

Title: Seasonal climate variation, gut microbiota dysbiosis, and rheumatoid arthritis disease activity: A cross-sectional clinical-biomarker analysis of the gut-joint axis

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ROUND 1

Reviewer A: Iftekhar Hussain Bandhan, **ORCID:** 0000-0002-3800-6155, **COI:** None, **AI disclosure:** None

1. **Comment** The term environmental exposome is a vast thing, it includes climate, urban/rural environments, green spaces, social capital, chemicals, pollutants, diet, alcohol, tobacco, metabolism, gut flora, oxidative stress, etc. In this study, environmental exposure assessment was limited to temperature, humidity, and gut microbiome only. So, the title should reflect the actual scope of exposure assessment (seasonal climate variation and gut microbiome).
Author response: We agree. 'Environmental exposome' is a much broader construct than what was actually measured (ambient temperature, relative humidity, and gut microbiome/fecal biomarkers only). We have retitled the manuscript to name the two exposure domains actually assessed and the gut-joint axis mechanism under study. This concern was also raised independently by Reviewer G (Comment G-1, below); both are addressed by the same title change.
2. **Comment**): Defined DAS28-CRP remission as ≤ 3.2 , which corresponds to low disease activity rather than remission. Please correct according to standard definitions (remission < 2.6).
Author response: DAS28-CRP ≤ 3.2 corresponds to the EULAR-defined low-disease-activity category, not remission. We have corrected the disease-activity-state definitions to reflect standard EULAR thresholds, explicitly stating all three bands (flare, low disease activity, remission) so they are unambiguous.
3. **Comment** Why can't the observed variations be attributed to genetic or individual differences instead of seasonal changes, since the same cohort wasn't followed up across all four seasons? This should be clearly mentioned in the limitation (with explanation). / Several important confounders should be accounted for, including glucocorticoid use, NSAIDs, dietary patterns, physical activity, socioeconomic conditions, etc. These factors could influence both gut biomarkers and disease activity; either adjust for these variables in the analysis or acknowledge them as limitations.
Author response: Both points are well founded. This cross-sectional study sampled different individuals within each seasonal stratum rather than following the same patients longitudinally through all four seasons; inter-individual or genetic variability is therefore a plausible alternative explanation for the observed seasonal differences that cannot be excluded by the present design, and we have made this explicit in the Limitations. Regarding confounders, glucocorticoid use, NSAID use, dietary pattern, physical activity, and socioeconomic status were not systematically captured or entered into the regression models; rather than retrofitting unmeasured covariates after the fact, which would not be scientifically defensible, we have explicitly acknowledged this as a limitation, consistent with the option the reviewer's comment allows.
4. **Comment** Tables should be self-explanatory. Please add appropriate acronyms in the footnotes. Table 1 doesn't look good. Remove the 'Test Statistic' column and 'Male', 'RF negative', and 'Anti-CCP negative' rows from Table 1. Remove 'Footnote' title from Table 3.
Author response: All three structural requests have been implemented exactly as specified.
5. **Comment** The manuscript (Table-3) compares fecal biomarkers with RF and anti-CCP in predicting disease activity and concludes superiority of fecal markers. However, RF and anti-CCP are not intended as markers of current disease activity, but rather as diagnostic markers. Please reframe to emphasize that fecal biomarkers reflect current inflammatory status rather than implying superiority over serological markers.
Author response: This is a scientifically important distinction and we agree fully. The original framing implicitly treated RF/anti-CCP as competing 'activity' biomarkers that fecal markers outperform, when RF and anti-CCP serve a categorically different clinical purpose (diagnosis/classification of autoimmune status) and are not expected, by clinical design, to fluctuate with current inflammatory activity. We have reframed every instance of this comparison - Key Messages, Results, the Table 3 caption, the Discussion subheading, and the Discussion body - to state that fecal biomarkers are more sensitive to current inflammatory status, without implying absolute superiority.
6. **Comment** Please avoid repetition of results (showing the exact values) in the discussion section.
Author response: We removed restated numeric results (r , β , R^2) from the Discussion paragraph that simply repeated Table 3, retaining only the interpretive content and a cross-reference to Results/Table 3.
7. **Comment** Limitations stated only the cross-sectional design. It should include all the explanations I mentioned in the design section.
Author response: Addressed jointly with Comment A-6 above: the Limitations paragraph now explicitly states the same-cohort/genetic-variability caveat and the unmeasured-confounder list (glucocorticoid use, NSAIDs, diet, physical activity,

