Reviewer's report

Title: Pre-radiotherapy plasma carotenoids and markers of oxidative stress are associated with survival in head and neck squamous cell carcinoma patients: a prospective study

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Reviewer: Matthias Kappler

Reviewer's report:

Major Compulsory Revisions
None

Minor Essential Revisions
1. Have you taken blood samples of patients and controls in the same way (in EDTA or heparin)? Have you measured the samples of patients and controls together in the same mensuration?

2. Could you explain the difference of total FRAP and modified FRAP (µmol/L)(Tab.2)? Because you have found 336 µmol/L modified FRAP in samples from healthy donor and 170 µmol/L in patients (pre-radiotherapy), which is a major difference of 166 µmol/L.

The sum of all FRAP (total FRAP) was 1055 µmol/L in the samples from healthy donor and 988 µmol/L in patients (pre-radiotherapy), which is a difference of 67 µmol/L. That means that modified FRAP is decreased in samples of patients (pre-radiotherapy) but on the other hand other FRAP (e.g. uric acid) is increased in patients (pre-radiotherapy) compared to healthy donors. Have you analysed uric acid in both groups separately?

3. Because of the interesting fact that d-ROM and total carotenoids are significant correlated (results, chapter HNSCC patients and controls) with stage of disease and your statement that total carotenoids as well as decrease in FRAP and increase in d-ROM is associated with progression-free survival, it would be more informative to show the adjusted Cox’s-regression analysis in Figures 1 than the Kaplan-Meier analysis.

4. Moreover in chapter results; pre-radiotherapy levels and change during radiotherapy it is written: “Similar results were observed for overall survival (data not shown)”. If the results are significant, you may show the calculations for overall survival too. Again, those results would be more powerful and interesting for the readers.

5. Eventually you may test in a two factor analysis if those patients with high total carotenoids and high increase in d-ROM have a better cumulative and overall survival compared to patients with low total carotenoids and low increase in d-ROM (Kaplan-Meier-Analysis and Cox’s regression analysis (uni-variate and adjusted)).
6. You should critically discuss the following literature in your manuscript:


Discretionary Revisions

1. In Chapter Method is written: “twenty-four received assisted nutrition during radiotherapy”. Have you found differences in the levels of antioxidants and oxidative stress biomarkers of those 24 patients compared to the other patients?

2. In Chapter results, change during radiotherapy is written: “Patients with a high relative decrease in the plasma levels of total antioxidant biomarker FRAP during radiotherapy have a prolonged progression-free survival as shown in figure 1B, that remained significant after adjusting for confounding factors (p = 0.05) (Table 5)”. That statement is critical, because p should be not rounded off (it must be p<=0.05).

3. In the same chapter:” The hazard rate ratio was as low as 0.3 showing that the patients with above median increase in d-ROMs during treatment period have 70% less risk of progression as compared to patients with under median increase in d-ROMs”. In general, it would be better for understanding to talk about relative risk. In that case it means that the patients with under median increase in d-ROMs during treatment period have 3.3-fold risk of progression as compared to patients with above median increase in d-ROMs.

Then it is written: “Similar results were observed for overall survival (data not shown)”. Those results would be more important and of interest for the readers. Please integrate those calculations for overall survival into the revised manuscript.

4. The conclusion:” Our results, thus, indicate that patients with an increase in systemic oxidative stress during radiotherapy had better progression-free survival suggesting that increased oxidative stress may be beneficial during treatment period. Our data shows that high pre-radiotherapy plasma total carotenoid status is beneficial for HNSCC patients. Thus, increased intake of carotenoids through fruits and vegetables and not supplements in the period before radiotherapy may be beneficial for HNSCC patients” is interesting. In my opinion you have another
very interesting conclusion. Although you demonstrated that d-ROM as well as
total carotenoids significantly correlated with the stage of disease
(pre-radiotherapy), you find out in adjusted Cox’s regression analysis, that high
total carotenoids as well as high increase in d-ROMs are good markers for
estimated progression free survival and as you mentioned overall survival. These
effects are independent of the stage of the patients and could help to detect
patients, which will not respond.

5. Why didn’t you adjust the stage of tumor in the calculation of survival risk for
the factors 8-ios PGF and increase FRAP (Tab. 5)?

6. In the abbreviation list the abbreviation for GGT should be in Latin.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a
statistician.

**Declaration of competing interests:**

I declare that I have no competing interests