Author's response to reviews

Title: Association of Fascin-1 with Mortality, Disease Progression and Metastasis in Carcinomas: a Systematic Review and Meta-analysis

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Author's response to reviews: see over
27.11.12
To: Dr. S. Alam, Editor-in-Chief,
BMC Medicine,
London.

Re: Association of Fascin-1 with Mortality, Disease progression and Metastasis in Carcinomas: a Systematic Review and Meta-analysis
First submitted as BMC Medicine MS: 1846584776729662

Dear Dr. Alam,

Please find here submitted a further revision of our manuscript “Association of Fascin-1 with Mortality, Disease progression and Metastasis in Carcinomas: a Systematic Review and Meta-analysis”. We would like to thank the reviewers for their constructive comments on the revised submission and were pleased that two of the three referees found the manuscript acceptable for publication as presented.

Further to the telephone conversation between Richard Martin and the Senior Editor (Dr Claire Barnard) on 5th November, we understand that the additional statistical comments made by the statistical reviewer, Ms Sarah Donegan, need to be addressed, whereas a need for changes in presentation would be decided by the editorial board. As explained in that conversation, we have reservations about making presentational changes that would affect the content of the article and negate the input of the three other expert referees who have already considered the article acceptable for publication. This revision therefore contains changes made in response to the statistical comments of the statistical reviewer, and we believe that we have carefully addressed these comments in full. Our full set of responses to the comments of statistical reviewer Sarah Donegan is given below.

We confirm that this manuscript is not under consideration elsewhere and that all authors have seen and approved the final revised version. There are no conflicts of interest to declare. We hope that with the included revisions our study will now be appropriate for publication in BMC Medicine. We look forward to hearing from you in the near future.

Yours sincerely,

Josephine C. Adams and Richard M. Martin, on behalf of the co-authors
Response to Reviewers comments on BMC Medicine MS: 1846584776729662

Revised title: Association of Fascin-1 with Mortality, Disease Progression and Metastasis in Carcinomas: a Systematic Review and Meta-analysis

Reviewer: Konstantinos Tsilidis

Discretionary Revisions
I agree with reviewer Cord Langner that in the Abstract the term "weak association" for associations where the confidence intervals contain the null value should be changed to "no associations"

Response: We have made the changes as recommended by the reviewer in the abstract.

Reviewer: Sarah Donegan (Statistical Reviewer)

Major comments:

1. Forest plots. The comparison ‘positive versus negative’ and the comparison ‘high versus low’ should be on separate plots. Data from the two comparisons should not be combined. This applies to the analysis within each carcinoma type and the analysis across carcinomas.

Response: We now present the results of the comparison ‘positive versus negative’ and ‘high versus low’ fascin-1 expression on separate plots (A and B panels within Figures 2, 4 and 5).

2. Choice of carcinomas (in ‘Data sources’ section). I think that the decision regarding which carcinomas to include should be based on whether the question is of interest rather than the number of available studies. The review authors could provide alternative justification.

Response: We focused on breast, colorectal, gastric, lung and oesophageal carcinomas as these are the most prevalent carcinomas that are major sources of morbidity and mortality, and therefore of high interest for new biomarkers. This is explained in the Abstract and on page 5.

3. In the last paragraph of the background, I disagree with the comment that the meta-analysis identifies potential sources of heterogeneity. A forest plot displays differences in results that can help to understand the causes of the difference. However, potential causes of heterogeneity should be considered prior to see the data and are therefore identified by consulting experts in the clinical area and searching published literature for proven effect modifiers. I actually think the last sentence in the background should be deleted.

Response: We have deleted the last sentence from the background.

4. I would describe the inclusion/exclusion criteria before describing the search strategy (i.e. ‘data sources’).

Response: We have made the changes and now describe the inclusion/exclusion criteria before describing the search strategy on page 5-7.
5. **Inclusion/exclusion criteria.** The eligibility criteria should be written as though you have not seen the included studies and therefore do not say what some included studies did or didn’t do. For example, ‘Studies had to meet the following inclusion criteria: (1) either randomised controlled trials, cohort or case-control studies; (2) in humans with carcinomas of the breast, colorectal, gastric, lung and oesophageal; (3) detected fascin-1 by immunohistochemistry and compared high versus low.... ’; and (4) reported outcome data for at least one of the following outcomes lymph-node or distal metastasis, time-to-disease progression, or mortality.’

Response: We have made changes to the Inclusion/Exclusion criteria on page 5-6 as follows “Studies had to meet the following inclusion criteria: (1) either randomised controlled trials, cohort or case-control studies; (2) in humans with carcinomas of the breast, colorectum, stomach, lung and oesophagus; (3) detected fascin-1 by immunohistochemistry and compared high versus low fascin-1 staining, negative versus positive fascin-1 staining, or negative, low and high fascin-1 staining; and (4) reported outcome data for at least one of the following outcomes: lymph-node or distal metastasis, time-to-disease progression, or mortality.”

6. The second paragraph of the inclusion/exclusion criteria is not about criteria and therefore should be under a different heading e.g. ‘selection of studies’.

Response: We have placed the second paragraph of the inclusion/exclusion criteria under a new subheading ‘Selection of studies’ (pages 6-7).

