declared, Maurizio Rossini Shareholder of: Abbvie, Amgen, Bms, Eli Lilly, Galapagos, Novartis, Pfizer, Sandoz, Theramex, Ucb.

**Disclosure of Interests:** have never received any personal payment from Industry.

Ailsa Bosworth Grant/research support from: NRAS

**Objective:** To evaluate the diagnostic performance of different combinations of imaging markers to derive data-driven imaging criteria for MRI in axSpA, separately for men and women.

**Methods:** A total of 1194 patients were included in the study. Considering the exclusion criteria (available MRI image data sets, confirmed diagnoses), 684 patients (379 axSpA and 305 control group) were included for further statistical analysis. Two trained readers scored the MRI images separately for the presence of ankylosis, as well as erosion, sclerosis, fat metaplasia, and bone marrow edema differentiated for 3 regions: support, ventral/mid/dorsal for sacro-ilial- and iliac-sided sacroiliac joint. Chi^2 test was applied to compare lesion frequencies per group. Contingency table analysis was performed to assess diagnostic performances. The diagnostic performances were compared using the diagnostic odd ratio (DOR).

**Results:** Overall, 136 female and 243 male axSpA patients were included. Higher prevalence for ankylosis (24.3 vs. 74%) and fat metaplasia (58.8 vs. 42.6%) was shown in male axSpA patients; in contrast, sclerosis was more common in female axSpA patients (75.0 vs. 57.6%). No sex differences in frequency were shown for bone marrow edema and erosion. In male axSpA patients, the most significant difference in individual parameters was shown for ankylosis (DOR 40.1) compared with females (DOR 4.7). The detection of erosion and fat metaplasia as markers was also better in male axSpA patients (DOR 17.6 vs. 11.1 and 18.6 vs. 6.3). Sclerosis and bone marrow edema were better suited in female axSpA patients (DOR 3.0 vs. 2.5 and 5.0 vs. 3.7). Overall, diagnostic accuracy was improved when only lesions in the middle and dorsal articular compartments were considered.

**Conclusion:** The diagnostic performance of established image markers on MRI is significantly lower in female axSpA patients. This is especially true for ankylosis, which provides the risk for false-positive findings in women. Based on these findings, future revisions of imaging criteria may include gender-specific recommendations to improve diagnostic accuracy for male and female axSpA patients.

**REFERENCES:**


**Disclosure of Interests:** Svetlog Tugce Ulas: None declared. Fabio Proft Speakers bureau: Novartis and UCB, as well as personal fees from AbbVie, AMGEN, BMS, Hexal, Janssen, MSD, Pfizer and Roche, Grant/research support from: Novartis, Eli Lilly and UCB, Torsten Diekhoff Speakers bureau: MSD, Novartis and Eli Lilly, Valeria Rios Rodríguez Speakers bureau: AbbVie and Falk e.V., Judith Rademacher Grant/research support from: Berlin Institute of Health (BIH) during the conduct of this study (Clinician Scientist Programme), Michael Podochny Grants/other support from: UCB3. Sarah Ohrndorf: None declared. Denis Poddubny Speakers bureau: AbbVie, Eli Lilly, MSD, Novartis, Pfizer, Bristol-Myers Squibb, Roche, UCB, Biocad, GlaxoSmithKline and Gilead, Grant/research support from: AbbVie, Eli Lilly, MSD, Novartis and Pfizer, Katharina Ziegeier Grant/research support from: Assessment of Spondyloarthritis International Society (ASAS) during the conduct of this study.

**DOI:** 10.1136/annrheumdis-2022-eular.1754
Background: Rheumatoid arthritis (RA) is characterised by relapsing joint and systemic inflammation, yet the immunopathological basis of these disease flares and their clinical prediction remain uncertain.

Objectives: Using mass cytometry and single cell RNA sequencing, we aimed to identify circulating lymphocyte subsets associated with RA flare, and identify potential cellular biomarkers to predict flare versus drug-free remission (DFR).

