

## **Authors' Response to the Review Comments**

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**Title:** Increased levels of circulating platelet-derived microparticles in psoriasis: Possible implications for the associated cardiovascular risk

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Dear Editor,

We are very pleased to resubmit for publication the revised version of our manuscript. We greatly appreciate the time and effort taken by you and the three reviewers to revise our paper. We have addressed all issues indicated in the review report and next, we offer detailed responses to your comments and those of the referees. The responses/changes are also highlighted in yellow in the revised manuscript.

- **Responses to the Editor's comments**

Thank you very much for all your valuable guidance in the edited manuscript. Following your comments #1,#2, #3, #4, we have now added the postcode required in the affiliations of all authors. This is now highlighted in the revised manuscript. Following your comments #5 and #6, we also provide signed PDF documents related to academic rules and norms and an audio core tip. As suggested (comment #7), we have put the reference numbers in square brackets in superscript across the text. We have also added the part of COMMENTS in our manuscript, just prior to the REFERENCES section (comment #8). Reference No 5 was duplicated (Comment #9), so we eliminated it once (i.e. Reference No. 33 in the original

manuscript was deleted). Finally, as requested (Comment #10), we offered total title for Figure 1.

- **Response to comments from Reviewer 00646537**

❖ Comment 1:

“The lesser number of controls and absence of age/sex matching is one of the limitations of the study.”

Response:

Thank you for your valuable comment. We admit that this is a limitation of our study and have now inserted this as such. Please refer to page 16, lines 1-2 of the revised manuscript (“The small number of controls and the absence of age/sex matching between patients and controls should be acknowledged as study limitations.”). However, we should note that we did not observe any significant difference in baseline characteristics, including age and sex, between the two groups, as shown in Table 1 (page 25).

❖ Comment 2:

“Was there any correlation between severity of psoriasis in terms of PASI/joint involvement with the levels of platelet activation markers?”

Response:

We appreciate the reviewer’s insightful comment. Based on your guidance, we also investigated a potential correlation between platelet activation markers and the PASI score. Indeed, a significant positive association was demonstrated between PASI score and both the levels of larger-size PMPs and the levels of CD63 expression. Please refer to page 11, lines 16-18 of the revised manuscript (“Increased PASI score was associated with increased levels of larger-size PMPs ( $r=0.45$ ;  $P=0.011$ ) and increased CD63 expression ( $r=0.47$ ;  $P<0.01$ )”). Apart from the section of the RESULTS, this finding has also been cited in the ABSTRACT of the paper (refer to page 3 of the revised manuscript), and added to the section of the DISCUSSION (on page 15, lines 13-14).

Concerning the matter of a potential correlation between platelet activation markers and joint involvement in psoriasis, please note that psoriatic arthritis was one of the exclusion criteria we had included from the beginning for patients with psoriasis, as marked on page 7, line 2 of the revised manuscript.

❖ Comment 3:

“Were the selected psoriatic patients on any medications which could have affected these (platelet activation markers) levels?”

Response:

Thank you for your comment. Please note that the use of anti-platelet drugs and systemic steroids, which are likely to influence the outcome of the study, was one of the exclusion criteria we had included for both patients and controls, as mentioned on page 6, lines 26-28, and page 7, lines 4-5 of the revised manuscript. Also, any systemic therapy for psoriasis, which might also influence platelet activation marker levels, had been interrupted for adequate wash-out period, as documented on page 6, lines 25-26 of the revised manuscript. Finally, the use of anti-hypertensive or lipid-lowering medication which might also affect platelet activity were similar between the two study groups, as stated on page 6, lines 21-22 and also shown in Table 1 (page 25). Nonetheless, all patients and controls had ceased antihypertensive treatment and statins 48h before blood sampling (page 7, lines 26-27).

❖ Comment 4:

“Did the psoriatic patient undergo a detailed cardio-vascular evaluation?”

Response:

Thank you again for your comment. We now explicitly state on page 7, lines 5-8 of the revised manuscript that “ All patients underwent exercise treadmill test and/or stress echocardiography as well as carotid and peripheral artery ultrasonography before blood sampling to exclude the presence of clinical significant CVD.”

- **Response to comments from Reviewer 00186131**

❖ Comment 1:

“ The bias is the small number of controls.”

Response:

We very much appreciate the comment. The same issue was also raised by Reviewer 00646537. We do recognize that this is a limitation of our study and now explicitly state this on page 16, lines 1-2 of the revised manuscript (“The small number of controls and the absence of age/sex matching between patients and controls should be acknowledged as study limitations.”).

