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ANA-arthritis: clinical and biomarker characterization of a population for basket trials

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Data Availability:
Data underlying this article will be made available on reasonable request to the corresponding author.

Ethics statement:
Research ethics approval was obtained from the UK Health Research Authority (IRAS ref. 60762). Written informed consent was obtained from all patients.
Abstract

Objectives:

ANA-associated RMDs (ANA-RMDs - SLE, pSS, scleroderma, inflammatory myositis, mixed connective tissue disease (MCTD) and undifferentiated connective tissue disease) are a disease spectrum with overlapping clinical and immunological features. Musculoskeletal inflammation is common and impactful across ANA-RMDs. We evaluated musculoskeletal inflammation (ANA-arthritis) prevalence in a multi-disease ANA-RMD study, assessed its clinical impact across ANA-RMD diagnoses, proposed new basket groupings of patients and evaluated immunological profiles in legacy and new basket contexts.

Methods:

An observational study enrolled ANA-RMD patients. Demographic variables, comorbidities, therapies, disease activity instruments (BILAG, SLEDAI, ESSDAI, physician-VAS), patient-reported outcomes (SF36, FACIT-Fatigue, EQ5D, ICECAP-A, WPAI, patient-VAS) and biomarker profile (6 gene expression scores, flow cytometry, autoantibody profile) were analysed. Reclustering utilized Gaussian Mixture Modelling (GMM). Clinical and immune features of new and legacy clusters were compared.

Results:

Inflammatory MSK symptoms were prevalent across ANA-RMDs, in 213/294 patients. In ANA-arthritis patients, most variables did not differ between diagnoses, excluding EQ5D-5L index and mobility domains (lower in MCTD/pSS, both p<0.05). Fibromyalgia and osteoarthritis prevalence were similar across diagnoses. Therapy use differed significantly, biologic use being greatest in SLE (p<0.05).

GMM yielded two multi-disease clusters; High-MSK disease activity (n=89) and Low-MSK disease activity (n=124). High-MSK disease activity contained all patients with active joint swelling and had significantly higher prednisolone usage, PGA and Sm/RNP/SmRNP/Chromatin positivity, Tetherin-MFI and Interferon Score-A activity; with numerically lower fibromyalgia and osteoarthritis prevalence.

Conclusion:

We define ANA-Arthritis, a more clinically and immunologically homogenous population than existing RMDs for trials, and a more prevalent population for therapies in the clinic.
Graphical abstract

**Key words:**
Arthritis, Autoimmune Diseases, SLE, Sjogren’s syndrome, Myositis

**Key messages:**

- Arthritis is common in each ANA-RMD but is treated differently according to disease specific therapy and treatment guidelines.
- ANA-arthritis has similar impact on patient and physician reported outcomes across ANA-RMDs.
- We define a more prevalent, active, clinically and immunologically homogenous ANA-arthritis population than existing diagnoses for clinical trials.
Introduction

ANA-associated RMDs (ANA-RMDs) are a spectrum of overlapping diseases characterized by autoreactivity to nuclear antigens encompassing systemic lupus erythematosus (SLE), primary Sjogren’s Syndrome (pSS), idiopathic inflammatory myopathies (IIM) and systemic sclerosis (SSc). Many patients have overlap syndromes, meeting classification criteria for multiple diseases, or undifferentiated forms of ANA-RMDs (UCTD) that are not easily classified (1–3). Despite distinct clinicopathological manifestations, such as specific antibodies, or skin fibrosis in SSc versus exocrine gland inflammation in pSS, ANA-RMDs also share features such as arthritis and immunopathogenic signatures (4–11).

Despite these shared features, treatment inequity exists between SLE and other ANA-RMDs. In SLE patients with arthritis, two targeted therapies are licensed while there are none for pSS patients with arthritis (12,13). Additionally, UCTD patients lack evidence-based treatment strategies and are ineligible for clinical trials. In single diseases such as SLE, diverse clinical and immunological presentations, such as cutaneous and musculoskeletal symptoms, pose challenges in defining trial populations, measuring outcomes, and assessing treatment effectiveness (14,15). Heterogeneity within SLE may partially explain how, despite encouraging clinical responses for certain disease manifestations, several studies failed to meet multisystem primary endpoints leading to programme discontinuation (12).

Reclassifying ANA-RMD patients into alternative “baskets” may address these issues, as exemplified by approaches to autoimmune disease-associated interstitial lung disease (16). Baskets may be defined as groups of patients from different legacy diagnoses into a new grouping that is suitable for a similar therapeutic intervention. Baskets may be based on shared pathogenic mechanisms (e.g. B-cells or Type-I interferon pathway activation) or a shared clinical problem, e.g. arthritis. Conducting clinical trials in a well-defined basket cohort could address unmet clinical needs and yield evidence-based interventions for patients across a wider spectrum of diagnoses. Furthermore, basket population trials may bolster effect sizes by utilizing more homogeneous study populations than existing trials, which cover multiple organ manifestations, biomarker subgroups, and background therapeutics. ANA-RMD arthritis may be a suitable basket for this strategy as it is common and significantly impacts quality-of-life, functional disability, work impairment, and health-economic outcomes (17,18).

