Author's response to reviews

Title: Prediction of melanoma metastasis by the Shields index based on lymphatic vessel density

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Author's response to reviews:

Dear Editor,

Thank you for your reviews of this article. We have made the changes requested by the reviewers as detailed below.

List of Changes

Reviewer One

1. Title to Figure 5 changed to read “Figure 5 Comparison of epi-tumoural lymphatic vessel density, lymphovascular invasion, shields index and sentinel lymph node biopsy.”

2. Lymphatic invasion was only assessed in the intra-tumoural area, as it was intra-tumoural LI that was identified as significantly increasing in metastatic compared to non-metastatic melanomas by Shields et al. (2004) in the initial identification of the Shields Index. This study was aimed at further examining the validity of this prognostic index and therefore focused on the same parameters as they had initially identified.

Reviewer Two

Minor Essential Revisions

1. Changed “then” for “than” in the sentence of Methods in the Abstract.

2. Abbreviation SLN defined fully for first time as sentinel lymph node in the Introduction, paragraph 2, line 6.

3. References specified for the increase in LVD around metastatic compared to
non-metastatic melanomas (beginning of second paragraph, page 4).

4. Lymphatic vessel density swapped for LVD at the bottom of page 4 and on page 8.

5. Specific components of the REMARK guidelines are now identified in the text by numbers in diamond not square brackets, so that it can be easily distinguished from other references.

6. Page 6, patient selection sentence re-written to read: “Melanoma tissues were randomly selected from 102 patients from the Registry, excluding any tumours that are only in situ and then selecting participants under the following criteria: clear of metastases at time of excision, with Breslow thickness < 8mm, with > 5-years of follow-up, and with no signs of ulceration #1,2#”

7. Reference 14 (now 16 after addition of new references), Skobe et al, is cited on page 15, the 3rd line of the second paragraph and reference 18 (now 20 after addition of new references), Harrell et al, is cited at the bottom of page 15, 3 lines from the end.

8. In the conclusions paragraph (page 16) the use of ‘was’ is swapped for ‘is’ in the sentence “…the use of the index described by Shields et al in 2004 was the most specific…”

Discretionary Revisions

1. Patients weren’t followed up routinely by the hospital after the first five years, unless they returned with a related complaint or symptom, and are assumed to be disease free unless they were seen again.

2. The specificity of LYVE-1 as a lymphatic marker is discussed in paragraph 2 of the discussion.

Major Compulsory Revisions

1. Metastatic outcome was further defined in the methods and results sections with: “(i.e. whether or not the patient went on to develop metastases)”.

2. “Randomly selected” in this instance means that the list of patients in the registry treated at least five years prior was used as a database. The database was not ordered by any parameter. The first 151 patients that fitted the criteria described above were selected.

3. Clinically occult metastases are those that are not accompanied by readily discernible signs or symptoms, but can only be detected by chemical or microscopic analysis. At the bottom of page 6, the use of “clinically occult metastases” has been exchanged for “clinically apparent metastases” as in the context we are referring to our non-metastatic patient cohort anyway.

4. The epi-tumoural LVD was calculated by dividing the total number of lymphatics in an epi-tumoural border of 350 by the total area of that border. When looking down the eyepiece of the microscope at 40x, the field of view is
known to have a diameter of 350µm. Therefore by tracing along the edge of the tumour border and counting all visible lymphatic vessels, the total number of vessels in a 350µm border around the tumour could be ascertained. From here, the entire tumour border along which the vessels were just counted was imaged, and from these calibrated images the total area (mm²) of the tumour border was calculated. Therefore, to calculate the overall epi-tumoral LVD, the number of lymphatics counted in the 350µm epi-tumoural border, was then divided by, the area of the very same border. Thus, an accurate calculation of the lymphatic vessel density was considered.

5. Differences in patient groups now represented as part of table 1.

6. The 45 patients that had metastasis all had lymphatic metastasis as far as we can tell from the registry. Some of them also had distant metastases, but the location was not always noted.

7. The range of LVD in normal dermis, was described by Joory et al., which is now referenced on page 11 with the normal range defined also.

8. Page 12, first line of paragraph after the heading ‘Hot Spot Analysis’ states that a subset of 24 patients were used to compare total LVD with hot spot analysis i.e. 24 patients underwent hot spot analysis.

9. The subset of 24 patients that were assessed for both hot spot analysis and total epi-tumoural lymphatic vessel density, were examined as part of an independent analysis i.e. analysis using either epi-tumoural LVD or hot spot analysis was compared directly within the group, except with respect to the AUC value for the ROC curve when AUC for Shields Index calculated by hot spot analysis was compared to the AUC for Shields Index calculated by total epi-tumoural LVD for all 102 patients (including this subset of 24).