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

Authors:

Lucie Sromova (lsrom@lf1.cuni.cz)
Petr Busek (busekpetr@seznam.cz)
Liliana Sedova (sedo@revma.cz)
Aleksi Sedo (aleksi@cesnet.cz)

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Author's response to reviews: see over
Dear Sir/Madam

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We would like to submit our MS “Intraindividual changes of dipeptidyl peptidase-IV in peripheral blood of patients with rheumatoid arthritis are associated with the disease activity” for consideration to be published in BMC Musculoskeletal Disorders as a research article.

The first speculations about the involvement of Dipeptidyl peptidase –IV in RA pathogenesis comes from the late eighties. Later studies demonstrated the role of DPP-IV in immunoregulations and led to the assumption of diagnostic and therapeutic potential of DPP-IV (for review Sedo et al., Arthritis Res Ther 2005, 7: 253-69).

In the submitted MS, we demonstrate that blood plasma DPP-IV enzymatic activity and concentration are decreased in RA patients during the active phase of the disease and the intraindividual comparison for the first time directly proves their increase in connection with the disease improvement. Concurrently, the DPP-IV/CD26 in peripheral blood mononuclear cells decreases. These results further support the possible role of DPP-IV in the pathogenesis of RA probably due to its participation in inflammatory processes.

The study has been approved by the local ethics committee of the Institute of Rheumatology in Prague under the code EK-C327LS/06.

The MS has not been published elsewhere. Its previous version was submitted to Arthritis Research & Therapy. In the current version, the majority of the changes suggested by the reviewers were implemented.

Response to the reviewers' comments:

Reviewer 2:
1) The running title “DPP-IV parallels RA activity” is misleading since there is differential DPP-IV response in plasma and mononuclear cells. It requires modification.

   The running title was removed.

2) Figure 1 legend needs modification. It can read as “The box plots of blood plasma DPP-IV-like enzymatic activity and concentration in RA and OA patients. The medians are depicted as small squares, interquartile range (25-75th centile) as boxes and bars represent the maximum and minimum values.”

   The figure legend was reformulated accordingly.

Reviewer 3:

However, their interpretation of the results is not supported by their data. In their cohort of patients, from data of medians, they state that there is an increase of the blood plasma DPP-IV-like enzymatic activity and DPP-IV concentration after the therapy while in contrast to the blood plasma, the DPP-IV expression in blood mononuclear cells was reduced in these patients as evidenced decrease in the cell surface DPP-IV-like enzymatic activity as well as the median fluorescence intensity of DPP-IV staining in lymphocytes.

The main message of the MS is based on the intraindividual comparison performed in RA patients (Wilcoxon pair test) and not on the comparison of the median values in patient groups. The results section of the MS was
slightly reformulated to make this more apparent for the readers. The medians (calculated from the relative change of the entry/active and follow-up values in the INDIVIDUAL patients, not as a characteristic of the whole groups of “active RA” versus “follow-up” patients) are shown in some cases just to illustrate the magnitude of change between the entry and follow-up values in the analyzed patient cohort.

*Most important: After watching their figures I see that there are 2 or 3 individuals that show the stated behavior with strong changes in the data. However, most of the patients in fact show the opposite behavior, so the statistic result is not biologically informative.*

The figures in the MS are consistent with our interpretation. For example, a rise in the blood plasma DPP-IV-like enzymatic activity of at least 20% compared to the patient’s entry values 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% in 79% patients, remained unchanged in 7% and increased in 14% of patients (see Figures 3 and 4).

Although the observed changes were variable (as can be expected in the clinical setting), the statistical analysis using the Wilcoxon pair test suggests that the observed differences are caused just by chance with a probability lower than the generally accepted value of 5% (the p values for individual comparisons are shown in the MS text).

*Moreover, there is no information about the type of therapy in the patients, which could be relevant to explain my commentary above.*

The information on the type of treatment administered was added to the discussion section. The variability of the various treatment types based on the clinical judgment of the attending physician precluded the analysis of the effect of individual treatment modalities. Nevertheless, we observed a significant intraindividual increase in the blood plasma DPP-IV and a decrease of DPP-IV in BMNC in patients with decreased RA activity.

*Finally, the flow cytometry results are too simple to support their conclusions. The behavior of CD26 staining in T lymphocytes is very complex as it can be checked in the following references: 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. Tejera-Alhambra M, Casrouge A, de Andrés C, et al. Low DPP4 expression and activity in multiple sclerosis. Clin Immunol 2014;150:170-83.*

We are aware of the by some authors hypothesized importance of CD26$^{\text{high}}$ T lymphocytes in the pathogenesis of autoimmune diseases including rheumatoid arthritis. Our flow cytometry data however did not allow a reliable distinction of the CD26$^{\text{dim}}$ and CD26$^{\text{high}}$ T cells in all patients, we therefore limited our conclusions to the overall positivity (as characterized by MFI) of the CD26$^{+}$ population.

There is neither link with commercial sources nor other financial interest or conflict of interests associated with the MS. All authors agree with the submission of the MS and its eventual publication in BMC Musculoskeletal Disorders.

Sincerely yours,

Prof. Aleksi Sedo, MD, PhD