

Reviewer 2

We thank the reviewer for her/his precious suggestions that will further improve our manuscript. We provide a point-by-point response to the reviewer's comments (marked in red).

The study also looked at the process of apoptosis (another form of programmed cell death) in these samples and found that the expression of BAX (a protein involved in apoptosis) was increased in CIA and RA compared to OA and PsA. On the other hand, the expression of Bcl-2 (an anti-apoptotic protein) was higher in CIA. caspase-3, an enzyme involved in the execution phase of apoptosis, was also found to be increased in SF of RA patients and associated with inflammatory and anti-inflammatory cytokines. Taken together, these results suggest that inflammatory SF is associated with genomic instability and aberrant cell subpopulations. Further research is needed to fully understand the mechanisms behind these observations and their potential implications for the treatment of inflammatory arthropathies.

1. One of the biggest problems with this paper is that there is insufficient supporting evidence for the conclusions, and the authors should have added differential expression at the protein level rather than just looking at it with PCR. The PCR results assess changes at the mRNA level only, which is not accurate enough.

We thank the reviewer for this important observation. Indeed, protein level should be determined to support the PCR analysis. We evaluated by western blot the proteins Bax and Bcl-2 on which we reported the most significant variations. The analysis was carried out on synovial fluids analyzed by PCR except for one OA fluid of which we won't have any more lisate to use. We reported the results in the supplementary file, integrating their description in the text.

2. Another problem with this paper is that it does not fully explore the molecular mechanisms. The analysis of cytokines, local inflammatory cell indices, and apoptotic genes obtained by the authors was predictable. Is it then possible that other pathways are responsible for these differences? For example, endoplasmic reticulum stress?

We agree with the reviewer that additional data on endoplasmic reticulum stress would add value to the conclusions. Unfortunately, the volume of SF collected was very variable between patients ranging from a few millilitres to maximum 10-12 ml and in case we perform WB to assess PCR results (query N.1), we won't have any more lisate to use. Furthermore, we couldn't use samples from new patients as it is essential to compare factors and pathways in the same samples. This mechanism is indeed very interesting in terms of unfolded protein response, and we will definitely consider this aspect for future studies.

The authors did not investigate this either. In addition, apoptosis should be coupled with the results of flow cytometry analysis to strengthen the credibility of the work.

We agree with the reviewer that results of flow cytometry analysis to strengthen the credibility of the work, however this type of analysis should be conducted on freshly extracted synovial fluid that are not available.

3. Evaluation at the cellular level would make the results of this paper more heterogeneous, which affects the cytological evaluation of leukocytes and weakens the reproducibility of the results of this paper. How did the authors address this issue?

As specified above, we agree and we performed additional analysis on Bax and Bcl-2 protein levels, but they were limited by the amount of the samples.