Reviewer G: Anonymous, COI: None, AI disclosure: None

- 8. Comment** The current title is quite appropriate; however, this study specifically investigates seasonal environmental change, gut dysbiosis, intestinal inflammatory biomarkers, and their association with RA disease activity. Thus, the current title may be too broad and does not adequately convey the novel gut-joint axis and seasonal aspects that are crucial to the results.

Author response: We agree; this concern converges with Reviewer A's Comment A-1. The title has been revised as described there to explicitly name seasonal climate variation, gut microbiome dysbiosis, and the gut-joint axis.

- 9. Comment** In Background or Methods, the authors should mention the study's cross-sectional design and the lack of causality implied by the association. The conclusion should be interpreted with caution since the study was cross-sectional.

Author response: Implemented in full. We added an explicit non-causality caveat to the Abstract Background and softened the causal language in the Abstract Conclusion.

- 10. Comment** The tables are well organized, accurately labeled, and effectively support the textual findings. For improved readability and consistency, minor edits to format and footnotes could be made.

Author response: Implemented. In addition to the structural Table 1 changes made in response to Reviewer A (Comment A-8a) and the Editor (Comments E-1/E-2), we added a footnote to Table 1 (previously absent, unlike Tables 2 and 3) and updated the Table 3 footnote to reference the new Figure 1.

- 11. Comment** There is some repetition of numerical results between the text and tables. The Results section could be streamlined by emphasizing key findings in the text while referring readers to the tables for detailed data.

Author response: Agreed and implemented. Two Results paragraphs that restated the full set of seasonal median/IQR values already presented in Table 2 have been condensed to state the key magnitude and direction of change with a table cross-reference, retaining quantitative anchors (fold-changes) only where needed for interpretability.

- 12. Comment** The Discussion is relevant and generally supports the main message of the manuscript by linking environmental and microbiome-related findings to RA disease activity, although some sections could be shortened to reduce repetition of results.

Author response: Addressed via the same Discussion-paragraph trimming described under Comment A-11 (removal of restated correlation/ β / R^2 values from the 'Differential Clinical Utility...' subsection), which was the principal locus of numeric repetition in the Discussion.

- 13. Comment** The strengths and limitations are well described, providing a balanced view of the study's contributions and constraints.

Author response: As with Comment G-6, the Limitations subsection has since been substantially expanded in response to Comment A-6 (same-cohort/genetic-variability caveat and unmeasured confounders); this strengthens, rather than contradicts, the reviewer's positive assessment.

- 14. Comment** The conclusions are generally supported by the presented data and analyses. However, causal language should be interpreted cautiously given the cross-sectional study design.

Author response: In addition to softening the Abstract Conclusion (Comment G-2), we identified and hedged one causally-worded sentence in the main-text Conclusion section that had not been explicitly flagged but falls squarely within the scope of this comment.

- 15. Comment** The overall length is appropriate for the scope and depth of the study, allowing sufficient detail without unnecessary repetition.

Author response: The word count has since increased modestly due to substantive additions required by other comments; see Comment A-16 for the updated figures (Abstract: 289 words; main text: 3,072 words), which remain appropriate for an original research article.

- 16. Comment** The English language in the manuscript is typically clear and understandable. Minor adjustments of grammar, style, and wording are suggested to increase readability and to fulfill the criteria of an international scientific journal.

Author response: Implemented. Beyond the two Introduction punctuation corrections described under Comment A-17, we performed a full manual read-through of the revised manuscript and did not identify further substantive grammar errors beyond those already corrected. As this comment did not include specific line references, we invite the reviewer to identify any remaining passages of concern in a subsequent round.

Responsible editor: M Mostafa Zaman, ORCID: 0000-0002-1736-1342

- 17. Comment** Table 1 is descriptive. No test statistic is necessary because there is no hypothesis testing, no group comparison.

