Author's response to reviews

Title: Effect of therapeutic plasma exchange on plasma levels and total removal of adipokines and inflammatory markers

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Author's response to reviews: see over
Response to Tom Barber

Major compulsory revisions:

As the authors acknowledge, this study only relates to those subjects who are undergoing TPE for clinical purposes due to some underlying condition that warrants this treatment. What about subjects with obesity and T2D? How does TPE affect sICAM-1 in these subjects? I think this important limitation needs to be made clear in the revised abstract, given that one cannot necessarily infer the same conclusions for an obese cohort based on the data on TPE being used for clinical purposes in patients with conditions such as MS for example.

We thank the reviewer for this important suggestion. As requested, we performed a BMI subgroup analysis. We included 10 patients with a BMI < 25 kg/m² and 11 patients with a BMI >25 kg/m². iCAM-1 reduction by TPE was highly significant in both groups (pre vs. post TPE levels of 502.7 ± 44.44 vs. 234.7 ± 20.84 ng/ml for BMI <25, p=<0.05 and 514.7 ± 62.88 ng/ml vs. 286.1 ± 42.72 ng/ml for BMI>25, p=<0.05), while none of the other investigated molecules showed a prolonged decrease during plasma exchange therapy. However, interestingly resistin pre TPE serum levels were significantly lower in the obese patient group. This is counterintuitive as resistin levels normally increase with BMI in healthy controls. One possible explanation is a higher prevalence of women in the lean patient group (7 of 10 patients were women). The limited amount of patients with diabetes allowed no valuable subgroup analysis in diabetic patients. We now state the variety of underlying conditions as a limitation in the manuscript.

There are grammatical errors throughout and some spelling errors which need to be corrected.

We thank the reviewer for this comment. The manuscript was revised accordingly.
The number of subjects (n=21) is relatively low. Does this provide sufficient power? Were power calculations performed a priori?

A sample size of 20 subjects was calculated to detect a 15% difference between pre- and post-TPE adipokine levels using a two sided t-test and an alpha of 0.05 with a power of 0.80.

**Minor essential revisions:**

The sexual dimorphism regarding resistin is interesting. Would the authors like to speculate on the possible reasons for this sex difference in the effects of TPE on resistin levels?

We were not the first research group that found gender specific differences in adipokine levels. Steppan et al. reported gender specific differences in rodents in their resistin description in 2001 [1]. The authors speculate in a later review that gender differences may be due to differences in body fat distribution or hormone levels [2]. Resistin levels may be influenced by hormone changes in the female body, as mean resistin levels are approximately two times higher during premenopause compared to peri- or postmenopause in a recent study [3]. However, estradiol administration or ovariectomy did not alter resistin serum levels in women [4]. In conclusion, the gender dependent difference in resistin levels requires further clarification, which our study cannot provide, as it was not the objective of our study.

Plasma levels of many of the adipokines analysed pre- and post-TPE did not change much. What is the explanation for differences in effects of TPE on plasma levels of sICAM-1 versus the other non-changed adipokines assessed? Is it related to molecular size? Some discussion of possible explanations for this phenomenon would be desirable.
Different pre and post treatment adipokine levels are more likely influenced by the different half-life times of the different molecules than by molecular size, as they are quite similar in a range of 12-89 kDa and far lower than the molecular cut-off of TPE (>1000 kDa). Especially as s-ICAM-1 (89 kDa) is the biggest as well as most strongly eliminated molecule. Leptin, as it is not significantly altered by TPE, has a half-life of 25 min in the human body [5]. Unfortunately, the half-life of sICAM-1, the most constantly influenced molecule, is not defined yet and most likely highly variable in different diseases. However, sICAM-1 release in human epithelial cells induced by TNF-alpha needs up to 24 hours to achieve a steady state sICAM-1 concentration [6]. Therefore the sICAM-1 serum dynamic is presumably slower than the one of leptin.
Respone to Milan Piya

This study was conducted in a population that had an immunological condition which is likely to result in a different baseline level of the adipokine before 1st TPE. Are the authors aware of how different the levels were compared to previous reported levels in the literature for ‘healthy’ populations? Please reference on line 198 and explain what the differences are. And although not powered for this, have the authors noticed a difference in these levels based on BMI? The reported BMI of subjects was 25.1±5, which suggests that some subjects were obese and some were lean?