The study objectives were: (i) to evaluate the prevalence of “ANA-arthritis”, defined as synovitis, tenosynovitis, enthesitis or other articular/periarticular inflammation in patients with ANA-RMD, in a multi-disease study; (ii) to test the hypothesis that ANA-arthritis is associated with a similar clinical impact across legacy diagnoses; (iii) to define new basket groupings of patients across the ANA-RMD spectrum for clinical trials; (iv) to evaluate immunological profile, and therefore suitability for therapies, of legacy diagnoses and new therapeutic baskets.
Methods

The DEFINITION cohort

Patient recruitment, variables collected, and use in statistical analyses are summarized in Figure 1. DEFINITION is a prospective, multi-disease ANA-RMD cohort study in Leeds, United Kingdom since May 2017. The primary aims were to better define the role of IFN-I and other biomarkers in ANA-RMD and refine the use of IFN-I targeted, conventional and other therapies. This analysis focused on patients with a history of inflammatory arthritis or currently active disease. Patients were identified through ultrasound documented synovitis at enrolment, the presence of inflammatory arthritis items on validated instruments (BILAG-2004 A-C articular/tendinopathy domains or ESSDAI), or any of the following terms in their medical documentation: arthropathy; arthritis; arthralgia; synovitis; tenosynovitis; joint tenderness; epicondylitis; polyarthralgia. All individuals provided informed written consent and this research was carried out in compliance with the Declaration of Helsinki. This study was approved by the National Health Service Health Research Authority (REC Ref: 17/YH/0166). Healthy control participants’ peripheral blood was collected under the study number 04/Q1206/107. All experiments were performed in accordance with relevant guidelines and regulations. University of Leeds was contracted with administrative sponsorship.

Demographics and comorbidity

We collected baseline age, gender, patient-identified ancestry, smoking status, index of multiple deprivation (IMD)(19), Charlson Comorbidity index (CCI)(20), clinical features of fibromyalgia, hypermobility and osteoarthritis. Diagnoses were recorded according to consultant physician review. This was preferred over diagnostic criteria which are absent in UCTD and often unfulfilled in pSS without tissue biopsy.

Laboratory measures

Full blood count, complement C3 and C4 levels and ANA subtypes, including anti-double-stranded DNA, Ro-52, Ro-60, La, Sm, SM/RNP, RNP, Scl-70, Jo-1, Centromere, Chromatin and Ribosomal-P antibodies (Bioplex multiplex analyser) were measured in a routine diagnostic laboratory. PBMC subsets were analysed using 8-colour flow cytometry as a proportion of total PBMC count (T-cells (CD3+CD56-), NK-cells (CD56+), NKT-cells (CD3+CD56+), Memory B-cells (CD19+CD27+, Plasmablasts (CD19+/CD27+CD38++), classical monocytes (CD14++CD16-) intermediate monocytes (CD14++CD16+)and non-classical monocytes (CD14+CD16+)). Tetherin (CD317) mean fluorescence intensity was quantified on each cell subset, with memory B-cell level as the primary biomarker(21).
Two validated interferon-stimulated gene expression scores (IFN Score-A and IFN Score-B) were analysed. PBMC were separated using density gradient method (Lymphoprep; Alere-Technologies, Norway) from EDTA-anticoagulated blood. Total RNA purification kit (Norgen-Biotek, Canada) was used followed by quantitative real-time reverse transcriptase-PCR (qRT-PCR) using TaqMan assays (Applied Biosystems, Invitrogen) for the selected 30 ISGs as previously described(22,23). Scores for genes annotated to plasmablast, myeloid lineage, inflammation and erythropoiesis function were included from previously described modules based on their known molecular function(24). We used untransformed dCT gene expression scores to preserve a normal distribution. For untransformed values in the figures and tables, numerically lower dCT values represent higher gene expression.

Clinical assessment
Disease activity was assessed at baseline using validated instruments applied to all diagnostic groups: EULAR Sjögren's syndrome disease activity index (ESSDAI); British Isles Lupus Assessment Group (BILAG) 2004 index; SLEDAI-2K. Rodnan skin score and MITAX were collected but not analysed due to limited relevance to clinical features and patient numbers. Physician global assessment (PGA) was also assessed. The validity of the articular component of the BILAG-MSK domain (excluding myositis) across non-SLE diagnoses was explored using association with PGA.

Patient-reported outcomes
Patient-reported disease impact was assessed using the following: 36-item Short Form Survey (SF36) – Composite and domain scores; Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-Fatigue); EuroQol-5 Dimension 5-level Score (EQ5D-5L) – Index and domain scores; ICECAP-A; Patient-reported visual analogue scales (VAS); Arthritis-VAS; Pain-VAS; Global-VAS; Fatigue-VAS; Global health-VAS and Early Morning Stiffness-VAS.

Machine learning
Model covariates were selected based on background evidence (MSK-BILAG Sm/SmRNP/RNP antibody status) and principle component analysis (PCA). PCA of 40 covariates, including Age, IMD-rank, prednisolone dosage, 15 ENA values, PGA, numeric MSK-BILAG, 8 gene-expression scores, 6 flow cytometry subsets, and 6 non-inflammatory features, identified 7 covariates for GMM. The primary variance was explained by IMD-rank, prednisolone dosage, numeric MSK-BILAG score, lymphocyte count, chromatin antibody positivity, Ro52/Ro60 antibody positivity, and Sm/SmRNP/RNP antibody positivity. Selected values explained >99.99% of data variance in the first 3 principal components within the 7-covariate model through singular value decomposition. Multiple imputation with chained equations (MICE) was utilized to address missingness, with 3.75% (n=8) of the IMD-rank data and 8.92% (n=19) of the lymphocyte count values being imputed. Hierarchical clustering, k-means clustering, and Gaussian Mixture Model (GMM) were trialled using the hclust and base-R packages.
Statistical analysis
Statistical analysis and data visualisation utilised the heatmap, corrplot, ggplot and tableone packages in R version 4.1.2. Multiple group comparison employed Kruskal-Wallis testing, while twin group comparisons of categorical and continuous variables utilized Chi-square and T-tests, respectively. For correlation analyses, Spearman’s rank correlation coefficient was used, considering correlations ≥0.3 or ≥-0.3 as substantive. Bonferroni correction was applied to compensate for multiple-hypothesis testing. Principle component analysis and Gaussian mixture modelling utilised the Mclust v6.0.0 packages. Data imputation used the MICE v3.15.0 package. Sankey plots were generated using SankeyMatic.

