Author's response to reviews

Title: Immunohistochemical detection improves the prognostic value of lymphatic and blood vessel invasion in primary ductal breast cancer

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Version: 6
Date: 1 July 2014

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01/07/14

BioMed Central Editorial,

Dear Dr Britta Weigelt,

MS: 2099120226114527 - Immunohistochemical detection improves the prognostic value of lymphatic and blood vessel invasion in primary ductal breast cancer.

Thank you for your e-mail on 19th of June. We acknowledge the detailed comments of the referee and would be grateful if you would reconsider this re-revised paper. We have considered carefully the referee’s comments and these have improved the manuscript. There follows our response;

Editor's comments:

1. The authors did not specify the cut-offs for ER, PR and HER2, which should be performed according to the ASCO/CAP guidelines.
Point taken. “ER, PR and HER-2 status were assessed visually using tissue microarrays as previously described (Mohammed et al 2012a; 2012b). The cut-off value for ER and PR status was taken as a #10% positive tumour nuclei, for Her2 status a score 3+ was regarded as positive and 0 and 1+ were regarded as negative and in those 2+ were regarded as equivocal, leading to referral for HER2 FISH.” Text amended accordingly in the method section.

2. In the revised version of the manuscript, it is crucial that the multivariate survival model includes the therapies the patients received as well (e.g. endocrine therapy yes vs no, chemotherapy yes vs no).

Treatment has now been included in the multivariate survival analysis.

3. Furthermore, the conclusions about the prognostic power of LVI and BVI ought to be toned down given that in the multivariate analysis of the cohort analyzed in this study, only tumor size of the clinicopathological factors included was found to be an independent prognostic factor, whereas grade, age or ER were not significant. This should also be addressed as one of the limitations of the study in the discussion.

Point taken. “The results of the present study show that LBVIH&E, LVID2-40 and BVIFVIII all predicted tumour recurrence and cancer specific survival in an observational cohort of patients with early breast cancer. However, only LVID2-40 and BVIFVIII independently predict cancer outcome. These results suggest that clinicopathological assessment of the lymphatic and blood vessel invasion by IHC of LVID2-40 and BVIFVIII may improve the prediction of outcome in patients with early breast cancer.”

And

“A limitation of the present study was that intra- and peritumoral LBVI foci were not separately analysed owing to small number of cases with intratumoural LBVIH&E (5%) compared to the (95%) of peritumoural LBVIH&E. This precluded meaningful analysis of each component but was unlikely to materially influence the concordance between the detection of LBVI-H&E and LBVI-IHC. Although, several previous studies have reported the prognostic significance of LBVI using H&E staining these studies have not discriminated between the types of vessel invasion whether lymphatic or blood vessel and have inconsistently used the terms vascular or lymphovascular invasion. For example, the American Joint Committee on Cancer (AJCC) staging guidelines (2005) has used the term lymphovascular invasion to indicate both lymphatic and vascular involvement
This clearly may be confusing as these terms may indicate involvement of lymphatic or lymphatic and blood vessels. This is largely a pragmatic approach to the limitations of the routine use of H&E slides to assess lymphovascular invasion. Another limitation was that the well established factors such as grade and ER status were not independently associated with cancer specific survival in all patients and in those with node negative or triple negative disease. This may suggest that the sample size was rather small for such multivariate analysis. Nevertheless, the results are of interest and make a case for further studies of routine clinical assessment of lymphatic and blood vessel invasion by IHC to ascertain LVI and BVI. Text amended accordingly in the discussion section.

Referee 1: Referee #1 (Comments to the Author):

Discretionary Revisions

1. The first paragraph of the Background is based on out-of-date statistics. I believe lung carcinoma is now the leading cause of cancer death in women and almost 50,000 women are diagnosed in the UK each year (refer to SEER data (US) or Cancer research UK data 2011 (available online).

Point taken. “Breast cancer is a common and one of the leading causes of cancer death in women. It accounts for approximately one tenth of all new cancers and a quarter of all female cancer cases (1). In the UK more than 49,000 women diagnosed with breast cancer in 2011 accounting for 30% of female cancer incidence. However, the survival rate has improved with 78% surviving 10 or more years (2).” Text amended accordingly in the introduction section.

2. Methods: I can’t see any data in the methods that documents the Stage of these patients. Are they all Stage I and II, or are there any Stage III patients? This would be useful to know. If you include stage in your multivariate analysis, is LBVI as statistically significant?

In patients with breast cancer there are a number of well established staging factors such as tumour size, grade, ER status and lymph node involvement that guide treatment and were included in the present analysis. In this context lymph node involvement defines TNM stage II and III disease and as a result has been examined in the present study.

3. Discussion: There is no review of the results of the survival analysis in the discussion section. I think a discussion of the significance of LBVI H&E vs LVI
D2-40 and BVI FVIII would be appropriate, e.g. why LBVI H&E was not found to be statistically significant on multivariate analysis in this cohort, but the LBVI IHC was significant. This is an important point, as it backs up the authors’ recommendation that the introduction of routine IHC for assessment of LBVI may be indicated.

“In summary, the results of the present study show that IHC for D2-40 and Factor VIII define lymphatic and blood vessel invasion with greater sensitivity and specificity than H&E, improving detection of LVI and BVI in early invasive breast cancer. Moreover, the prognostic significance of the LVID2-40 and BVIFVIII was superior to that of LBVIH&E and this was consistent throughout analysis of sub-cohorts. Therefore, these results make the case for their assessment in routine clinicopathological practice.” Text amended accordingly in the discussion section.

4. Discussion: I think that the Discussion is very much improved since the last draft but it still needs some stylistic editing. The first sentences of paragraphs two and three should be re-drafted as they lack coherence. Likewise the syntax in paragraph twelve needs to be reworked. Overall, I think the whole discussion could be more succinct.

Where appropriate, changes have been made to the discussion to improve the grammar and syntax and clarity of text. Where possible, the discussion text has been shortened.

Minor Essential Revisions

1. In the Abstract, abbreviations such as LBVIH&E etc need to be written as subscripts, like in the rest of the paper.

Point taken. Text amended in the abstract accordingly.

2. Background paragraph 2 sentence 3 – re-write.

Point taken. Text re-written.

3. Results, paragraph 7 sentence 2 – I think you mean “younger age” rather than older age?
Point taken. Text amended accordingly to “younger age”.

4. Discussion paragraph 13, sentence 1 – insert the words “detection of” between the words “of” and “LBVI”.

Point taken. Text amended accordingly in paragraph 13, sentence 1

5. Figure 1 – I think that the figure legend is wrong or images E, F, G and H may be in the wrong order, as image F is referred to in the legend as a positive Factor VIII stain, when it is actually negative, and G is called a negative stain when it appears to be positive.

Point taken. This has now been corrected.

We look forward to your reply in due course.

Yours sincerely

Fadia JA Gujam

Donald C McMillan