Author's response to reviews

Title: Prognostic significance of lymphangiogenesis in laryngeal carcinoma patients

Authors:

Dario Garcia-Carracedo (dagaca@hotmail.com)
Juan Pablo Rodrigo (juanpablo.rodrigo@sespa.princast.es)
Aurora Astudillo (astudillo@hca.es)
Carlos Suarez (carlos.suarez@sespa.princast.es)
Maria Victoria Gonzalez (gonzalezvictoria@uniovi.es)

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Author's response to reviews: see over
Robin Cassady-Cain, PhD
In-house Editor
BMC Cancer
RE: Revised MS: 2104702080323541 submission.

MARCH 2\textsuperscript{nd} 2010

Please accept the revised version of manuscript entitled \textbf{Prognostic significance of lymphangiogenesis in pharyngolaryngeal carcinoma patients} for review and publication in BMC Cancer.

The manuscript has been modified according to the reviewers' comments. A point-by-point respond to the reviewers' suggestions is provided.

Panayiotis Kyzas

<table>
<thead>
<tr>
<th>Title: Please add “pharyngeal” in the Title, since the population was not entirely homogeneous of laryngeal cancer patients.</th>
<th>Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>In the Abstract/Results: What was the outcome for which poor prognosis was recorded? (Death/recurrence/response to treatment/other?).</td>
<td>Specified</td>
</tr>
<tr>
<td>Page 4/Introduction: Podoplanin is indeed a very specific lymphatic endothelial marker. Its specificity has been recently quantified; therefore the proper reference should be cited. Another referee (David G Jackson) says: Page 4. The statement that podoplanin has been proposed as the most specific stain for lymphatics is an inaccurate one. There is no indication that podoplanin is really more 'specific' than other markers such as D6, LYVE-1, PROX-1 etc</td>
<td>The text has been modified and the proper reference cited</td>
</tr>
<tr>
<td>Page 5/Methods: Has the study been registered prior to commence? Was there any formal protocol submitted?</td>
<td>The study was not registered since there were no therapeutic implications.</td>
</tr>
<tr>
<td>Page 5/Methods: How many assessors have evaluated the staining? Were they blinded to the outcome?</td>
<td>Specified</td>
</tr>
<tr>
<td>Page 6/Statistical analysis: I cannot understand the dichotomization of the stage to I vs. II-IV. A more appropriate one would be I-II vs. III-IV. I suspect this</td>
<td>Redone according to stage I-II vs III-IV classification and also to pT1-2 vs pT3-4. Figure 3 has been modified</td>
</tr>
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</table>
may have introduced significant bias in the analyses and I suggest re-doing the analyses with the proposed clustering.

Pages 6-7/ Statistical analyses: Using only disease specific deaths is hazardous – Overall survival has been shown to be the safest outcome as being the least amenable to biases.

Discussion, page 10: This is not the first report on the impact of the presence of tumour emboli; references that the authors cite have already examined this issue.

The conclusion may be too bold – there is still a long way until re-validation and replication will allow implication of lymphangiogenesis into clinical practice.

In the KM figures, I would like to see the number of patients for each group in every time point (i.e. every 10 months).

Rephrasing of the relative sections of the manuscript in a way that fits the REMARK criteria, and discuss the limitations of their work, citing the proper articles referred above.

David G Jackson

<table>
<thead>
<tr>
<th>Page 5 top line Pathology not Pathologic</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Page 5 Methods. The authors have omitted to provide details of the immunohistochemical staining protocol for D2-40. Presumably this used peroxidase conjugated Abs.</td>
<td>Completed</td>
</tr>
<tr>
<td>The analysis of LVD for each tumour type is rather superficial and not sufficiently comprehensive. It would be more informative if the authors included an analysis of ILD v nodal status ILD and survival presence of intravessel emboli v nodal status</td>
<td>Tables are included</td>
</tr>
<tr>
<td>Figure 1. What are the tumour types depicted in each panel?</td>
<td>Specified</td>
</tr>
<tr>
<td>Figure 2. These data are not very convincing. Because the numbers of proliferating Ki67 positive cells is not very high the authors need to show more examples. Inset can be used for this purpose to save space.</td>
<td>Insets (for the sake of space) of another example were added</td>
</tr>
<tr>
<td>In evaluating their data and comparing it with those of others the authors should take into account the different methods used for quantitating LVD and how this can be a source of variation.</td>
<td>Discussed</td>
</tr>
<tr>
<td>The authors have also referred to previous reports on HNSCC with which their own current data disagree as being &quot;controversial&quot;. This is misleading and highly prejudicial. Such wording should be changed.</td>
<td>Changed</td>
</tr>
</tbody>
</table>
Fernando Schmitt

