Author's response to reviews

Title: Changed adipocytokine concentrations in colorectal tumor patients and morbidly obese patients compared to healthy controls

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Version: 2 Date: 28 July 2012

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

Please find attached a revised version of our manuscript entitled "Changed adipocytokine levels in colorectal tumor patients and morbidly obese patients compared to healthy controls" (MS no 1912167671706131) which we submit for consideration for publication in BMC Cancer. We have addressed the reviewer’s issues in our rewritten manuscript.

We appreciated the stimulating and helpful comments from the reviewers. We list, below, our responses to the issues raised on a point-by-point basis. These are in the format where I present the reviewer’s comments (verbatim) in italics and follow each point with our response in plain text. Actions taken are highlighted in bold.

Please do not hesitate to contact me in case of any question arising. Thank you very much indeed for your efforts.

Yours faithfully
Andreas Hillenbrand
General:
Final version of the manuscript was proofread by a professional editor / native speaker.

Reviewer 1

Major Compulsory Revisions:

1. Please state your objective in the abstract.
   We now clearly state our objective in the abstract.

2. Please state the conclusion of your abstract in one succinct sentence so that it answers your objective. Right now, your conclusion is a summary.
   The conclusion of our abstract is now written in one succinct sentence.

3. Throughout the manuscript, please combine one or two sentence paragraphs so that each paragraph has at least 3 sentences.
   Apart from method section, shorter paragraphs are now combined.

4. Please revise your objective and hypothesis in the last paragraph of the Introduction to make it more clear and concise.
   The last paragraph of the introduction has been rewritten with a clear definition of the objective.

5. Please do multivariate logistic regression with adjustment for age and gender for examining the effect of tumor stage and tumor grading on serum concentration and show your results in tables. Please remove Figure 2.
   In the new version of the manuscript Figure 2 has been removed. We performed a multivariate logistic regression with adjustment for age and gender regarding the effect of tumor stage and tumor grading on resistin concentration in the plasma. As proposed by our statistic division, the case numbers are too small to receive significant results after adjustment for age and gender. Nevertheless, we consider the correlation of resistin with tumor size and tumor grading to be of great interest and are therefore confident, that even without significance after adjustment for age and gender this is still to be considered an important result.

6. PAI-1 is a serine protease and not a cytokine. Please correct it throughout the manuscript.
In the new version this mistake is now corrected.

7. *MCP is a chemokine and not a cytokine. Please correct it throughout the manuscript.*
In the new version this mistake is now corrected.

8. Please provide the inter- and intraassay CV’s for your multiplex analysis kits.
The inter- and intraassay CV’s are listened in the revised version of the manuscript in Table 2.

9. Please state in the Statistical analysis section if and how you adjusted for multiple statistical comparisons.
The following sentence was added: No adjustments were made for multiple statistical comparisons.

10. Please rewrite completely your Discussion.
The discussion has been completely rewritten according to the reviewers’ suggestions.

11. Conclusion section: The goal of a conclusion is to answer your objective. Please revise accordingly.
The conclusion has been rewritten according to the reviewers’ suggestions.

*Minor Essential Revisions:*

1. In the manuscript you switch between plasma and serum samples. Please specify whether you used plasma or serum samples.
*We used plasma samples. This has been rectified.*

2. Throughout the manuscript, please change “levels” to “concentrations”.
*Throughout the whole manuscript “Levels” has been changed to “concentrations”.*

3. Statistical Analysis last Sentence: Please change the last sentence to “Statistical significance was declared at *P < 0.005* and a tendency at *0.05 < P < 0.10*”.
The last sentence of these paragraphs has been rewritten according to the reviewers’ suggestions.
4. Results 6th line. Please close the parenthesis at the end of the sentence. Please also induce the P-values for the gender differences.

Here, comparisons are drawn between six groups. The table shown below provides the p values thereby indicating that all p values except those of female MO vs. male MO are significant.