Patient and public involvement (PPI)
The NIHR Leeds Biomedical Research Centre PPI group have regular insight and input into planning and conduct of local ANA-RMD research. A workshop when designing the study identified arthritis as a key problem of interest.
Results

Prevalence of inflammatory joint and tendon disease in ANA-RMDs.
Of 294 patients with ANA-RMDs recruited to DEFINITION, 213 with inflammatory articular features were included. Key baseline features are detailed in Table 1. The SSc and pSS groups had a higher median age and were more comorbid than other ANA-RMDs with a higher baseline CCI (p=0.021 and 0.033 respectively). SLE and myositis groups had higher proportions on long-term prednisolone therapy. MSK inflammation was common, and most prevalent in SLE and MCTD patients (87% and 77% respectively).

No significant differences were observed in physician-defined fibromyalgia features among diagnostic groups. No significant differences were found in the prevalence of nodular osteoarthritis (on clinical examination), x-ray confirmed osteoarthritis, or hypermobility syndrome.

Validity of MSK-BILAG across ANA-RMDs
To compare disease activity across ANA-RMDs, we explored the concurrent validity of articular scores within the MSK component of BILAG-2004 Index. This demonstrates face validity across all RMDs, relying on the presence of inflammatory pain or swelling to categorize arthritis/tenosynovitis severity. Its definition mirrors the articular MSK assessment in MITAX and ESSDAI, each encompassing mild, moderate, and severe grades for MSK inflammation. BILAG-2004 articular MSK grades A-D were significantly associated with PGA across both SLE (F=14.43, p<0.001) and non-SLE patients (F=11.62, p<0.001), supporting the use of this measure in classifying arthritis patients with various ANA-RMDs, pending further validation.

Clinical impact of joint and tendon inflammation in ANA-RMDs
To compare the clinical impact of articular symptoms across ANA-RMDs, we assessed physician-reported outcomes. Numeric BILAG-2004 values, ESSDAI total and physician global assessment did not differ significantly between diagnoses (Table 1). Overall disease activity, as per the BILAG score and individual domains, did not significantly differ across groups except for BILAG gastrointestinal domain activity, which was highest in SLE (p>0.05, Supplementary Table S1). SLEDAI-2K scores also differed significantly, being highest in the MCTD group (p<0.001, Table 1).

We compared patient-reported outcomes for symptoms (pain-VAS, EMS-VAS, arthritis-VAS, fatigue-VAS and global health-VAS) quality-of-life (SF36-MCS, SF36-PCS, EQ5D-5L), participation (ICECAP-A) and fatigue (FACIT-Fatigue) across ANA-RMDs (Figure 2).

Patients reported similar disease impact on their quality-of-life across all SF36 domains and 5 visual analogue scores (Pain, early morning stiffness, arthritis, global health and fatigue). There were numeric but non-significant differences in FACIT-Fatigue scores (Highest in MCTD patients, F=1.767, p=0.12). Significant differences were observed in EQ5D-5L index scores between RMD
groups (F=2.564, p=0.03) which were lowest in MCTD patients (0.43) and the EQ5D mobility
domain (F=2.611, p=0.03) which was lowest in pSS patients.

We then assessed whether patient-reported impact was associated with disease activity
(Supplementary Figure S2). Patient-reported VAS scores for pain, arthritis and early morning
stiffness correlated well with BILAG-MSK scores when comparing BILAG A/B and D/E disease
(p<0.05 in all). FACIT-fatigue scores also showed a significant correlation (p<0.05). EQ5D and
SF36 domains scores were not associated as tightly with articular MSK-BILAG scores, likely due to
confounding in composite scoring tools covering several domains.

Current therapeutics in ANA-arthritis

We assessed whether the similar clinical and immunological features of ANA-arthritis across
diagnoses were matched by therapeutic use. This significantly differed across diagnoses
(Supplementary Table S3). Current and previous biologic use was significantly associated with
diagnosis ($\chi^2=11.933$ and 12.335 respectively and p<0.05 both). Biologic use was greater in the
SLE group (18/90 (20%) previously received, 12/90 (13%) currently receiving) and MCTD group
(3/10 (30%) previously received, 2/10 (20%) currently receiving) compared to other diagnoses
(combined, 6/113 (5%) previously received, 5/113 (4%) currently received). Previous azathioprine
use was significantly greater in the SLE group and current mycophenolate use was greater in the
SSc group (p<0.001 in both). Among those currently on prednisolone, doses were notably higher in
the IM group (7.83mg, p<0.05). These differences may reflect current guideline impact on practice
but may be unjustified given the relative homogeneity in immunological and patient-reported
aspects across diagnoses.