Methods: We analysed peripheral blood mononuclear cells (PBMCs) from patients recruited to the BioRRA study (Figure 1), a prospective clinical trial of conventional synthetic disease-modifying anti-rheumatic drug (csDMARD) cessation.[1] Patients with RA in clinical (DAS28-CRP < 2.4) and ultrasound (absence of power Doppler signal in 7 joints) remission stopped csDMARDs, with flare defined as DAS28-CRP ≥ 2.4 during 6 month follow-up. A 44-marker mass cytometry panel was used to profile PBMCs from 36 patients (20 flare, 16 DFR) at two time points (baseline, and flare onset / month 6 DFR). In a subset of patients (n = 12: 8 flare, 4 DFR), fluorescence-activated cell sorting of T and B cells was followed by single cell sequencing (n = 81,923 cells) incorporating 320 immune genes, 43 oligo-tagged surface protein antibodies, and TCR/BCR CDR3 sequence. Clones were defined as ≥2 cells with identical CDR3 nucleotide sequence, and clonal expansion as a significant increase in proportion from baseline to final study visit. Statistical significance was assessed after Benjamini-Hochberg multiple test correction (adj p < 0.05).

Results: Mass cytometry revealed 31 distinct cell clusters: notably, greater proportions of memory (CD45RO+/PD1+) CD4+ and CD8+ T cells, and memory (CD27+/CD21-) B cells, were observed at onset of flare versus baseline (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Cluster</th>
<th>Median %</th>
<th>Adj. p (GLMM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flare onset vs baseline</td>
<td>Flare patients</td>
<td>CD4+/CD45RO+/PD1+ memory T cells</td>
<td>2.14 vs 0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD8+/CD45RO+/PD1+ memory T cells</td>
<td>6.64 vs 0.07</td>
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<tr>
<td></td>
<td></td>
<td>CD19+/CD27+/CD21- memory B cells</td>
<td>2.39 vs 0.03</td>
</tr>
</tbody>
</table>

Single cell RNAseq (n = 8 flare + 4 DFR)

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Cluster</th>
<th>Median %</th>
<th>Adj. p (Wilcoxon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flare onset vs baseline</td>
<td>Flare patients</td>
<td>IgA+ plasma cells</td>
<td>0.37 vs 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD4+/CD25+/Foxp3+ Treg cells</td>
<td>0.55 vs 1.27</td>
</tr>
</tbody>
</table>

To better characterise these flare-associated subsets, single cell sequencing of CD45RO+/PD1+ CD4+ and CD8+ T cells, and CD19+ B cells, was performed and identified 21 distinct clusters. CDR3 sequencing revealed significant clonal expansion (Fisher exact, p < 0.05) at flare onset within five unique CD8+ clones (4 patients), one CD4+ clone (1 patient), and no B clones. Overall, there was a significantly greater proportion of IgA+ plasma cells at flare onset versus baseline. In contrast, a significantly lower proportion of CD25+/Foxp3+ regulatory T cells were present at csDMARD cessation (baseline) in subsequent flare versus DFR patients (Table 1), suggesting biomarker potential.

To further assess the predictive performance of CD4+ Tregs as a biomarker for flare versus DFR, we analysed PBMCs from an independent cohort of 50 patients (25 flare, 25 DFR) stopping csDMARDs in the ongoing BIO-FLARE study.[2] By flow cytometry, we confirmed a lower proportion of CD4+CD25hi Tregs at baseline in flare vs DFR (median 4.74 versus 6.37%; Wilcoxon p = 0.037; AUC: 0.67). In this cohort, stopping csDMARDs only in patients with elevated (>6.11% total CD4) baseline Tregs would have prevented drug cessation in 18/25 (72%) of flare patients; 9/25 (36%) of DFR patients would have continued csDMARDs unnecessarily.

Conclusion: We present a detailed longitudinal characterisation of circulating lymphocyte surface phenotype, gene expression, and clonal expansion in RA flare vs DFR. Furthermore our data, across two independent cohorts, suggests a role for CD4+ Tregs in promoting drug-free remission meriting further investigation, with potential for future clinical biomarker development.