<table>
<thead>
<tr>
<th>Adipokine</th>
<th>Subgroup vs. Subgroup</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>BD female vs. BD male</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>CRC female vs. CRC male</td>
<td>p = 0.012</td>
<td></td>
</tr>
<tr>
<td>MO female vs. MO male</td>
<td>p = 0.007</td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>BD female vs. BD male</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>CRC female vs. CRC male</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>MO female vs. MO male</td>
<td>p = 0.147</td>
<td></td>
</tr>
</tbody>
</table>

5. Results 15th line: The p-value does not correspond with the p-value in Table 2. Please correct the p-value to “p<0.001”.

P-value has been corrected.

6. Results 19th line: Please change “were elevated just below the level of significance” “tended to be elevated”.

The sentence has been changed.

7. Table 2 Row 1 columns 6 and 7: Before ng/ml are <18 and <7. What does that mean? Please remove them.

These two values are the normal values for male / female leptin. There are no normal values for the other cytokines/adipkines. In order to avoid misunderstanding we therefore removed these two normal values.
Reviewer 2

**Major Compulsory Revisions:**

1. **Missing information on BMI in control group.**
   
   We have added a strengths and limitations section in our discussion. Therein, we listed several limitations.

2. **What is the rationale for comparing MO patients with CRC patients?**
   
   Obesity has been associated with increased incidence for colorectal cancer. A possible link between obesity and cancer is that deregulation of adipocytokines contribute to carcinogenesis. The aim of this study was to demonstrate that both colorectal cancer patients and morbidly obese patients have a deregulated cytokine / chemokine profile

3. **Abstract:** The 2nd last sentence in Conclusion is actually a result. Same sentence: The authors use the word “increased”, which should be replaced by “higher because the study is cross-sectional and the authors don not study changes in biomarkers over time.

   The abstract has been completely rewritten according to reviewers’ suggestions.

4. **Methods, p6:** The results on BMI difference between CRC and MO would be better placed in the Results section. The same is true for the results on age in CRC group. Even though there are several ways to present the anthropomorphic data, we believe it would be more appropriate to present these data in Methods and Patients section.

5. **Results, p. 9, 2nd parag.** “A gender specific difference…”. Did the author test it this differences is significant? Does this refer to results presented in Table 2?

   This refers to results presented in Table 2. We feel, however, that the presentation of all p values is excessive. The following table provides the P values:

<table>
<thead>
<tr>
<th>Adipokine</th>
<th>Subgroup vs. Subgroup</th>
<th>p value</th>
</tr>
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<tr>
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</tr>
<tr>
<td>CRC female vs. CRC male</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>
6. Results, p.9, 4th parag. “…(p>=0.01; …” Is p>=0.01 correct?
Thank you for your cautionary advice, in the new version of the manuscript it has been changed to p≤0.01.

7. Results, p. 10, 2nd last sentence. This sentence does not make much sense. Please rephrase.
This sentence has been rephrased:
Initial version: “There was no correlation of colon cancer with tumor size based T staging and tumor grading.”
New version: “In patients suffering from colon cancer, there was no correlation of resistin levels with tumor size based T-staging and tumor grading.”

8. Results p. 11, last sentence of results: how is proportionally elevated define? How do the authors decide if something is more or less proportionally elevated?
This sentence has rephrased:
Initial version: “Active PAI-1 is proportionally more elevated in MO compared to CRC. MCP-1, however, is proportionally more elevated in CRC compared to MO.”
New version: “While TNF-α and IL-1α plasma concentrations are comparable in MO and CRC, active PAI-1 reaches high concentrations in MO, MCP-1, however, reaches high concentrations in CRC.”

9. Figure 2 shows concentrations by stage, which should be tested by a test for trend or variance analysis. However, computing a correlation coefficient is not sufficient.
According to suggestion of reviewer 1 Figure 2 has been removed.

10. Table 2: The word “change” is not appropriate. Better use difference.
Change was removed. Due to lack of space it was not replaced with difference.

11. Figure 1: Please show n for groups (as done in Fig. 2).
In Figure 1 number of patients have been added.

12. P.12, last sentence in 2nd parag.: Reference 27 might be more appropriate than ref. 21.
Former reference 21 is now replaced by former reference 27.
13. P.12, 2nd last sentence: What does “gender specific homologous BMI” mean?

