Reviewer's report

Title: Immunostaining with D2-40 improves evaluation of lymphovascular invasion, but may not predict sentinel lymph node status in early breast cancer

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Reviewer: Gabor Cserni

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Authors: Anna V Britto, André A Schenka, Natália G M Schenka, Marcelo Alvarenga, Júlia Y Shizato, José Vassallo and Laura S Ward

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Reviewer: Dr Gabor Cserni, Bacs-Kiskun County Teaching Hospital (cserni@freemail.hu)

Summary of the content as the reviewer understands it

The authors attempt to look for correlation or association between either lymphatic vascular density or invasion (detected by D2-40 immunostaining) or microvessel density (reflected by CD34 immunostaining) or angiogenesis (shown by VEGF-A immunostaining) and sentinel lymph node status, other prognostic factors or the latest St Gallen risk categories. One of the findings is an increased rate of detected lymphatic invasion with the use of the D2-40 monoclonal antibody. On this basis the conclusion worded in the abstract is that the routine use of D2-40 immunohistochemistry (IHC) has a potential impact in determining risk groups; on the other hand lymphatic invasion and microvessel density failed to predict prognosis or sentinel lymph node status.

I do not think that all these conclusions are adequate.

Some experts have agreed that distinction between lymphatic vessels and blood capillaries cannot be accurately made on hematoxylin and eosin (HE) stained slides. I agree with this notion and therefore favor the non-committed term (lympho-)vascular invasion (LVI). (It seems that the authors are able or believe to be able to distinguish between lymphatics and blood capillaries on HE, and use the LVI abbreviation for lymphatic vessel invasion – referred to as LI here, keeping the LVI for HE detected (lympho-)vascular invasion.)

In other reports, LVI was not surprisingly found to be associated with nodal metastasis, sentinel nodal (SN) metastasis and non-SN metastasis in patients with involved SNs. As the authors report (and this is in keeping with cited papers) the use of lymphatic endothelium specific markers can increase the detection rate of LI. As lymphatic vessels drain to the SNs, it would be logical to hypothesize that an increased rate of LI would be associated with a higher rate of
SN involvement. The findings of the study are against this logical hypothesis (which is worded in the introduction), and the authors fail to give sufficient explanation for possible causes. As they mention, D2-40 also stains myoepithelial cells, and therefore ductal carcinoma in situ with a separation artifact may mimic LI, although if one is aware of this possible pitfall (and the authors are aware of that), this is an unlikely explanation. Another possible explanation may be that LI identified only by IHC could be ignored as it was not associated with SN metastasis, and therefore probably not associated with nodal metastasis at all. Since many publications support that LVI detected on HE is a predictor of nodal tumor burden, but this study suggest that D2-40 IHC detected LI is not, maybe the enhanced detection of LI is more harmful than useful and could shift patients to higher risk groups without substantial evidence. (Analogy to cytokeratin detected nodal isolated tumor cells can be remembered).

Another condition that suggests that LVI might be of importance in influencing disease free intervals is the possibility of local recurrences stemming from lymphatic emboli, but the authors have not looked at this issue, and again, lack of data do not support that increased detection of LI by D2-40 IHC would be useful. These considerations would merit some major revisions.

Other comments:
Consider abbreviating sentinel lymph node as SN or SLN instead of SL; that would be more adherent to common practice. (Discretionary revision)

In the methods there is no need to start with 126 patients, just start with the 92 patients with invasive breast cancer (excluding microinvasive cases). (Discretionary revision)

Further in the methods, it is unclear what you mean by clinicopathological stage. Stages are defined on the basis of given TNM categories, which can be derived from clinical investigations (including imaging studies): cTNM - or can be derived from pathological assessment: pTNM. Generally this is a combination of pT pN and cM, but please, be more specific. (Minor essential revision)

For SN biopsy please specify the site of injection of both tracers, and state firmly what you mean by serial HE stained sections. State the distance between levels, and specify whether serial sectioning is gross slicing of the lymph node or is a sectioning done with a microtome (i.e. step-sectioning). (Minor essential revision)

Although there is a reference to the 6th edition of the AJCC Cancer staging Manual, the statement that only nodal tumor foci greater then 2 mm (i.e. greater than micrometastasis) were considered positive findings. Mistyping? Should it be 0.2 mm? (Minor essential revision)

There are no data about the number of slides stained for the mentioned markers. If staining of a single slide with D2-40 increased the LI rate by 23%, staining of multiple slides of the tumor would be expected to result in an even higher rate. If multiple slides were stained, provide median or mean and SD. (Minor essential revision)
The study seems to be the test of finding a statistical relation (e.g. association or correlation) between different prognostic markers, and the results of D2-40, CD34 and VEGF IHC, but prognosis (e.g. disease free or overall survival) was not directly assessed.

I would strongly recommend to reduce the manuscript to describe only findings related to MVD, LVI, VEGF and SN status.

There are many test used, including the chi square one (please use the greek letter instead of X or write chi) and the kappa statistics (please provide the number of observers here too, not only in the results section.) (Major revision)

In the results you mention “risk”. This is somewhat obscure. I believe you are referring to the St Gallen risk categories. Than it is not surprising that the risk category was related to grade (one of the definer of low and intermediate risk) and stage (as nodal category influencing stage is also a definer of the intermediate and high risk group). (Major revision is necessary)

Table 1: For tumor sizes please provide further details. Are these medians (and ranges) or means (and SDs)? (Minor essential revision)

Table 1 would benefit from the addition of further rows: e.g. Mean MVD, LVD, presence of LVI on HE, IHC, VEGF results... (Minor essential revision)

Table 2 is not really necessary. This can be mentioned in the text. As a strange finding this would have merited more discussion. (Minor essential revision)

References 12 (Capital H for Hausmaninger), 24 (funny symbols after some “o” and “u” letters imitating the German letters, and lack of full title and source) and 32 (again the symbols after Heikkika) need some corrections. (Minor essential revision)

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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