Regarding therapeutic confounders, among the 7 model covariates, only 3 exhibited significant
associations: Chromatin antibody positivity correlated with higher hydroxychloroquine use (27.0%
vs 14.3%, p=0.04), lower mean lymphocyte counts with increased azathioprine prescription (1.0 vs
1.42, p=0.01), and current mycophenolate treatment with higher previous rituximab therapy rates
(34.6% vs 9.1%, p<0.01) and lower mean IMD-rank (15582.72 vs 8990.35, p<0.01).

Alternative predictors of disease outcomes

Statistical analysis with paired t-tests revealed several ENA subtypes linked to increased disease
activity (defined by PGA). Sm, SmRNP, and RNP antibody positivity were all associated with
significantly PGA scores (p-values 0.016, 0.008, and 0.005, respectively; Supplementary Figure
S4).

Machine-learning reclassification of ANA-arthritis

Existing SLE trial designs recruit active disease within individual diagnoses. We estimated the
proportion of patients with disease activity likely to be suitable for immunosuppressive therapy
(BILAG A/B) among the 213 patients with MSK symptoms. 16 patients (7.5%) were identified with
a diagnosis of SLE and BILAG-MSK A/B disease. 30 patients (13.0%) had BILAG-MSK A/B disease irrespective of their diagnosis. These values indicate prevalent baskets of patients with active arthritis across the ANA-RMD spectrum, which we explored using machine learning. Gaussian mixture modelling identified 2 clusters (Table 2). Kmeans and hierarchical clustering were also trialled but were poorer identifiers of high BILAG-MSK disease activity patients than GMM. Overall, cluster 1 contained more patients with inflammatory features (High MSK Activity Cluster) and cluster 2 contained more patients with non-inflammatory causes of joint pain (Low MSK Activity Cluster). Cluster 1 comprised 89 patients (41.8%) including all patients with BILAG A/B MSK disease. Cluster 1 patients were younger with a lower proportion of UCTD and pSS. They included a higher proportion of SLE and MCTD patients with a lower mean Charlson Comorbidity Index (p = 0.002). Cluster 1 contained substantively lower numbers of patients with nodal OA (3.4% for cluster 1 vs. 11.3% for cluster 2, p = 0.065); radiographic evidence of OA (10.1% for cluster 1 vs. 19.4% for cluster 2, p = 0.10); fibromyalgia symptom pain and stiffness (9% for cluster 1 vs. 19.4% for cluster 2, p = 0.058); fibromyalgia alldynia (3.4% vs. 10.5%, p = 0.093). RNP/SmRNP/Sm antibody positivity was significantly greater in Cluster 1 patients. Numeric BILAG, ESSDAI, SLEDAI and physician global assessment scores were all significantly higher in Cluster 1 patients (p <0.001 in all). Memory B-cell tetherin and Interferon score A expression was also significantly higher in Cluster 1 (p =0.018 and p = 0.021, note that with untransformed gene expression scores, numerically lower values represent higher gene expression). Cluster 1 patients received more frequent and higher dose prednisolone. PCA plots formed from the 7 GMM covariates are shown in Figure 3.

Key potential confounders, including IMD rank, mucocutaneous, renal, neurological, and gastrointestinal BILAG scores, as well as Rodnan skin score, showed no significant differences between the GMM-derived clusters. Interestingly, Cluster 1 patients exhibited higher MSK disease activity despite significantly higher rates of previous treatment with rituximab (14.6% vs. 5.6%) and mycophenolate (19.1% vs. 8.9%) (p<0.05 for both).

The distribution of patients between legacy diagnoses and new GMM clusters is shown in Figure 4 to illustrate potential trial stratification strategies. Conventional trial designs recruit patients with SLE and swollen joints. After reclassification, the High MSK Activity Cluster (cluster 1) included all those patients as well as larger numbers from other RMDs. All patients in the High MSK Activity cluster with swollen joints would be eligible for an ANA-arthritis trial design and represent twice as many patients as a conventional SLE trial design. The remaining patients in the High MSK Activity Cluster lacked swollen joints at assessment but were similar in terms of immune biomarkers and clinical impact. These patients may be hypothesised to have a higher rate of joint inflammation when assessed over a longer time period, under different glucocorticoid or other immunosuppressive medications, or with musculoskeletal imaging, which has been shown to detect joint inflammation in a larger percentage of symptomatic populations (25). Therefore, these
patients in the High MSK Activity Cluster may be additional candidates for therapy licensed for ANA-arthitis in clinical practice.


**Discussion**

This is the first work assessing the clinical impact and immune profile of arthritis across multiple RMDs in a systematically collected, richly-phenotyped, multi-disease cohort. We demonstrate that ANA-positive RMD patients with musculoskeletal symptoms contain a High MSK Disease Activity population that is homogenous in clinical features, patient-reported impact and immune profile. The existing classification had previously distributed ANA-arthritis patients into other groups based on their additional disease features, potentially resulting in unjustified variations in therapy. Instead, we suggest and define a novel classification that consolidates all ANA-arthritis patients into a single group. This classification can facilitate basket trials, new therapy indications and inform routine clinical care guidelines.

We identified few differences between patients with musculoskeletal symptoms across RMD diagnoses, including physician and patient-reported clinical outcomes and biomarkers, with the exception that MCTD was generally worse. As expected from previous work, patients with musculoskeletal symptoms contained a mixture of patients with objective disease activity along with patients with low disease activity and non-inflammatory explanations for pain collectively, or for each legacy diagnosis.