This sentence has been rephrased:

Initial version: “Since both patient groups have a gender specific homologous BMI”
New version: “Since both patient groups have approximately the same BMI”

14. Plasma adiponectin levels, like those of leptin, are more elevated in females than in males. Do the authors want to say that women have higher levels than men? If yes, just state like this.

Plasma adiponectin levels and leptin levels are higher in women compared to men (Fonseca-Alaniz MH et al.; Adipose tissue as an endocrine organ: from theory to practice. J Pediatr (Rio J). 2007 Nov;83(5 Suppl):S192-203)
Furthermore a negative correlation has been clearly demonstrated between adiponectin levels and degree of obesity. Leptin levels in obese are elevated. Gender specific differences and influence of obesity on adiponectin levels and leptin levels are well documented and is therefore not discussed further in this manuscript.

15. P. 14: “In further clinical studies….” Reference 31 is not a clinical study.

Refers number 31 referrers to Wei et al. in the former manuscript as in now resubmitted manuscript. Wei EK et al evaluated the association between adiponectin and colorectal cancer among 18 225 men in the Health Professionals Follow-up Study who provided blood samples. They present a prospective nested case-control study (Wei EK, Giovannucci E, Fuchs CS, Willett WC, Mantzoros CS: Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study. J Natl Cancer Inst. 2005 Nov 16;97(22):1688-94).
We have changed our sentence:
Initial version: “In several clinical studies”
New version: “In several further studies”


This refers to the previous reference (Gonullu G et al., ). In the new version of the manuscript we have added the references and the following reference: Kumor A, Daniel P, Pietruczuk M, Malecka-Panas E, Serum leptin, adiponectin, and resistin concentration in colorectal adenoma and carcinoma (CC) patients. Int J Colorectal Dis. 2009 Mar;24(3):275-81)
However, a recent study (Danese E et al, 2012) analyze gender specific, further recent studies analyzed only blood samples of female patients (Ho GY, et al, Cancer research 2012) or male patients (Otake S, et al., World J Gastroenterol. 2010). However, none of the above mentioned studies differentiated between colon and rectal cancer.

17. For resistin, there appears to be a difference by tumor grade, but no difference between rectal cancer cases and blood donors. Any idea why?

This question is very interesting and we have addressed this aspect to. However, at present we can only speculate. Further analyses will be necessary to address this issue in detail.

18. p 18, paragraph starting with “The relationship…” What is the connection between the first and the second sentence of this paragraph?

This part of discussion is rearranged in the new version of the manuscript.

In general:

The authors often use the word “increased”, “decreased”, and even “changed”, which are not appropriate in cross-sectional studies. Better use “higher, lower, and different”.

This issue has now been addressed.

Are there similar clinical studies? If yes, what are their results?

Recently, there are some studies published. Many of them are cited in the manuscript (Ref: 28, 29, [31] and 33). Most of them have similar result, some studies, however, report higher resistin levels in colorectal cancer patients (Salageanu et al, 2010; Gonullu et al, 2010, Kumor et al, 2009)

Median age of cancer patients is 20 years older than of the two other groups. Does age affect the level of the measured biomarkers? If yes, might it explain some of the observed differences?

As reported previously, ageing doesn’t seem to have a stronger impact on adipokine levels (Rouen Pa et al; Biol Res Nurs. 2010).

Strengths and limitations of this study?

At the end of the discussion, as section strengths and limitations was inserted.
The authors hardly discuss the results of the MO Patients. Why were they included? What is the benefit of this group? MO patients have much higher BMI than CRC patients and probably blood donors. How does this affect the results?

Most Adipokines are first described in MO patients and adipokines are well examined and described in morbidly obese. Further is the influence of adipokines in obesity related diseases like Heart Disease, High Blood Pressure, High Cholesterol, Type 2 Diabetes well described. MO are included to demonstrate parallels in the adipokine profile of MO and CRC.

Adipokine levels of BD are - largely independent of age - close to reported adipokine levels reported for healthy individuals. Of course, one of the main limitations of our study is the missing BMI level of BD.

The authors do not discuss that they did not take into account BMI and age as a confounder in their analysis.

We did a multivariate logistic regression with adjustment for age and gender, as stated above, ageing doesn't seem to have a stronger impact on adipokine levels (Rouen Pa et al; Biol Res Nurs. 2010).