Author’s response to reviews

Title: Serum 20S proteasome levels are associated with disease activity in MPO-ANCA-associated microscopic polyangiitis

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We thank the Reviewer for the critical comments and useful suggestions; they helped us improve our manuscript. As indicated in the responses that follow, we have taken all comments and suggestions into account in the revised version of our manuscript. Modified and new text is underlined in the revised manuscript.

A point-by-point response

Reviewer 1 (Dr. Massimo Radin):
The authors have replied to all the comments.

Response: Thank you for your thoughtful remarks about our manuscript.

Reviewer 2 (Dr. Roberta Fenoglio):
The authors have answered all the questions requested. Now the paper is suitable to be published

Response: Thank you for your thoughtful remarks about our manuscript.
Reviewer 3 (Dr. Michael Gingold):
This article outlines the possible use of a novel serum biomarker, 20S proteasome, in monitoring disease activity in Microscopic Polyangiitis (MPA) and provides a platform for further research in this area. This study would need to be replicated in a larger, more heterogeneous vasculitis population that would also include patients with Granulomatosis with Polyangititis and would need to address some further key areas that are not sufficiently addressed in this study.

Major point 1:

The role of prednisolone in influencing results has not been adequately addressed. Supplementary file 1 outlines that the 30 patients with active MPA were on no treatment. The 30 patients with inactive MPA were all on some form of immunosuppression but specifically all 30 were on high doses of Prednisolone - none less than 30mg daily. Were they all on Prednisolone at the time of testing? If so, this needs to be explicitly mentioned in the text. If not, what dose of Prednisolone were they on at time of analysis? This needs to be clearer in the submitted text and if it has not been or cannot be addressed statistically this should be clearly commented on in the limitations section. This is likely to be an important confounder in the study.

Response: Thank you for your thoughtful remarks about our manuscript. As you mention, the described treatments of inactive vasculitis patients in Supplementary file 1 had been initial treatments of them. At the testing, no patients with inactive vasculitis had treated with any immunosuppressant. All those patients had treated with oral corticosteroids at the testing, and the mean dosage of prednisolone in those patients was 5.00 ± 1.97 mg daily. We additionally described treatments at the testing in text, and rewrite treatments at the testing from initial treatments in Supplementary file 1. In addition, we have additionally described the influence of treatments as a study limitation.

Major point 2:

The other concern is that serum proteasome 20S is a non-specific marker of an acute phase response or cell turnover and it is uncertain how its elevation adds to the assessment of a patient with active vasculitis. Having an additional comparator of other patients with stable autoimmune disease (for example rheumatoid arthritis or SLE) who were not taking Prednisolone at the time would be useful.

Response: Thank you for your thoughtful remarks about our manuscript. We could not prove the mechanism of elevated serum 20S-proteasome in active AAV. As you mention, we had preliminary more investigated serum 20S-proteasome levels in several patients with lupus nephritis before the corticosteroid therapy as possible (there is unfortunately no rheumatologist in our institute, so we could not investigated those levels in patients with rheumatoid arthritis), but it is difficult to investigate more new patients with lupus nephritis in the limited time (by May 3, 2020) under the outbreak of COVID-19. Among only three patients with lupus nephritis before treatments, there was no elevation of serum 20S-proteasome levels in those patients (410.18 ± 146.29 ng/mL) compared to those levels in active AAV patients (3414.6 ± 2738.9 ng/mL). Although elevated serum 20S-proteasome in active AAV may be simply reflected an acute phase response, we described several potential
mechanisms of elevated those levels as you mentioned because sample size was marked small in additional investigation. Moreover, we also described the necessity of further examination as a study limitation in the discussion part.

Minor point 1:

Further to major point 1 - average and/or Prednisolone dosing between the two groups with vasculitis should be illustrated in Table 1.

Response: Thank you for your thoughtful remarks about our manuscript. As you mention, we added the mean (and standard deviation) dosages of prednisolone in Table 1.