Reviewer's report

Title: Prognostic significance of lymphangiogenesis in laryngeal carcinoma patients

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Reviewer: David G Jackson

Reviewer's report:

The manuscript by Garcia-Carracedo et al reports on the association between various measurements of lymphatic vessel density / invasion by tumour and parameters of stage, metastasis and disease-free survival in HNSCC, based on immunodetection with the podoplanin mAb D2-40. The authors make various observations from their data and conclude that a measurement of vessel invasion and global vessel density shows best correlation with such parameters in node negative patients. The manuscript covers territory that has been studied in the past with other lymphatic markers and unsurprisingly does not add a huge amount to what is already in the literature.

Minor essential revisions

Page 4. The statement that podoplanin has been proposed as the most specific stain for lymphatics is an inaccurate one. More recent reviews of lymphatic staining have ruled that the use of more than a single marker is required for unequivocal identification and distinction from blood vessels (see eg. First international consensus on the methodology of lymphangiogenesis quantification in solid human tumours. " Van der Auwera I, Cao Y, Tille JC, Pepper MS, Jackson DG, Fox SB, Harris AL, Dirix LY, Vermeulen PB. (2006) Br J Cancer. 95,1611-1625. There is no indication that podoplanin is really more "specific' than other markers such as D6, LYVE-1, PROX-1 etc.

Page 5 top line Pathology not Pathologic

Page 5 Methods. The authors have omitted to provide details of the immunohistochemical staining protocol for D2-40. Presumably this used peroxidase conjugated Abs.

Major compulsory revisions

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 as well as ILD and survival and of the presence of intravessel emboli v nodal status.

Figure 1. What are the tumour types depicted in each panel? The authors give no information and hence this Figure is of little value as it stands. The number of different examples is very limited and should be increased, with annotation as to
the region of the tumour being shown.

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.

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.

The authors have also referred to previous reports on HNSCC with which their own current data disagree as being "controversial". This is misleading and highly prejudicial. Such wording should be changed.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.