Author response: This concern converges with Reviewer A's Comment A-8a (below), which independently requested removal of the same column.

18. Comment What are the values given in the parenthesis? Clearly indicate in the title or footnote.

Author response: Conclusion has been reduced from a 240-word paragraph to a 56-word, three-line summary that states only the main finding and the policy recommendation

19. Comment Tables and figures: total ≤ 6 and placed at end of manuscript.

Author response: Implemented. Table 1 previously had no footnote at all (unlike Tables 2 and 3); we added one that defines the parenthetical values and also documents the absence of a test-statistic column (Comment E-1).

20. Comment Table 3: Beta coefficients with their 95% CIs would suffice to transmit the message. The correlation coefficients values could be plotted in a graph having 11 panels. The graph should indicate r values in texts.

Author response: Implemented, with one transparent adaptation that we flag explicitly for the Editor's review. The Spearman r [95% CI] column has been removed from Table 3, leaving adjusted β (95% CI) and adjusted R^2 as the primary tabulated statistics, exactly as requested. For the graphical display, we created Figure 1, a single composite forest-style plot containing all 11 predictor-outcome associations from Table 3, grouped by category (gut biomarkers / serological markers / climate-biomarker associations), each line annotated with its exact r value and significance level in text, with 95% CIs shown where available, rather than as 11 physically separate panel frames.

ROUND 2

Responsible editor: M Mostafa Zaman, ORCID: 0000-0002-1736-1342

21. Comment Table 1: The final column for range (min-max) is not necessary; remove it.

Author response: Implemented. The Range (Min-Max) column has been removed from Table 1 in its entirety (column and all 11 data-row entries). A sentence was added to the Table 1 footnote noting the removal for transparency, consistent with the treatment of the Table 2 change below.

22. Comment Table 2: The final column has the Kruskal-Wallis test statistic and P values. P values alone would suffice.

Author response: Implemented. The final column header was changed from "H (p-value)" to "P-value", and the Kruskal-Wallis H statistic was removed from all 11 data rows, leaving only the (FDR-corrected) P-value in each cell. The footnote sentence describing the H statistic was rewritten accordingly.

23. Comment Table 3: Values are beta (95% CI). Therefore, the table title needs to be revised (it indicates correlation coefficients). Adjusted R-square needs clarification also. Is it model R-square? If so, many R-squares are very small. Those models are hardly informative.

Author response: The table title was revised from "Spearman Correlations and Adjusted Regression Estimates..." to "Adjusted Multivariable Regression Estimates (β , 95% CI)...", removing the inaccurate "correlation" wording since every value shown is an adjusted standardized β coefficient, not r. The footnote was rewritten to state explicitly that Adjusted R^2 is the overall (model-level) adjusted R^2 for each regression model as a whole, not a partial R^2 for the single predictor shown. We agree with the Editor's underlying concern: for the three climate-biomarker models (Temperature→Shannon diversity, Humidity→RF positivity, Climate Stress Factor→Fecal lactoferrin), Adjusted R^2 is ≤ 0.01 . The footnote now states this explicitly and characterizes these three models as explaining negligible outcome variance and as weak/exploratory rather than clinically informative, so readers are not misled by the significant beta coefficients alone.

24. Comment Figure 1 footnote refers to Table 3. Values in Table 3 are beta coefficients. Therefore, these are two different issues. If Table 3 and Figure 1 present the same information, we need to keep either one, not both. 95% CI values are not visible for r values close to 1.0. Kindly prepare the graph in landscape to make the 95% CI values visible. There is confusion about the legends shown in colours. These should be placed in a blank space within the graph to avoid any misunderstanding that they are r-values.

Author response: Implemented, with the rationale documented here for the Editor's consideration. (a) Table 3 and Figure 1 are retained as two separate, complementary displays because they report two different statistics, not the same information: Table 3 reports confounder-adjusted multivariable β coefficients, while Figure 1 reports bivariate (unadjusted) Spearman r. The Figure 1 legend and the Table 3 footnote were both rewritten to state this distinction explicitly and remove the earlier cross-reference that incorrectly implied they showed the same values. If the Editor prefers a single display rather than two complementary ones, we are glad to remove Figure 1.