❖ Comment 2:

“ I suggest to discuss the role of IL-17 and Th-17 cells (see and add as reference Murdaca et al. concerning the topic).”

Response:

We appreciate this suggestion and find this very interesting, as well as the reference you urged us to add which was very clarifying on the role played by Th17 cells in various inflammatory diseases. We have therefore inserted the following part in the Discussion section concerning the role of IL-17 and Th17 cells: “IL-12 leads to the differentiation of type 1 T helper (Th1) lymphocytes, whereas IL-17A and IL-17F, secreted by type 17 T helper (Th17) cells, activate keratinocytes and induce the production of antimicrobial peptides. Notably, recent interest has focused particularly on IL-17-producing Th17 cells <sup>[40]</sup>. This cell type is specialized in immunosurveillance of epithelium, and it also secretes interleukin 22, a key cytokine linking adaptive immune effectors and epithelial dysregulation in psoriasis. Amelioration of epidermal hyperplasia during successful anti-TNF treatment is associated with reduced Th17 responses. Based on the current knowledge, it appears that Th17 cells are responsible for many of the inflammatory and autoimmune responses once attributed to Th1 lymphocytes.” (Please refer to page 14, lines 10-20). The relevant reference is listed as reference No. 40 in the revised manuscript (Please refer to page 23).

• **Response to comments from Reviewer 01016438**

❖ Comment 1:

"In this manuscript, authors establish a correlation between some markers of platelet activation (PMP), increase of inflammatory cytokines (IL-12, IL-17) and risk of cardiovascular diseases. Indeed the authors suggest that the cardiovascular risk of patients with psoriasis is linked to increased inflammation and increased PMP. However, all data reported by the authors are already known as the increase of IL-12, IL-17, and PMP in patients with psoriasis. The most interesting part of the manuscript remains the one in which speculate that the increase in PMP may be cause of heart disease."

Response:

Thank you for your comment. We totally agree with you that the increase of IL-12, IL-17, and PMP in patients with psoriasis was already known. Our study, however, adds to those findings by demonstrating for the first time, to the best of our knowledge, a positive relationship between PMP concentrations and cytokines IL-12 and IL-17, suggesting a close association between PMPs and high inflammatory disease burden. Given the ability of IL-17A to promote platelet function, this finding is in favor of the view that inflammation and platelet activation may perpetuate each other culminating in the development of atherosclerosis. Furthermore, this is the first study, to the best of our knowledge, to report a higher level of larger-size PMPs, additionally to small-size ones, in psoriasis patients. It is now accepted that PMPs are separated into four size classes with different active components and different functional effects on platelets and endothelial cells, and therefore, elucidation of the size class(es) involved in psoriasis can help clarify PMP involvement in the disease and the mechanisms implicated in exertion of their effects in future studies. But surely, we agree with you that the most interesting part of the manuscript concerns the speculation that the association between psoriasis and atherosclerosis may be related to excessive PMP formation.

❖ Comment 2:

"Minor suggestion: Authors can change the setting of the work, which can become a point of view or a brief review or a commentary, otherwise if they want to continue to present an

“Observational Study”, they must demonstrate that the increase of PMP induces a PASI higher and / or a greater number of cardiac pathologies.

Response:

We very much appreciate your direction towards examining a potential relationship between PMPs and PASI score. The same issue was raised by Reviewer 00646537. Background information on the topic was ambiguous, since in some of the previous studies, PMPs were shown to correlate with the PASI score, while in others, they did not (Please refer to Page 6, lines 5-7 of the revised manuscript regarding the topic). Based on your guidance, we therefore endeavoured to investigate a possible association between platelet activation markers and the PASI score. Indeed, a significant positive association was demonstrated between PASI score and both the levels of larger-size PMPs and the levels of CD63 expression. Please refer to page 11, lines 16-18 of the revised manuscript (“Increased PASI score was associated with increased levels of larger-size PMPs ( $r=0.45$ ;  $P=0.011$ ) and increased CD63 expression ( $r=0.47$ ;  $P<0.01$ )”). (Apart from the section of the RESULTS, this finding has also been cited in the ABSTRACT of the paper (refer to page 3 of the revised manuscript), and added to the section of the DISCUSSION (on page 15, lines 13-14)).

Unfortunately, the study design did not allow us to examine a potential relationship between PMPs and a greater number of cardiac pathologies. This issue could be addressed in a next-step research.

**Closing comments to the Editor:**

We again appreciate the opportunity you gave us to revise our work. We hope that the reviewed version can meet the journal publication requirements.