During machine learning (ML) analysis patients with MSK symptoms were not sorted according to legacy diagnosis. Instead, the GMM approach generated High and Low MSK Disease Activity clusters, showing greater homogeneity compared to diagnoses like SLE and undifferentiated CTD. The High Disease Activity cluster contained every patient with joint swelling, and other features such as higher physician global assessment, prednisolone dose and IFN-Score. The Low Disease Activity cluster contained more patients with features of fibromyalgia and osteoarthritis. Patient-reported outcomes like pain, fatigue, and quality-of-life didn't vary between clusters, as expected, given that both inflammatory and non-inflammatory causes of pain may equally affect patient experience. The High Disease Activity cluster included many patients without joint swelling, grouped based on other features such as prednisolone dose, Sm, RNP, Sm/RNP, Chromatin and Ro antibodies, lymphocyte count and IMD-rank. Although lacking documented joint swelling on the study visit day, these patients might exhibit joint inflammation on ultrasound imaging (as we previously demonstrated with the same antibody subtypes) or would present with joint swelling if the prednisolone dose were reduced or if assessed over a longer duration (25).

The High MSK Disease Activity group may be suitable for basket clinical trials. Current SLE clinical trials involve patients with diverse organ manifestations, requiring complex disease activity instruments to compare severity and response across different presenting complaints. These trials may also necessitate different standard-of-care therapies. These factors may have contributed to inconsistent trial results(14,15). Conversely, clinical trials in ANA-arthritis would recruit a more homogeneous population, despite different legacy diagnoses. This enables the use of robust
organ-specific outcome measures, such as the LAMDA, which combines swollen joint count, patient and physician VAS, and acute phase markers. In SLE, this principle is shown by a litifilimab phase-2 trial meeting its primary endpoint of joint counts, or baricitinib trials achieving MSK-specific secondary and exploratory variables(14,26). Multisystem disease activity tools would only be required to monitor for worsening in other organs. ANA-arthritis trials could recruit more patients, and possibly incorporate musculoskeletal imaging. The resulting evidence base would be relevant to a larger patient population than SLE, addressing a healthcare inequality.

Importantly the Low MSK Disease Activity group still had a significant symptom burden, but with less evidence of active inflammation amenable to immunosuppression. These patients could potentially be offered more appropriate non-immunosuppressive therapy modalities. Our data suggests a large patient population whose needs may not be met by current research and guidelines.

SLE management guidelines have been published, covering diverse areas including diagnosis, assessment, care delivery and therapeutics(27). Many recommendations in these guidelines are not specific to MSK manifestations. However, for other patients with ANA-arthritis there are no guidelines. Further research on the described population could enhance patient outcomes in routine practice. In DEFINITION, notably, a higher proportion of non-SLE patients had ANA-arthritis compared to SLE patients in terms of pure numbers.

Biomarker analysis can help determine whether a population is immunologically homogenous and appropriate for similar therapies. The biomarker results in this study are more consistent and logical than others reported in SLE patients with arthritis. In SLE, IFN-I Scores correlate with increased skin disease activity, but not always with increased MSK disease activity—in certain studies, MSK disease activity appeared lower in IFN high patients(13,22). In our study, the High MSK Disease Activity cluster showed significantly higher tetherin and IFN Score A expression.

While our study comprised a large and extensively phenotyped cohort, certain limitations persist. Notably, the sample sizes for some diagnoses were small, thereby limiting the generalizability of findings within these groups. Consequently, validation in other cohorts along with prospective studies are essential. Additionally, the diversity of the cohort was limited by the regional population from which it was recruited. South Asian patients were better represented in our study than many other cohorts, but other groups were under-represented. Longer follow-up and imaging data were unavailable in our study, future research should investigate these. The articular component of the BILAG MSK appears valid across these diagnoses but better instruments in development, such as the LAMDA and joint counts, should be validated.(28). Finally, although we measured a wide range of gene expression and flow cytometric biomarkers, there are others emerging in autoimmunity(11).
As patient age is included as a covariate in our model, we can explore an interesting concept regarding disease stratification. Patients with an index presentation such as lupus nephritis or interstitial lung disease are treated according to established guidelines. If these patients later develop a predominant musculoskeletal (MSK) manifestation, it may be appropriate to categorize them within the arthritis basket. Therefore, inclusion in a basket is not static for a patient throughout their disease duration; but dependent on their predominant issue at the time of assessment.

In conclusion, these data indicate that the ANA+ arthritis basket has more unifying than dividing aspects in terms of quality-of-life impact, therapeutic usage, and biomarker variables. We describe an alternative means to classify patients with arthritis across ANA-RMDs. Clinical trials in this population could generate larger effect sizes and make new guidelines and interventions available to more patients.

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Conflict of interest statement:

M.Y.M.Y. has received speaker fees from Roche and Novartis and consultancy fees from Aurinia Pharmaceuticals and UCB. E.M.V. has received honoraria from Novartis, AstraZeneca, Otsuka, Roche, UCB, Aurinia, Lilly, Alumis, BMS, GSK, Pfizer and research grants paid to his employer from AstraZeneca and Sandoz. All other authors declare no competing interest related to the work described in this manuscript.