REFERENCES:
[2] Rayner et al; BMC Rheumatology; 5:22

Acknowledgements: This work was funded by research grants from Wellcome Trust [102595/Z/13/A to KFB], Newcastle NIHR Biomedical Research Centre [B136167P/D00045 to KFB], British Society for Rheumatology [KFB], Academy of Medical Sciences [SG022/1074 to KFB], Newcastle University Wellcome Trust Translational Partnership [KFB], Newcastle Hospitals Charity [18033 to KFB], and a National Institute for Health Research Clinical Lecturership [CL-2017-01-004 to KFB]. Our work is supported by the Research into Inflammatory Arthritis Centre Versus Arthritis (RACE) (grant number 20298), and Rheuma Tolerance for Cure (European Union Innovative Medicines Initiative 2, grant number 777357), AGP and JDI are named as inventors on a patent application by Newcastle University ("Prediction of Drug-Free Remission in Rheumatoid Arthritis"; International Patent Application Number PCT/GB2019/050902). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health and Social Care.

Disclosure of Interests: Kenneth F Baker Consultant of: Modern Biosciences Ltd, Grant/research support from: Pfizer, Genentech, Fiona Rayner: None declared, Henrique Lemos: None declared, David McDonald: None declared, Gillian Hulme: None declared, Rafiquil Hussain: None declared, Jonathan Coxhead Speakers bureau: Tesaro, Arthur Pratt Grant/ research support from: Pfizer, Gilead, Amy E. Anderson: None declared, Andrew Filby Grant/research support from: Becton Dickinson, John Isaacs Speakers bureau: Abbvie, Gilead, Roche, UCB, Grant/research support from: GSK, Janssen, Pfizer.

DOI: 10.1136/annrheumdis-2022-eular.1341

OP0075

FIBROBLAST/MACROPHAGE CROSSTALK VIA LACTATE: NEW THERAPEUTIC TARGET IN RHEUMATOID ARTHRITIS

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Background: The synovial membrane is the principal site of inflammation in rheumatoid arthritis (RA) and distinct subsets of fibroblasts and macrophages, with different effector functions, have been described within it.[1] Inflammation renders the RA synovial microenvironment hypoxic and acidic, with increased levels of lactate, the end product of glycolysis. Lactate acts as an immunomodulatory molecule within the synovium, interacting with lactate transporters present on fibroblasts and macrophages to regulate their function, movement and metabolism.

Objectives: To test whether dysfunctional crosstalk between fibroblasts and macrophages, driven by lactate, promotes the persistence of synovial inflammation.

Methods: Synovial tissues (n = 8) from patients fulfilling the 2010 ACR/EULAR RA criteria were obtained by ultrasound-guided synovial biopsy. Osteoarthritis (OA) synovial tissues of subjects undergoing joint replacement were used as control group. Monocarboxylate transporter 1 (MCT1) and MCT4 expression on fibroblasts and macrophages was assessed via confocal microscopy. We used RA synovial fibroblasts and monocyte-derived macrophages to test the effect of lactate in vitro. Migration was assessed in trans-well plates or via scratch test assays. Seahorse was used to evaluate metabolic pathways. IL6 production was measured by ELISA. Biinformatic data were confirmed on publicly available scRNAseq datasets.

Results: We showed that: i) The expression of MCT1 and MCT4 which regulate lactate import and export respectively, is up-regulated upon inflammation. ii) Fibroblasts preferentially express MCT1, while MCT4 is more highly expressed by macrophages. iii) Lactate, at the concentration found in RA synovial fluid (10 mM), has divergent effects on the effector functions of these two cell types. In fibroblasts, lactate promotes IL6 production and cell motility; these effects are reduced by pre-treatment with a pan-lactate transporter inhibitor. In contrast macrophages respond to lactate by reducing migration, IL6 secretion and glycolysis.

Conclusion: The contrasting effects of lactate on macrophage and fibroblast migration, IL6 production and metabolism suggest that lactate represents a key