Author's response to reviews

Title: Intraindividual changes of dipeptidyl peptidase-IV in peripheral blood of patients with rheumatoid arthritis are associated with the disease activity

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Author's response to reviews: see over
Cover Letter- response to the reviewer

MS: 101331487160766 - Intraindividual changes of dipeptidyl peptidase-IV in peripheral blood of patients with rheumatoid arthritis are associated with the disease activity

Dr. James Mockridge
Executive Editor
BMC Musculoskeletal Disorders
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Dear Dr. Mockridge,

Thank you for the ongoing support and for all reviewer comments. We did our best to benefit from them and to improve the manuscript accordingly. The responses made to the questions are given below. We implemented the suggestions into the revised manuscript and marked them with yellow background.

Answers to the reviewers’ comments and the respective changes made in the manuscript:

1. The question posed by the authors is well defined. It is currently unclear whether the changes of DPP-IV in blood plasma and peripheral mononuclear cells relate to this or other disease activity on the intra-individual basis. Current knowledge is based in longitudinal studies.
2. The methods are appropriate and well described.
3. The data are sound.
4. The figures appear to be genuine, i.e. without evidence of manipulation.
5. The manuscript adheres to the relevant standards for reporting and data deposition.
6. The discussion and conclusions are NOT YET so well balanced and adequately supported by the data.

The Discussion part of the manuscript was widely reformulated with respect to the reviewer comments.

7. Limitations of the work are NOT so clearly stated.

A separate section “Limitations of the study” was added to the manuscript.

8. Authors clearly acknowledge any work upon which they are building, both published and unpublished (more can be cited, along with a longer discussion) if the word limit of the journal allows it.
9. The title and abstract accurately conveys what has been found.
10. The writing is acceptable.

- Major Compulsory Revisions

+ In my opinion, the results do support the possible role of DPP-IV in RA
We agree that our data suggesting the role of DPP-IV in RA associated pathophysiology only indirectly indicate a possible pathogenetic role of DPP-IV in RA. Thus the formulations were rephrased and made “less penetrant”.

+ It remains unexplained why are not differences in the DPP-IV-like enzymatic activity and DPP-IV expression in blood mononuclear cells between the RA and OA groups (while there are differences in the soluble serum DPP-IV) and, however, after therapy there are changes in CD26 expression on BMC of RA patients.

The Discussion section of the manuscript was modified. Increased DPP-IV/CD26 expression, but not enzymatic activity, on blood mononuclear cells of RA patients in comparison to healthy individuals was suggested by some reports. Muscat et al [1] suggested that higher RA disease activity was associated with increased DPP-IV expression in peripheral blood T cells, while patients with less active disease had DPP-IV expression comparable to healthy subjects. Ellingsen et al observed a significant increase of the DPP-IV/CD26 antigen expression in CD4+ T cells only in patients with chronic (median disease duration 11.5 years) RA [2], while in patients early after diagnosis (< 6 months), there were no differences in comparison to healthy controls [3]. Thus, the direct comparison of the so far published studies is problematic due to the variance of the disease activity within the experimental groups as well as different methodologies used for the disease activity assessment, different control groups (osteoarthritis patients vs. healthy individuals) used and variable duration of the illness in individual studies.

Together, the differences in the “DPP-IV phenotype” of blood mononuclear cells might be more easily identified on the basis of intraindividual comparison during the disease course, while they may be masked by the rather wide variability when comparing different patient cohorts.

In more general terms, are the authors suggesting a mechanism relating blood soluble and T cell surface DPP-IV expression? If so, extend on this (see below).

The Discussion was amended with respect to the reviewers suggestions. T cells from peripheral blood were suggested as the most likely source of plasmatic DPP-IV [4], but this remains speculative. We observed the simultaneous rise of the plasmatic DPP-IV and the decrease of the DPP-IV expression in T cells in 45% of the patients with the disease improvement. It remains currently unclear, whether these changes are directly related to each other or whether they are part of different biological processes occurring during the disease course. Different experimental and analytical strategies would have to be used to determine the mechanism(s) relating plasmatic DPP-IV to the expression of DPP-IV on the surface of T cells in general as well as during RA pathophysiology. This however was not the aim of the current study.

- Minor Essential Revisions
  + Check the order of cites in the text.
  + Check the style of the References section.
  + State the n= in the Figure 3 and 4 legends.
  + Systemic lupus erythematosus in discussion.

All minor essential revisions were implemented in the revised manuscript.

- Discretionary Revisions
  + Authors state in the Abstract that dipeptidyl peptidase-IV (DPP-IV) is a marker of activated lymphocytes but thats not true: There are many activated lymphocyte populations that do not express CD26 (check + Krakauer M, Sorensen PS, Sellebjerg F. CD4(+) memory T cells with high CD26 surface expression are enriched for Th1 markers and correlate with clinical severity of multiple sclerosis. J Neuroimmunol 2006;181:157, 64; or + Tejera-Alhambra

Instead, authors may state that CD26 is a marker of Th1 cells, as they do in the introduction.

+ In the introduction, DPP-IV functions as a T cell co-stimulatory molecule, is involved in the T cell activation and proliferation, and these effects seem to be dependent on its intrinsic enzymatic activity?.
There are many effects independent of the enzymatic activity described in the literature.

The Abstract, Introduction and Discussion sections of the manuscript were modified according to the reviewers comments.

+ As summary, the authors state that rise in the blood plasma DPP-IV-like enzymatic activity of at least 20% compared to the patients entry value is seen in 13 patients (72%) in association with the disease improvement. The enzymatic activity remained unchanged in 3 patients (17%) and decreased in 2 (11%). Similarly, 62% patients showed an increase of at least 20% of the entry DPP-IV concentration as determined by ELISA, 23% patients remained unchanged and decreased concentration was observed in 15% patients.

Regarding the enzymatic activity in blood mononuclear cells (BMNC), 13 (72%) patients exhibited at least a 20% decrease compared to the entry DPP-IV-like enzymatic activity. In 3 (17%), the enzymatic activity remained unchanged and in 2 (11%) it increased. Accordingly, MFI of DPP-IV/CD26 in lymphocytes decreased by at least 20% in79% patients, remained unchanged in 7% and increased in 14% of patients (see Figures 3 and 4)?.

For the putative model mentioned above, it could be interesting to know whether positive, negative or no changes in one measure correlate with similar changes in the other measures at the individual level.
In addition it could be interesting to know if these positive, negative, or without changes are related or not to particular therapies.

We agree with referees interpretation of data plotted in Figures 3 and 4. Regarding the enzymatic activity in blood mononuclear cells (BMNC), 12 (66%) patients exhibited at least a 20% decrease compared to the entry DPP-IV-like enzymatic activity. In 3 (17%), the enzymatic activity remained unchanged and in 3 (17%) the activity increased.

Thus the attenuation of RA disease activity was accompanied by a rise of the blood plasma DPP-IV and a reduction of DPP-IV expression in blood mononuclear cells in the majority of patients. At a cut-off level of a 20% change in comparison to the entry value, 45 % of the patients exhibited both of these changes concurrently-the information on the overlap of the changes was added to the Results and Discussion sections of the manuscript.

The effects of particular therapies cannot be reliably determined due the heterogeneity of treatment in our RA patient cohort.

References:

We are thankful for the helpful comments received.

Sincerely yours,

Prof. Aleksi Sedo, MD, PhD