(World J Surg Oncol 2007; 5:140). This paper is more recent than all references cited by the authors related to LVD and HNSCC and should be included in the paper.

Included

The main question of the present paper is the discrepancy between laryngeal and pharyngeal tumours concerning prognosis and lymphatic vessel and LVD. This finding is very important and deserves more discussion by the authors that just mention previous reports. Why oral carcinomas and laryngeal carcinomas are prognostically related to LVD and pharyngeal tumours not? Is this an anatomic problem?

Discussed

Other issue that needs to be clarified is the criteria used to select cases for double staining (Ki-67 and D2-40) and to state how many cases were studied.

Clarified

Reda Saad

1. The **abstract**, particularly the result section… The question, what the purpose of mention HNSCC group? Please delete and focus on both groups (not the total).

Deleted

2. The sentence: The lymphangiogenic process correlated with agressive tumour features ...etc. sites. This is a conclusion not result. The authors should state their findings regarding laryngeal and pharyngeal carcinoma without any conclusion remarks. Also, please try to add some statistical data (like P value) to show the reader how much it is significant.

Focused of laryngeal and pharyngeal groups and p values added.

3. In the **result** section, there is no detailed statistical results. The authors should include a new table include their statistical results of PLD, ILD (or global) in relation to other prognostic parameters (tumor size, LN status, lymphovascular invasion, etc). The table should include the raw statistical value (for example r= etc, if they used correlation and adding the P value). The authors can add 3 categories: Total group of HNSCC, laryngeal group and pharyngeal group versus global lymphatic density (or ILD and PLD).

Tables are included

4. The authors measure lymphovascular invasion using D2-40. Are all the lymphovascular invasion measured outside or inside the tumor? Some studies have suggested that LVI outside the tumor is more significant than just inside the tumor. Please make this clear in your article (where you measured LVI in this study).

Lymphatic invasion was recorded irrespective of its location relative to the tumour.

5. (Page 9): How many pharyngeal versus laryngeal. Also please give more details about the pN0 group.

Specified

6. There is no problem to claim that global LVD has a prognostic implication in their study, but this does not deny the importance of ILD and PLD also in the

Tables and discussion incorporated
I really recommend to add the statistical results of all groups (ILD, PLD, and global versus different prognostic parameters).

Also, is there any correlation between LVI using D2-40 and other prognostic parameters, including survival or not? Please include.

| 7. Last, the authors show us that active lymphangiogenesis (using Ki67) is happening in HNSCC tumors. It will be interesting if they show us if the presence of active lymphangiogenesis has any correlation with clinicopathologic prognostic parameters including survival. | Double D2 40 + ki67 IF was not performed in a number of cases sufficient to assess this question |

Total number of tables (5)

Total number of figures: 3 (colour, 2): the originally numbered “Figure 3” has been removed since it would be now redundant with the detailed information contained in tables 2-4.

According to one of David G Jackson’s suggestions, figure 2 has been changed, and smaller images of an additional example of a proliferative lymph vessel have been included as insets (for space sake). Figure 3 (formerly 4) has also been modified as suggested by Panayiotis Kyzas.

If any further suggestion is supplied, we will be willing to fulfil it.

Thanking you for considering this manuscript as potentially acceptable, we would be pleased if this modified version could be considered now for publication.

Yours sincerely,

Maria Victoria Gonzalez, PhD.