Author's response to reviews

Title: Revisiting the technical validation of tumour biomarker assays: How to open a Pandora's box

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Author's response to reviews: see over
16th March 2011

Dr Lin Lee
Editor-in-Chief
BMC Medicine
The BioMed Central Editorial Team

Dear Lin,

Re: MS: 2041071702513335 2 Revisiting the technical validation of tumour biomarker assays (or how to open a Pandora's box)

Many thanks for your email from 14th March on the above manuscript.

We thank you and the reviewers for the constructive criticisms and suggestions, which have been incorporated in the manuscript.

Below please find a point-by-point response to the reviewers’ and editorial comments:

Reviewer #1
Minor Essential Revisions
In this mini-review Marchiò et Al draw attention to potential pitfalls in using biomarkers in medicine, with emphasis on assay validation by using immunohistochemical techniques. The paper is well-written, raises valid points and could undoubtedly contribute to improving the use of biomarkers in clinical practice by better validation of the analytical procedures used. I have a few suggestions to improve the manuscript by referencing relevant work previously published on this and related fields:
1. A commentary that could complement reference 5 regarding mass spectrometry-based serum proteomic analysis can be found in JNCI 2003;95:489-90
Authors: This work has now been quoted.
2. Another JNCI Editorial has drawn attention to various pitfalls in using biomarkers, and provides suggestions for future improvements. This work should be referenced: JNCI 2010;102:1462-7.
Authors: This work has now been quoted.

3. An important paper providing practice guidelines for use of tumour markers in the clinic, with special emphasis on quality requirements (pre-analytical, analytical and post-analytical) has recently been published (Clin Chem.2008;54:E1-E10 by Dr. Sturgeon and colleagues). This is a very relevant paper dealing with similar issues raised in this manuscript and should be referenced.
Authors: These guidelines and other two sets of guidelines also published in Clin Chem 2008 have now been quoted.

Reviewer #2
This review is interesting and should be accepted with discretionary revisions. Here are my suggestions:
1. In the introduction, the authors should briefly mentioned the new or other available biomarkers such as in blood (CTC, circulating DNA) and emphasize that not only tumor-derived biomarkers are to be submitted to careful validation and accurate protocols.
Authors: We have now mentioned in the Introduction the possibility to measure a biomarker not only on tissue samples but also on peripheral blood and other bodily fluids.

On page 3, para 1, we have added the following passage: “As the definition suggests, biomarkers can be used for multiple purposes in cancer research and measured on distinct types of specimens, such as tissue samples as well as peripheral blood (see for example circulating tumour cells) by using several assays.”

On page 3, last para, and page 4, para 1, we have added the following passage: “. It should be noted, however, that the concepts discussed in this review are applicable to biomarkers based on other types of samples (e.g. circulating tumour cells, blood, serum, urine and other bodily fluids).”
2. At the end of the last paragraph of “Validation: when and why?”, regarding internal quality control, I think it is important to give examples “breast cancer based” such as ER and PR and HER2 for which the normal glands should be respectively positive for ER/PR and negative for HER2 before the marker is interpreted and signed out.

Authors: This has now been incorporated on page 5, para 1, which now reads: “…It should also be noted that internal controls in individual samples, represented by the presence of normal tissue adjacent to the tumour, can be of great support to define the validity of a test. For example, in the assessment of ER, PR and HER2, it is crucial to select a tumour block that in addition to the tumour areas also contains adjacent normal ducts or lobules, which can be used as internal controls.

3. I personally don’t like the term "hazards" in the second paragraph of "Validation assay for novel biomarkers". I would favor "multiple steps in tissue preparations difficult to be perfectly standardized"?

Authors: To accommodate the reviewer’s request, we have now changed the word “hazards” with “pitfalls”. The pitfalls illustrated in the second sentence of that paragraph, Figure 1 and Table 1 transcend the issues related solely to “tissue preparation”, they are also related to choice of antibodies and later stages of immunohistochemical analysis. Therefore, we have chosen to keep this sentence more general and employed the term “pitfalls”; all issues are discussed in the subsequent paragraphs.

