractory RA [9,51].

Conclusions

This study highlights the necessity of phenotypic classification through UGSTB in refractory RA to guide treatment decisions.

Competing interests

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CRediT authorship contribution statement

Alessandro Giollo: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mariangela Salvato:** Writing – review & editing, Visualization, Investigation, Data curation. **Francesca Frizzera:** Writing – original draft, Investigation, Data curation. **Kiren Khalid:** Writing – review & editing, Investigation, Data curation. **Lorenzo Di Luozzo:** Writing – original draft, Investigation, Data curation. **Maria Capita:** Writing –

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Patient consent for publication

Complete written informed consent was obtained from the patients for the publication of this study.

Ethics approval

The study was approved by 2 territorial ethics committees (CET-ACEV 6029/AO/24; 698/2020/Sper/AOUFe) in compliance with the principles of the Declaration of Helsinki as revised in 2000.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

The participants in this study did not give written consent for their data to be shared publicly. The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.07.023.

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REFERENCES

[1] Winthrop KL, Mease P, Kerschbaumer A, Voll RE, Breedveld FC, Smolen JS, et al. Unmet need in rheumatology: reports from the Advances in Targeted Therapies meeting, 2023. Ann Rheum Dis 2024;83:409–16.
[2] Alivernini S, Firestein GS. McInnes IB. The pathogenesis of rheumatoid arthritis. Immunity 2022;55:2255–70.
[3] Najm A, Orr C, Heymann MF, Bart G, Veale DJ. Le Goff B. Success rate and utility of ultrasound-guided synovial biopsies in clinical practice. J Rheumatol 2016;43:2113–9.
[4] Rivellese F, Humby F, Bugatti S, Fossati-Jimack L, Rizvi H, Lucchesi D, et al. B cell synovitis and clinical phenotypes in rheumatoid arthritis: relationship to disease stages and drug exposure. Arthritis Rheumatol 2020;72:714–25.
[5] Roodenrijs NMT, van der Goes MC, Welsing PMJ, Tekstra J, Lafeber FPJG, Jacobs JWG, et al. Difficult-to-treat rheumatoid arthritis: contributing factors and burden of disease. Rheumatology (Oxford) 2021;60:3778–88.
[6] Roodenrijs NMT, van der Goes MC, Welsing PMJ, van Oorschot EPC, Nikiphorou E, Nijhof NC, et al. Non-adherence in difficult-to-treat rheumatoid arthritis from the perspectives of patients and rheumatologists: a concept mapping study. Rheumatology (Oxford) 2021;60:5105–16.
[7] Roodenrijs NMT, de Hair MJH, van der Goes MC, Jacobs JWG, Welsing PMJ, van der Heijde D, et al. Characteristics of difficult-to-treat rheumatoid arthritis: results of an international survey. Ann Rheum Dis 2018;77:1705–9.
[8] Bertsias A, Flouri ID, Repa A, Avgoustidis N, Kalogiannaki E, Pitsigavdaki S, et al. Patterns of comorbidities differentially affect long-term functional evolution and disease activity in patients with 'difficult to treat' rheumatoid arthritis. RMD Open 2024;10:e003808.
[9] Buch MH, Eyre S, McGonagle D. Persistent inflammatory and non-inflammatory mechanisms in refractory rheumatoid arthritis. Nat Rev Rheumatol 2021;17:17–33.
[10] Najm A, Costantino F, Alivernini S, Alunno A, Bianchi E, Bignall J, et al. EULAR points to consider for minimal reporting requirements in synovial tissue research in rheumatology. Ann Rheum Dis 2022;81:1640–6.
[11] Novella-Navarro M, Cabrera-Alarcón J, López-Juanes N, Villalba A, Fernández Fernández E, Monjo I, et al. Patient and physician assessment in difficult-to-treat rheumatoid arthritis: patterns of subjective perception at early stages of b/tsDMARD treatment. RMD Open 2023;9:e003382.
[12] D'Agostino MA, Terslev L, Aegerter P, Backhaus M, Balint P, Bruyn GA, et al. Scoring ultrasound synovitis in rheumatoid arthritis: a EULAR-OMERACT ultrasound taskforce-Part 1: definition and development of a standardised, consensus-based scoring system. RMD Open 2017;3:e000428.
[13] Humby F, Kelly S, Hands R, Rocher V, DiCicco M, Ng N, et al. Use of ultrasound-guided small joint biopsy to evaluate the histopathologic response to rheumatoid arthritis therapy: recommendations for application to clinical trials. Arthritis Rheumatol 2015;67:2601–10.
[14] Just SA, Humby F, Lindegaard H, Meric de Bellefon L, Durez P, Vieira-Sousa E, et al. Patient-reported outcomes and safety in patients undergoing synovial biopsy: comparison of ultrasound-guided needle biopsy, ultrasound-guided portal and forceps and arthroscopic-guided synovial biopsy techniques in five centres across. Europe. RMD Open. 2018;4:e000799.
[15] Humby F, Romão VC, Manzo A, Filer A, Bugatti S, Vieira-Sousa E, et al. A multicenter retrospective analysis evaluating performance of synovial biopsy techniques in patients with inflammatory arthritis: arthroscopic versus ultrasound-guided versus blind needle biopsy. Arthritis Rheumatol 2018;70:702–10.
[16] Kelly S, Humby F, Filer A, Ng N, Cicco MD, Hands RE, et al. Ultrasound-guided synovial biopsy: a safe, well-tolerated and reliable technique for obtaining high-quality synovial tissue from both large and small joints in early arthritis patients. Ann Rheum Dis 2015;74:611–7.
[17] Najm A, Le Goff B, Orr C, Thurlings R, Cañete JD, Humby F, et al. Standardisation of synovial biopsy analyses in rheumatic diseases: a consensus of the EULAR Synovitis and OMERACT Synovial Tissue Biopsy Groups. Arthritis Res Ther 2018;20:265.
[18] Krenn V, Morawietz L, Burmester GR, Kinne RW, Mueller-Ladner U, Muller B, et al. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. Histopathology 2006;49:358–64.
[19] Humby F, Lewis M, Ramamoorthi N, Hackney JA, Barnes MR, Bombardieri M, et al. Synovial cellular and molecular signatures stratify clinical response