We now provide a brief BMI subgroup analysis. However, in our point of view the patient numbers are to small to find any conclusions. Adipokine levels of healthy volunteers differ highly from our patient population (e.g. mean leptin levels of 16 ng/mL are reported for healthy subjects [7], while we found a mean pre TPE leptin level of 178.5 ng/ml). sCD40L levels in former reported control groups are about fourfold lower as in our patient collective [8], likewise the serum levels of sTNF-R [9], sICAM [10] and MCP-1 differ to those in the healthy population. Resistin levels in our patient population are comparable to healthy volunteers [11]. This comparison is now stated in the manuscript.

The time duration between TPE sessions was 25±5 hours. Did the authors notice a difference for the longer intervals compared to the shorter ones? The authors need to mention this as a limitation as ideally the TPE sessions should have been done with the same interval if it was a clinical trial, but as the authors mention, it was performed as part of their clinical care.

The variation in time duration between TPE sessions is due to practical clinical reasons, as all patients were occasionally occupied with concurrent diagnostic and therapeutic procedures. We
agree with the reviewer and state this limitation now in the respective section. We were not able to find significant pre TPE II serum level differences in patients with a treatment interval of more or less than 24 hours.

The sample size is small and should be mentioned in the limitations section of the paper. Also, is there enough power to detect differences between gender, given that the total sample size is already quite limited?

We agree with the reviewer and state this important limitation now in the limitations section. However, gender specific differences of resistin blood levels are described before and therefore plausible despite the small and patient number in this trial.

The eBiosciences FlowCytomix Human Obesity 9plex kit was used to analyse the plasma samples. Have the authors validated these measurements compared to single plex assays, or are the authors aware of any such validations in the literature or done by the manufacturer? Often, multiplex assays will measure some of the proteins accurately and other proteins less accurately and this would influence results, especially in small sample sizes like in this study. Please reference.

This methodological remark is often discussed when measuring multiple cytokines or chemokines with Multiplex /Luminex kits. The kits differ in sensitivity and reproducibility [12]. eBiosciences performed Simplex and Multiplex analyses and state that no cross-reactivity could be observed with the ‘Human Obesity 9plex kit’. In another project we compared the sensitivity of the 9plex kit as compared to a standard Elisa in terms of leptin measuring. We also found comparable results with both approaches.
Have the authors considered the effect of molecular weight on why some adipokine levels reduced with TPE while others did not? Please include in discussion. Or did the authors feel it was a different factor like solubility/charge/affinity etc? And why did the authors choose to measure sTNFR instead of the more commonly measured inflammatory markers TNFalpha or IL-6?

As mentioned in the response to the first reviewer, different pre and post treatment adipokine levels could be influenced by the different half times of the different molecules more likely as to their size characteristics.

TNF-receptors have been found on adipocytes. The soluble form of this receptors is released in the systemic circulation by proteolytic cleavage at the cell surface. In obese subjects amounts of sTNFR have been shown to be highly elevated [13]. Thus, apart from ‘classical’ adipose tissue derived cytokines the sTNFR is of specific interest as a possible marker of fat mass amount.

Minor comments:

There are a large number of spelling and grammatical errors, please revise. In line 60, the authors use the word ‘apprehended’. What does this mean here? In line 112-114, please revise the second sentence of the statistical analysis section as it is not clear what the authors are trying to say. Please revise conclusion as it is not clear what the key points of the study are.

We thank the reviewer and corrected the outlined mistakes accordingly.
References: