Author's response to reviews

Title: The sweet and sour of serological glycoprotein tumor biomarker quantification

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Author's response to reviews: see over
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Ursula D'Souza PhD
Senior Editor
BMC Medicine

Dear Dr. D’Souza,

Please find attached, our responses to the reviewers’ comments. We hope that our manuscript now meets with your approval.

Yours sincerely,

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REVIEWER 1 (Dr. Jong Shin Yoo)

General Response:

The authors find the Reviewer’s comments correct and helpful. The majority of the comments focused on the absence of information on analytical mass spectrometry-based methods used for characterization of glycans and glycoconjugates. We intentionally left out some of these studies by trying to focus on works that dealt directly with the quantification of glycoproteins and their glycan moieties with clinical potential, going beyond analysis and characterization, as the title of manuscript suggests. Also, we have aimed this review at a wider audience (suitable to BMC Medicine), which may not be interested in the intricacies and details of MS-based analysis of glycans resulting in a broader and less detailed work (when considering the aspect of MS), than other reviews that can already be found in the literature. We try and refer the reader to a number of reviews in the text that deal with different MS-based characterization and analysis methodologies for study of glycoproteins (more have been added in the revision). However, we agree with the Reviewer that mention of some of the major works by the groups proposed by the Reviewer would benefit the manuscript as a whole. Therefore, the corrections and additions suggested by the Reviewer have been made.

Individual Comments:

1. Additional text and references have been added to the manuscript. Bottom of pg 14 and top of pg 15.
2. Additional text and references have been added to the manuscript. We would like to remind the reviewer that HILIC and PGC are methods for “enrichment and separation” of glycans (as stated in his comments), not for direct quantification (although they can be used for sample preparations) which is the focus of the review. Top of pg 15, 2nd paragraph pg 20.
3. References were added to reflect the Reviewer’s concerns. Pg 18, second paragraph.
4. The text in the manuscript has been changed and references have been added. Top of pg 19.
5. Text and references have been added to other examples in the literature which followed later in the section that the reviewer might not have noticed. The majority of these examples actually do utilize LC-MS. Pg 19, 2nd paragraph.
REVIEWER 2 (Dr. Yehia Mechref)

General Response:
We fully agree with the comments by the reviewer and required corrections have been made.

Individual comments:

Abstract, line 6, what are the reasons behind “spares and varied success”?
we believe that the abstract does not provide enough space to present these reasons, which are listed in detail in the main body of the manuscript.

Page 3, line 7, although the term “cellular” is correct, it might be better suited to use a different term, such as “cell”
correction made

Page 3, line 9, does this number include linkages and anomericity. This number is not really true.
correction made to reflect variability due to linkage type and monosaccharide anomers. Pg 3.

Page 3, bottom of page, the authors should also include that glycosylation is also dependent on the activity of the enzymes involved in trimming and addition of monosaccharides.
corrections made in the text. Pg 3.

Page 4, bottom of page, the activity of transferases and exoglycosidases also play a role in alteration of glycosylation in tumors. More references are needed in this part.
corrections made, references added. Bottom of pg 3 and top pg 4.

Page 5, lines 12-14, the names of the different glycoprotein need to be included here as well as valid references. This is also true on page 6 line 11.
references are found in Table 1 to which these sentences point to.
Page 5, last line, the two references included here are not the most recent. The authors are encouraged to include recent reviews pertaining to the subject matter.

references added. Top of pg 6.

Page 6, line 19, a reference related to the CA 19-9 assay is needed.

Reference added.

Page 7, the section entitled “The Potential and the Pitfalls” need to be included at the end of the manuscript, not this early.

We prefer for this section to remain in its current place, because we feel it is necessary to introduce the major subject and importance of the subsequent sections. We leave it to the discretion of the editor where to place this section.

Page 10, line 14, please replace “precious” with another term.

Correction made.

Pages 15-16, this review is focused on glycomics and glycoproteomics in cancer studies, it is not clear how references 72 and 73 are relevant to this manuscript.

References removed.

Page 17, there are two recent manuscripts describing the MRM of glycopeptides which should be described here.

We have included the paper by the reviewer’s group (Rapid Commun Mass Spectrom. 2012 Sep 15;26(17):1941-54), but we feel that the other suggested paper (Anal Chem. 2011 Jan 1; 83(1):240-5) is not relevant considering the fact that, if the article is examined closely, the MRMs performed were on fully de-glycosylated glycopeptides (ie. peptides, not glycopeptides). This paper is actually referred to earlier in the manuscript. Pg 19, second paragraph.

Page 18, bottom of the page, this is not totally true. The use of oxonium ions for MRM or SRM quantification appear to overcome the limitation listed. Please see Rapid Commun Mass Spectrom. 2012 Sep 15;26(17):1941-54.

Corrections have been made in the text. Top of pg 19.