to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. Ann Rheum Dis 2019;78:761–72.
[20] D'Onofrio B, van Mulligen E, Bugatti S, van der Helm-van Mil A. Can the patient global assessment of disease activity help to discriminate inflammatory and non-inflammatory refractoriness in early rheumatoid arthritis? Joint Bone Spine 2025;92:105803.
[21] Bugatti S, De Stefano L, D'Onofrio B, Nicrosini A, Mauric E, di Lernia M, et al. Inflammatory correlates of the Patient Global Assessment of Disease Activity vary in relation to disease duration and autoantibody status in patients with rheumatoid arthritis. Ann Rheum Dis 2022;81:1206–13.
[22] McDermott GC, DiIorio M, Kawano Y, Jeffway M, MacVicar M, Dahal K, et al. Reasons for multiple biologic and targeted synthetic DMARD switching and characteristics of treatment refractory rheumatoid arthritis. Semin Arthritis Rheum 2024;66:152421.
[23] Paudel ML, Li R, Naik C, Shadick N, Weinblatt ME, Solomon DH. Prevalence and characteristics of adults with difficult-to-treat rheumatoid arthritis in a large patient registry. Rheumatology (Oxford) 2025;64:1102–10.
[24] RA-MAP Consortium. Characterization of disease course and remission in early seropositive rheumatoid arthritis: results from the TACERA longitudinal cohort study. Ther Adv Musculoskelet Dis 2021;13 1759720X211043977.
[25] Bai Z, Bartelo N, Aslam M, Murphy EA, Hale CR, Blachere NE, et al. Synovial fibroblast gene expression is associated with sensory nerve growth and pain in rheumatoid arthritis. Sci Transl Med 2024;16:eadk3506.
[26] Wallace BI, Moore MN, Heisler AC, Muhammad LN, Song J, Clauw DJ, et al. Fibromyalgianess and glucocorticoid persistence among patients with rheumatoid arthritis. Rheumatology (Oxford) 2022;61:1556–62.
[27] David P, Di Matteo A, Hen O, Dass S, Marzo-Ortega H, Wakefield RJ, et al. Poly-refractory rheumatoid arthritis: an uncommon subset of difficult to treat disease with distinct inflammatory and noninflammatory phenotypes. Arthritis Rheumatol 2024;76:510–21.
[28] Tan Y, Buch MH. Difficult to treat' rheumatoid arthritis: current position and considerations for next steps. RMD Open 2022;8:e002387.
[29] Cincinelli G, Maioli G, Posio C, Favalli EG, Ingegnoli F, Caporali R. Truth unveiled by time and the marbled definition of D2T-RA: retrospective analysis on the persistence of the difficult-to-treat status among refractory RA patients. Arthritis Res Ther 2024;26:161.
[30] Lliso-Ribera G, Humby F, Lewis M, Nerviani A, Mauro D, Rivellese F, et al. Synovial tissue signatures enhance clinical classification and prognostic/treatment response algorithms in early inflammatory arthritis and predict requirement for subsequent biological therapy: results from the pathobiology of early arthritis cohort (PEAC). Ann Rheum Dis 2019;78:1642–52.
[31] Giollo A, Zen M, Larosa M, Astorri D, Salvato M, Calligaro A, et al. Early characterization of difficult-to-treat rheumatoid arthritis by suboptimal initial management: a multicentre cohort study. Rheumatology (Oxford) 2023;62:2083–9.
[32] Yoshii I, Sawada N, Chijiwa T. Clinical characteristics and variants that predict prognosis of difficult-to-treat rheumatoid arthritis. Rheumatol Int 2022;42:1947–54.
[33] Weisenfeld D, Zhang F, Donlin L, Jonsson AH, Apruzzese W, Campbell D, et al. Associations between rheumatoid arthritis clinical factors and synovial cell types and states. Arthritis Rheumatol 2024;76:356–62.
[34] Nerviani A, Di Cicco M, Mahto A, Lliso-Ribera G, Rivellese F, Thorborn G, et al. A pauci-immune synovial pathotype predicts inadequate response to TNFα-blockade in rheumatoid arthritis patients. Front Immunol 2020;11:845.
[35] Wang J, Conlon D, Rivellese F, Nerviani A, Lewis MJ, Housley W, et al. Synovial inflammatory pathways characterize anti-TNF-responsive rheumatoid arthritis patients. Arthritis Rheumatology 2022;74:1916–27.