Data availability statement:

Requests for original data may be made to the corresponding author on request.
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<td>Current Smoker (%)</td>
<td>10 (11.1)</td>
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<tr>
<td>IMD Rank (mean (SD))</td>
<td>12998 (10263)</td>
<td>15767 (10511)</td>
<td>18573 (9456)</td>
<td>15709 (9346)</td>
<td>17691 (13968)</td>
<td>9890 (11320)</td>
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<td>CCI Total (mean (SD))</td>
<td>2.00 (1.38)</td>
<td>2.04 (1.34)</td>
<td>2.91 (1.47)</td>
<td>2.10 (1.97)</td>
<td>1.67 (1.21)</td>
<td>3.29 (2.87)</td>
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<tr>
<td>FMS Pain/stiffness (%)</td>
<td>19 (21.1)</td>
<td>10 (13.0)</td>
<td>3 (13.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0.221</td>
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<tr>
<td>FMS Allodynia (%)</td>
<td>9 (10.0)</td>
<td>5 (6.5)</td>
<td>1 (4.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (14.3)</td>
<td>0.698</td>
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<td>Hypermobility syndrome (%)</td>
<td>3 (3.3)</td>
<td>3 (3.9)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0.883</td>
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<tr>
<td>Nodal OA (%)</td>
<td>6 (6.7)</td>
<td>4 (5.2)</td>
<td>3 (13.0)</td>
<td>1 (10.0)</td>
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<td>2 (28.6)</td>
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<td>X-ray proven OA (%)</td>
<td>11 (12.2)</td>
<td>13 (16.9)</td>
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<td>Lymphocyte count x 10^9/L (mean (SD))</td>
<td>1.27 (0.59)</td>
<td>1.53 (0.73)</td>
<td>1.43 (0.65)</td>
<td>1.13 (0.64)</td>
<td>1.15 (0.70)</td>
<td>1.32 (0.31)</td>
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<td>BILAG Numeric (mean (SD))</td>
<td>4.96 (6.01)</td>
<td>3.09 (4.45)</td>
<td>2.83 (2.81)</td>
<td>5.80 (5.73)</td>
<td>5.50 (7.18)</td>
<td>4.86 (4.38)</td>
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<td>ESSDAI Total (mean (SD))</td>
<td>2.46 (3.54)</td>
<td>1.86 (3.58)</td>
<td>2.52 (3.49)</td>
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<td>SLEDAI Total (mean (SD))</td>
<td>5.60 (4.10)</td>
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<td>2.83 (2.15)</td>
<td>6.00 (4.97)</td>
<td>5.33 (4.13)</td>
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<tr>
<td>PGA Q2 (mean (SD))</td>
<td>2.84 (2.17)</td>
<td>2.49 (1.88)</td>
<td>2.86 (1.81)</td>
<td>4.05 (2.36)</td>
<td>4.37 (3.87)</td>
<td>3.77 (1.91)</td>
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</table>

MSK inflammation was defined as current/previous active MSK disease as defined by the BILAG-2004 and ESSDAI criteria or any documentation of joint or tendon inflammation within the medical notes.

P values refer to Bonferroni-corrected ANOVA for continuous variables and Chi Squared test for categorical variables.
Table 2: characteristics of ANA-RMD clusters

