

December 28, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6862-review.doc).

Title: Biomarkers for pancreatic cancer: recent achievements in proteomics and genomics through classical and multivariate statistical methods, a review

Author: Emilio Marengo, Elisa Robotti

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6862

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 References and typesetting were corrected

3 Revision has been made according to the suggestions of the reviewers:

REFEREE 00521168

- 1) *Cox regression is a general procedure for modeling the time to an event, not necessarily limited to survival.*

A comment was added to clarify.

- 2) *Some statistical methods that are described later in the review may be less familiar to some readers (Bayes factors, metropolis algorithm, etc) and could have been covered in more detail.*

The methods were included in the statistical section.

- 3) *The review does not follow a theme to keep it interesting and informative for the readers.*

The general scheme was changed according also to the comments by the other referees.

- 4) *In order to justify the search for biomarkers, the authors refer to the imaging methods for pancreatic cancer such as CT and MRI as 'invasive techniques' while in fact, these are not invasive. Inconvenient perhaps, but not invasive.*

The sentence was reformulated.

- 5) *Some terms used are unconventional or unknown, such as 'exiguous', 'monovariate', 'p-level', 'omoskedasticity' etc.*

Unconventional terms were clarified in the text or eliminated when possible.

- 6) *The authors also refer to 'deepening the mechanism of action of the disease' (top of page 4), which doesn't make sense as written. Maybe one could say 'to better understand the etiology of pancreatic cancer,' or something similar.*

The sentence was changed accordingly.

- 7) *I am sure the authors are aware of the difference between 'multivariable' and 'multivariate', but they do not seem to make this distinction.*

A comment was added to the text to specify how the term "multivariate" is used.

- 8) *The authors could perhaps distinguish between prognostic biomarkers and early diagnosis biomarkers – and also the biomarker types themselves (proteins, microRNAs, methylation, expression studies, etc) this might help to better organize the review which seems to jump from topic to topic.*

The markers were presented as suggested by the referee.

- 9) *The examples given are interesting and could be informative, but animal studies and cell studies are also covered and seem to be given equal weight to studies of human specimens – these could perhaps be separated more clearly as well.*

Studies on cell lines and animals were presented separately.

- 10) *The manuscript lacks critical analysis of the methodologies reviewed. Which methods work best, and in what situations, and for which type of biomarker?*

A comment was added in the conclusions.

REFEREE 02544938

1. *Structure of the manuscript is a little bit confusing. The authors used section “Discussion” for examples of biomarker analysis. I would skip the word “Discussion”.*

The section Discussion was removed.

2. *In Introduction the authors discussed severity of pancreatic cancer and claimed the importance of diagnostic markers for this disease. But the following examples of biomarker analysis are based on prognostic and predictive biomarker. The authors should give some examples of studies dealing with diagnostic biomarkers.*

Examples were provided.

3. *The authors discussed only statistical analysis of potential biomarkers, but did not point the important problem of biomarker development – namely – biomarker validation in the prospective studies, where the number of patients and biomarker cut-offs should be determined. Biostatistics design of such studies is very complicated and it would be nice to have in this review some ideas and opinions to this point from the authors as specialists in the field.*

A comment was added in the conclusions.

4. *The review is poor illustrated. Some discussed materials can be summarized in tables which will also facilitate reading of the manuscript. For example, the statistic methods and examples of biomarker studies can be summarized in tables.*

Tables were added.

5. *The provided information about pancreatic carcinoma is confusing: please refer new paper of Jemal “Cancer Statistic”. Please, structure the information as following: (i) how many patients could be operated at time of diagnosis (20%), how many with distant and local metastasis (not-operable); based on this please (ii) provide the 5-y survival for not-operable patients (about 1%), the 5-y survival after surgery (about 20% without an adjuvant therapy and about 25-30% with the therapy).*

The information requested was included.

6. *In Introduction the authors discuss only diagnostic markers, please provide general information about predictive and prognostic biomarkers.*

A comment was added in the text.

7. *There is too much background information about basic statistic. It is ok for a student book, but not for a review, please compress this and avoid the mathematical equations.*

Basic statistics was shortened and equations removed.

8. *Please, summarize the methods in a Table for better visualization.*

A table was added.

9. *Some methods are not mentioned in the section, but are discussed in “Discussion”: Monte Carlo simulation and OPLS, these are not trivial, please provide little information about these methods in the Section.*

The methods were included in the statistical section.

10. *Please skip the name of the section.*

The name was eliminated.

11. *In this section many different examples are presented in a mixture: (i) all marker types, (ii) sources of antigens: serum, tissues, other fluids, (iii) methods: ELISA, immunohistology, microarrays and so on. It is poor structured and very difficult to follow. Please, find a better structure for the section. I would suggest: refer the information based on the biomarker types: diagnostic, predictive and prognostic.*

A new structure was presented based on the referee's suggestion.

12. *Please, delete from the review information and examples for IPMN, this is far from pancreatic ductal adenocarcinoma.*

Information for IPMN was removed.

13. *I cannot understand the subtitle "Identification of biomarkers of pathology".*

The section was removed and the studies presented in other sections.

14. *Conclusion Please make real conclusions and give you statement about biostatistics for biomarkers in pancreatic cancer as specialists in the field.*

Considerations were included in the conclusions section.

REFEREE 02544224

1) *The single maker or combined makers by statistics methods seems not be fit for the clinical use in the future. How to solve the problems?*

A comment was added in the conclusions.

2) *Much of the manuscript is discussing the statistics and it is difficult for doctors to understand.*

The statistical section was revised according also to the comments of the other referees.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely your:



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April, 21, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6862-review2.doc).

Title: Biomarkers for pancreatic cancer: recent achievements in proteomics and genomics through classical and multivariate statistical methods, a review

Author: Emilio Marengo, Elisa Robotti

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6862

The manuscript has been improved according to the suggestions of reviewers:

REFEREE 02544990

- 1) *“However the statistical details have at the same time a risk of double edged sword for the readership of this journal’s specialty. The mere description of statistical methodology is quite difficult to follow.”*

The description of the statistical procedures was reduced and simplified. The section was not completely removed to fulfill also the first three referees’ suggestions.

- 2) *“Moreover, each of the established markers published based on the independent multivariate statistical methods are too descriptive”*

The description of the single studies was shortened.

- 3) *“The future perspective and cross-sectional approaches as the authors suggests in this papers should be more indicated in the latter part of the paragraphs”*

Comments were added, when possible, to compare different studies.

- 4) *“It would be strengthen the focus of this manuscript and future direction for exploratory biomarker search.”*

Some comments were added in the text.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