[36] Rivellese F, Nerviani A, Giorli G, Warren L, Jaworska E, Bombardieri M, et al. Stratification of biological therapies by pathobiology in biologic-naive patients with rheumatoid arthritis (STRAP and STRAP-EU): two parallel, open-label, biopsy-driven, randomised trials. Lancet Rheumatol 2023;5:e648–59.
[37] Zhang F, Jonsson AH, Nathan A, Millard N, Curtis M, Xiao Q, et al. Deconstruction of rheumatoid arthritis synovium defines inflammatory subtypes. Nature 2023;623:616–24.
[38] Roodenrijs NMT, Kedves M, Hamar A, Nagy G, van Laar JM, van der Heijde D, et al. Diagnostic issues in difficult-to-treat rheumatoid arthritis: a systematic literature review informing the EULAR recommendations for the management of difficult-to-treat rheumatoid arthritis. RMD Open 2021;7:e001511.
[39] Kelly S, Bombardieri M, Humby F, Ng N, Marrelli A, Riahi S, et al. Angiogenic gene expression and vascular density are reflected in ultrasonographic features of synovitis in early rheumatoid arthritis: an observational study. Arthritis Res Ther 2015;17:58.
[40] Bellis E, Agugliaro F, Garulli C, Perrone S, Liperoti G, Gatto M, et al. The role of musculoskeletal ultrasound in difficult-to-treat RA: insights from a systematic literature review. Autoimmun Rev 2025;24:103694.
[41] Qi W, Robert A, Singbo N, Ratelle L, Fortin PR, Bessette L, et al. Characteristics of patients with difficult-to-treat rheumatoid arthritis: a descriptive retrospective cohort study. Adv Rheumatol 2024;64:55.
[42] Das D, Choy E. Non-inflammatory pain in inflammatory arthritis. Rheumatology (Oxford) 2023;62:2360–5.
[43] Sciacca E, Surace AEA, Alaimo S, Pulvirenti A, Rivellese F, Goldmann K, et al. Network analysis of synovial RNA sequencing identifies gene-gene interactions predictive of response in rheumatoid arthritis. Arthritis Res Ther 2022;24:166.
[44] Nerviani A, Boutet MA, Ghirardi GM, Goldmann K, Sciacca E, Rivellese F, et al. Axl and MerTK regulate synovial inflammation and are modulated by IL-6 inhibition in rheumatoid arthritis. Nat Commun 2024;15:2398.
[45] Micheroli R, Elhai M, Edalat S, Frank-Bertoncelj M, Bürki K, Ciurea A, et al. Role of synovial fibroblast subsets across synovial pathotypes in rheumatoid arthritis: a deconvolution analysis. RMD Open 2022;8:e001949.
[46] Garaffoni C, Tamussin M, Calciolari I, Lanza G, Bortoluzzi A, Scirè CA, et al. High-grade synovitis associates with clinical markers and response to therapy in chronic inflammatory arthritis: post hoc analysis of a synovial biomarkers prospective cohort study. Front Immunol 2023;14:1298583.
[47] Su QY, Luo J, Zhang Y, Li Q, Jiang ZQ, Wen ZR, et al. Efficacy and safety of current therapies for difficult-to-treat rheumatoid arthritis: a systematic review and network meta-analysis. J Transl Med 2024;22:795.
[48] Sebastiani M, Zabotti A, Biasi B, Cacioppo S, Sandri G, Giovannini I, et al. Factors associated to long-term retention rate of Janus kinase inhibitors in a multi-failure rheumatoid arthritis population. Clin Exp Rheumatol 2024;42:1416–20.
[49] Svensson MND, Zoccheddu M, Yang S, Nygaard G, Secchi C, Doody KM, et al. Synoviocyte-targeted therapy synergizes with TNF inhibition in arthritis reversal. Sci Adv 2020;6:eaba4353.
[50] Roodenrijs NMT, Hamar A, Kedves M, Nagy G, van Laar JM, van der Heijde D, et al. Pharmacological and non-pharmacological therapeutic strategies in difficult-to-treat rheumatoid arthritis: a systematic literature review informing the EULAR recommendations for the management of difficult-to-treat rheumatoid arthritis. RMD Open 2021;7:e001512.
[51] Fitton J, Melville A, Naraghi K, Nam J, Dass S, Emery P, et al. Single-centre experience of refractory rheumatoid arthritis. Rheumatol Adv Pract 2022;6 rkac057.