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<th>Demographics</th>
<th>GMM Cluster 1</th>
<th>GMM Cluster 2</th>
<th>p-value</th>
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<tr>
<td>n</td>
<td>89</td>
<td>124</td>
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<td>Sex = M (%)</td>
<td>13 (14.6)</td>
<td>13 (10.5)</td>
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</tr>
<tr>
<td>Age (mean (SD), years)</td>
<td>44.28 (14.46)</td>
<td>53.01 (12.83)</td>
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<tr>
<td>Diagnosis (%)</td>
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<tr>
<td>SLE (n, % of Cluster)</td>
<td>48 (53.9)</td>
<td>42 (33.9)</td>
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<tr>
<td>UCTD</td>
<td>23 (25.8)</td>
<td>54 (43.5)</td>
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<tr>
<td>pSS</td>
<td>3 (3.4)</td>
<td>20 (16.1)</td>
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<tr>
<td>MCTD</td>
<td>9 (10.1)</td>
<td>1 (0.8)</td>
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<tr>
<td>IIM</td>
<td>4 (4.5)</td>
<td>2 (1.6)</td>
<td></td>
</tr>
<tr>
<td>SSc</td>
<td>2 (2.2)</td>
<td>5 (4.0)</td>
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</tr>
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<td>Other demographics</td>
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<tr>
<td>Charlson Comorbidity Index (mean (SD))</td>
<td>1.78 (1.16)</td>
<td>2.42 (1.65)</td>
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<tr>
<td>Non Inflammatory</td>
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<tr>
<td>FMS pain/stiffness (%)</td>
<td>8 (9.0)</td>
<td>24 (19.4)</td>
<td>0.058</td>
</tr>
<tr>
<td>FMS allodynia (%)</td>
<td>3 (3.4)</td>
<td>13 (10.5)</td>
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<td>Hypermobility syndrome (%)</td>
<td>2 (2.2)</td>
<td>4 (3.2)</td>
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<td>Nodal OA (%)</td>
<td>3 (3.4)</td>
<td>14 (11.3)</td>
<td>0.065</td>
</tr>
<tr>
<td>XR proven OA (%)</td>
<td>9 (10.1)</td>
<td>24 (19.4)</td>
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<td>Current Therapies</td>
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<tr>
<td>Current prednisolone (%)</td>
<td>41 (97.6)</td>
<td>14 (11.3)</td>
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<tr>
<td>Current prednisolone dose (mean (SD))</td>
<td>5.53 (7.70)</td>
<td>0.58 (1.68)</td>
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<td>Current HCQ (%)</td>
<td>50 (56.2)</td>
<td>65 (52.4)</td>
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<tr>
<td>Current MTX (%)</td>
<td>18 (20.2)</td>
<td>25 (20.2)</td>
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</tr>
<tr>
<td>Current MMF (%)</td>
<td>11 (12.4)</td>
<td>15 (12.1)</td>
<td>1</td>
</tr>
<tr>
<td>Current AZA (%)</td>
<td>9 (10.1)</td>
<td>12 (9.7)</td>
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<tr>
<td>Current RTX (%)</td>
<td>13 (14.6)</td>
<td>7 (5.6)</td>
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<td>Previous Therapies</td>
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<td>Previous MTX (%)</td>
<td>20 (22.5)</td>
<td>19 (15.3)</td>
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<td>Previous MMF (%)</td>
<td>17 (19.1)</td>
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<tr>
<td>Previous RTX (%)</td>
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<td>12 (9.7)</td>
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<td>GMM Cluster 1</td>
<td>GMM Cluster 2</td>
<td>p-value</td>
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<td>--------------</td>
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<td><strong>Previous Cyclo (%)</strong></td>
<td>10 (11.2)</td>
<td>14 (11.3)</td>
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<td><strong>Selected other clinical features (see supplement)</strong></td>
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<td>Raynauds (%)</td>
<td>32 (36.0)</td>
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<td>Alopecia (%)</td>
<td>25 (28.1)</td>
<td>14 (11.3)</td>
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<td><strong>Immunology</strong></td>
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<td>dsDNA (%)</td>
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<td>Ro60 (%)</td>
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<td>Ro52 (%)</td>
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<td>La (%)</td>
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<td>Sm (%)</td>
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<td>SmRNP (%)</td>
<td>33 (37.1)</td>
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<td>RNP (%)</td>
<td>19 (21.3)</td>
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<td>Chromatin (%)</td>
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<td><strong>Clinical/Lab tests</strong></td>
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<tr>
<td>Lymphocyte count x 10^9/L (mean (SD))</td>
<td>1.30 (0.69)</td>
<td>1.44 (0.63)</td>
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<td><strong>BILAG Scores</strong></td>
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<td>BILAG Numeric (mean (SD))</td>
<td>6.62 (6.64)</td>
<td>2.30 (2.76)</td>
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</tr>
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<td>BILAG Total (%)</td>
<td></td>
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</tr>
<tr>
<td>A</td>
<td>28 (31.5)</td>
<td>4 (3.2)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>16 (18.0)</td>
<td>21 (16.9)</td>
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</tr>
<tr>
<td>C</td>
<td>41 (46.1)</td>
<td>82 (66.1)</td>
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<tr>
<td>D/E</td>
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<td>3 (2.4)</td>
<td></td>
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<tr>
<td>B</td>
<td>15 (16.9)</td>
<td>16 (12.9)</td>
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<tr>
<td>C</td>
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<td>10 (8.1)</td>
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<td>D/E</td>
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<td>95 (76.6)</td>
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<td>BILAG MSK (%)</td>
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<td>22 (24.7)</td>
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</tr>
<tr>
<td>B</td>
<td>8 (9.0)</td>
<td>0 (0.0)</td>
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<tr>
<td>C</td>
<td>44 (49.4)</td>
<td>91 (73.4)</td>
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<tr>
<td>D/E</td>
<td>15 (16.9)</td>
<td>33 (26.6)</td>
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<tr>
<td>BILAG General (%)</td>
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<td>6 (6.7)</td>
<td>2 (1.6)</td>
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</tr>
<tr>
<td>C</td>
<td>5 (5.6)</td>
<td>1 (0.8)</td>
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<td>GMM Cluster 1</td>
<td>GMM Cluster 2</td>
<td>p-value</td>
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<td>High MSK Activity</td>
<td>Low MSK Activity</td>
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<td>N = 89</td>
<td>N = 124</td>
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<td>D/E</td>
<td>D/E</td>
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<td>121 (97.6)</td>
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<td>29 (32.6)</td>
<td>25 (20.2)</td>
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<td>D/E</td>
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<td>99 (79.8)</td>
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<td>BILAG Renal (%)</td>
<td>3 (3.4)</td>
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<tr>
<td>B</td>
<td>1 (1.1)</td>
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<tr>
<td>C</td>
<td>29 (32.6)</td>
<td>25 (20.2)</td>
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</tr>
<tr>
<td>D/E</td>
<td>59 (66.3)</td>
<td>99 (79.8)</td>
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<tr>
<td>Other physician disease activity measurements</td>
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<tr>
<td>ESSDAI Total (mean (SD))</td>
<td>3.79 (4.56)</td>
<td>1.12 (2.07)</td>
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</tr>
<tr>
<td>SLEDAI Total (mean (SD))</td>
<td>5.81 (3.97)</td>
<td>3.31 (2.56)</td>
<td>&lt;0.001</td>
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<td>Physician global assessment (mean (SD))</td>
<td>3.86 (2.39)</td>
<td>2.13 (1.55)</td>
<td>&lt;0.001</td>
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<td>Patient reported outcome scores</td>
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<tr>
<td>Pain VAS (mean (SD))</td>
<td>5.40 (2.49)</td>
<td>4.90 (3.07)</td>
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<td>EMS VAS (mean (SD))</td>
<td>6.10 (2.65)</td>
<td>5.51 (3.01)</td>
<td>0.204</td>
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<td>Arthritis VAS (mean (SD))</td>
<td>5.85 (2.93)</td>
<td>5.89 (2.91)</td>
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<td>Global VAS (mean (SD))</td>
<td>5.79 (2.59)</td>
<td>5.61 (2.86)</td>
<td>0.675</td>
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<tr>
<td>Fatigue VAS (mean (SD))</td>
<td>6.78 (2.62)</td>
<td>6.38 (3.05)</td>
<td>0.373</td>
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<tr>
<td>EQ5D-5L Index (mean (SD))</td>
<td>0.63 (0.20)</td>
<td>0.65 (0.23)</td>
<td>0.509</td>
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<tr>
<td>EQ5D Self Care (mean (SD))</td>
<td>1.99 (1.18)</td>
<td>1.69 (1.06)</td>
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<tr>
<td>ICECAP Total (mean (SD))</td>
<td>0.70 (0.22)</td>
<td>0.68 (0.22)</td>
<td>0.432</td>
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<td>SF36 Physical Component Score (mean (SD))</td>
<td>45.64 (7.32)</td>
<td>45.09 (6.23)</td>
<td>0.653</td>
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<tr>
<td>SF36 Mental Component Score (mean (SD))</td>
<td>36.44 (5.76)</td>
<td>36.15 (5.50)</td>
<td>0.778</td>
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<tr>
<td>FACIT Fatigue Total (mean (SD))</td>
<td>30.19 (12.39)</td>
<td>28.18 (13.85)</td>
<td>0.365</td>
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<tr>
<td>Biomarkers</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Memory B cell tetherin MFI (mean (SD))</td>
<td>52882 (31936)</td>
<td>41979 (20287)</td>
<td>0.018</td>
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<tr>
<td>Interferon Score A dCt (mean (SD))</td>
<td>4.33 (1.95)</td>
<td>4.92 (1.66)</td>
<td>0.021</td>
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<tr>
<td>Erythropoiesis Score dCt (mean (SD))</td>
<td>7.21 (1.38)</td>
<td>6.87 (1.19)</td>
<td>0.064</td>
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<tr>
<td>Inflammation Score dCt (mean (SD))</td>
<td>5.15 (1.41)</td>
<td>5.29 (1.14)</td>
<td>0.453</td>
</tr>
<tr>
<td>Memory B cells/PBMCs (mean (SD))</td>
<td>0.009 (0.01)</td>
<td>0.013 (0.01)</td>
<td>0.057</td>
</tr>
</tbody>
</table>
Figures
Figure 1: Study schematic