4. To be added in the list of pre-analytical variables "dehydration steps in the V.I.P. device " added before paraffin embedding.

Authors: This has been added on page 6 para 1 which now reads “…dehydration steps, and conditions for paraffin embedding…”

5. At the end of the paragraph listing the key issues with immunohistochemistry, it should be added that when a biomarker is used in routine, internal statistics have to be performed to be sure that the lab's results are in agreement with others;

Authors: This has been mentioned on page 7 para 1 which now reads “…Finally, when dealing with biomarkers used in routine diagnostic practice, audit of the results from the annual workload can provide supporting evidence of the actual performance of an optimised assay used in routine practice.”
6. Finally in figure 2, I would add for FFPE tissues: use of multi-tissue blocks for positive and negative controls.

Authors: Figure 2 has been modified in order to incorporate the reviewer’s suggestion.

Editor’s comments:

1. Title: I liked this title as it is both informative and also entices the reader to read the whole article. I have removed the parentheses to make it slightly snappier.

Authors: We are grateful for the amendment made to the title.

2. Introduction: This gave good background to biomarkers, an overview of the process of biomarker development and the importance of validation. Reviewer 2 notes that, as a discretionary revision, you should briefly mention the new or other available biomarkers such as those in the blood (CTC, circulating DNA) and emphasize that it is not only tumour derived biomarkers are to be submitted to careful validation and accurate protocols.

Authors: This point has now been mentioned.

3. Validation: when and why?: This section gave a clear discussion of the importance of early biomarker validation. Reviewer 1 suggests you add an additional reference to citation 5 as detailed in comment 3. Reviewer 2 suggests that you add a paragraph at the end of this section focusing on breast cancer based biomarkers in reference to internal validation strategies, which would also then give further weight to the last sentence of your introduction.

Authors: The suggested references have been quoted, and the suggestions have been incorporated.

4. Validation of assays for novel biomarkers: This section was also very clear and concise. Both reviewers have requested extra details that will strengthen the manuscript. Reviewer 1 suggests the inclusion of a citation that also touches upon similar points to this manuscript. I have also replaced the term “hazard?” with the preferred alternative term in accordance with Reviewer 2. Finally, although this section focuses on assays for novel markers, Reviewer 2 suggests that you should add a few sentences at the end of this section to discuss internal validation of routinely used biomarkers, as detailed in comment 9.
Authors: The suggested references have been quoted. The text was amended in a slightly different fashion. To accommodate the reviewer’s request, we have now changed the word “hazards” with “pitfalls”. The pitfalls illustrated in the second sentence of that paragraph, Figure 1 and Table 1 transcend the issues related solely to “tissue preparation”, they are also related to choice of antibodies and later stages of immunohistochemical analysis. Therefore, we have chosen to keep this sentence more general and employed the term “pitfalls”; all issues are discussed in the subsequent paragraphs.

As for the addition of comments on routinely used biomarkers, on page 5, para 1, we have added the following passage: “…It should also be noted that internal controls in individual samples, represented by the presence of normal tissue adjacent to the tumour, can be of great support to define the validity of a test. For example, in the assessment of ER, PR and HER2, it is crucial to select a tumour block that in addition to the tumour areas also contains adjacent normal ducts or lobules, which can be used as internal controls.

5. Figure 1: Please add a brief legend to accompany this schematic.
Authors: A legend has been added.

6. Figure 2: To strengthen this figure, Reviewer 2 suggests that you include the use of multi-tissue blocks for positive and negative controls.
Authors: The figure has been modified accordingly.

7. Tables 1 and 2: At the moment, the titles of these tables read like legends. Therefore, leave the titles as they are but have them as legends instead, and please add a short and snappy title to these legends to make it easier for the audience to read.
Authors: The titles and legends of the tables have been modified accordingly.

All authors have read and approved the revised version of the manuscript.

This manuscript has not been submitted to and is not under consideration by any other journal.
Should you have any queries, please do not hesitate to contact us.

Many thanks for this great opportunity.

I look forward to hearing from you in due course.

Best wishes,

Prof Jorge Reis-Filho, MD PhD FRCPath
Professor of Molecular Pathology
Breakthrough Breast Cancer Research Centre