Healthy controls

SLE, pSS, UCTD, IM, MCTD, SSc

DEFINITION Cohort (n = 294)

Current or previous history of inflammatory joint/ tendon disease

ANA+ Non Arthritis (n = 79)

ANA+ Arthritis Cohort (n = 215)

Demographics

Age
Ethnicity
Smoking status
Charlson comorbidity index
Non-inflammatory features
Index of multiple deprivation
Current/past therapies
Cardiovascular risk factors

Clinical features

BILAG Scores
Physician global assessment
Other disease activity scores
Autoantibodies
Lymphocyte count

Patient reported outcomes

VAS - Arthritis
VAS - Global
VAS - Pain
VAS Early Morning Stiffness
EQ5D
ICECAP-A
SF36
FACT-Fatigue
WHQ

Gene Expression Scores

IFN Score A
IPN Score B
Myeloid Score
Inflammation Score
Erythropoiesis Score
Plasmablast Score

Flow Cytometry

Memory B cell subtypes
Memory B cells
Plasmablasts
NK1T cells
NK cells
Classical/nonclassical monocytes

Input covariates

Machine learning models (GMM, Hdbas, KNN)

Characterisation variables

New classification
SLE (Systemic Lupus Erythematosus), UCTD (Undifferentiated Connective Tissue Disease), pSS (Primary Sjögren's Syndrome), MCTD (Mixed Connective Tissue Disease), IM (Idiopathic Myositis), and SSc (Systemic Sclerosis)
Figure 2: PRO and biomarker data by diagnosis
SLE (Systemic Lupus Erythematosus), UCTD (Undifferentiated Connective Tissue Disease), pSS (Primary Sjögren’s Syndrome), MCTD (Mixed Connective Tissue Disease), IM (Idiopathic Myositis), and SSc (Systemic Sclerosis)
Figure 3: Collated PCA plots

A – Diagnosis

B – SLE with high total BILAG

C – SLE with high MSK BILAG

D – GMM based stratification
Figure 4: Sankey plot showing makeup of GMM high and low groups

SLE (Systemic Lupus Erythematosus), UCTD (Undifferentiated Connective Tissue Disease), pSS (Primary Sjögren’s Syndrome), MCTD (Mixed Connective Tissue Disease), IM (Idiopathic Myositis), and SSc (Systemic Sclerosis)
Figure 1: Study schematic

782x613mm (118 x 118 DPI)
Figure 2: Patient-reported outcome and biomarker data by diagnosis.

451x307mm (300 x 300 DPI)
Figure 3: Collated PCA plots showing different trial population stratification methods

640x360mm (302 x 302 DPI)
Figure 4: Sankey plot showing makeup of GMM high and low groups

338x190mm (600 x 600 DPI)
**Graphical abstract**

855x481mm (38 x 38 DPI)