7. **Data extraction section is quite long and could be condensed.** For example the section could be ‘Data were extracted onto standardized pro forma by one assessor (VYT) and then double checked by two others (RMM and SJL). For dichotomous outcomes (i.e. mortality, ..., Lymph Node Metastasis, Distant Metastasis) the number of patients with the event of interest and the total number of patients in each category group (i.e. high, low, positive or negative) were extracted. For time to event outcomes (i.e. time-to-Disease progression), a hazard ratio was extracted from the study report where possible, otherwise a hazard ratio was estimated from Kaplan Meier curves using the method described by Tierney et al (ref). When a measure of effect (e.g. relative risk or hazard ratio) was adjusted for covariates, it was also extracted along with details of the corresponding covariates.’

Response: We have shortened the data extraction section as suggested by the reviewer on pages 7-8.

8. In the statistical analysis section, describe whether hazard ratios were analysed that were unadjusted for confounders, or adjusted hazard ratios, or both.

Response: In the previous version of our manuscript on page 9, we had included a description of whether unadjusted hazard ratios or adjusted hazard ratios were analysed as follows “….to calculate summary RR estimates by combining fully adjusted hazard ratios, crude hazard ratios and/or relative risks.” In the latest revised manuscript, we have described this on page 9 as follows “We combined fully adjusted effect-estimates if these were available, otherwise we used the unadjusted estimates.”

9. Avoid describing the included studies in the methods. Methods of systematic reviews should be pre-specified.

Response: We have removed the description of the included studies from the methods.
10. I think that the results text should have three distinct sections: description of the characteristics of the included studies; quality results; results of the analysis in this review.

Response: The Results text is now divided into four sections: Identification and selection of included studies, Characteristics of the included studies, Quality of the studies, and Meta-analysis results.

11. Some of the cells in the quality assessment table are blank. Please provide a reason for the missing information e.g. ‘unclear information’ or ‘not reported’.

Response: We have now added the reason for the missing information into the Quality assessment table (Additional file 1).

12. A short summary of the quality of the studies should be given after the description of included studies and results of the search. Refer to the quality results table in the summary. Delete the text and quality from page 16. Move the information about the quality of the studies to the summary. Results of your analyses regarding quality should be presented in the subgroup analysis section instead.

Response: We have now provided a summary of the quality of the studies after the description of included studies on pages 11-12. Results of the subgroup analyses by methodological quality scores for each outcome are now presented in the subgroup analysis section on pages 18-20.

13. The quality of all 26 included studies should be assessed.

Response: We have now assessed the quality of all 26 included studies. The results of the quality assessment are shown in additional file 1 and a summary of the quality of the studies is given on page 11. As stated in the Discussion (page 23), “…the high quality studies showed a positive association between fascin-1 expression and mortality (pooled fixed effects HR: 1.43 (1.26-1.63; p<0.001), that was similar in magnitude to the association observed when all 18 studies were pooled (pooled random effects HR: 1.44 (1.24-1.68; p<0.001)).”

14. The ‘sensitivity analysis’ for ‘definition of mortality’ is a subgroup analysis. If you delete the results for the subgroup without the definition, the analysis becomes a sensitivity analysis.

Response: We have deleted the results for the subgroup without the definition of mortality from this Table. Sensitivity analysis was carried out only for the studies with a definition that mortality referred to death from cancer (Page 20 and Table 2A).

15. When there is significant heterogeneity (chi-square p-value <0.1), use the random effects model i.e. figures 2, and 3 (overall). The result from the random effects model is equivalent to the result from the fixed effects model when there is no heterogeneity, so if there is a difference between the results from each model, you could use the random effects model.

Response: We have made the changes according to the reviewer’s request. Because the decision to carry out fixed or random effects meta-analysis is an area of considerable controversy, we have presented both the fixed and random effects results in the forest plots (when the I² value was more than 50% or when the chi-squared p-value was less than 0.1) to allow readers to make their own judgement.
16. When there is significant heterogeneity, do not report the fixed effects meta-analysis in the forest plot because the confidence intervals are artificially narrow.

Response: As stated above, we have now presented both the fixed and random effects results in the forest plots when the I^2 value was more than 50% or when the chi-squared p-value was less than 0.1, to allow readers to make their own judgement.

17. I don’t understand the need to ‘recalibrate’ some scoring systems for these studies. By doing this, do all the studies have the same cut-offs for high, low, positive, negative. Can you clarify the reasoning in the additional file?

Response: Takikita et al and Pang et al had categorised an immunostaining score of ≤3 as negative fascin-1 expression within the tumour in their studies. Because an overall score of 3 could possibly include fascin-1-positive cells, for these two studies, we categorised a score of ≤3 as low fascin-1 expression and a score of ≥4 as high fascin-1 expression. We have clarified this in Additional File 1. We removed the word “recalibrate” as it is potentially confusing.

18. The cutoffs of studies seem to be very different, is a meta-analysis across these studies giving a meaningful result? I suggest that this should be a discussion point in the discussion.

Response: We discuss the limitations of meta-analysing studies with different cutoffs on page 23 as follows “The scoring of fascin-1 by immunohistochemistry is a continuous measurement and in most publications researchers categorised tumour specimens into high/positive fascin-1 or low/negative fascin-1 based on different semi-quantitatively assessed cut-off points. For example, two studies [58,66] categorised 0-8 as low expression and 9-12 as high expression. On the other hand, another study [9] dichotomised low expression as a score of <75% immunoreactive tumour cells and high expression as >75% immunoreactive tumour cells. These differences could possibly discard potentially important quantitative information and reduce statistical power to detect real associations [73].”

Despite these different cut-offs for studies, there was generally little evidence of heterogeneity across studies (exceptions were when studies compared positive versus negative fascin-1 in colorectal and lung carcinomas or when studies were pooled across all carcinomas), indicating that the different cutoffs were unlikely to affect the results. Thus, we consider that the pooled data do give meaningful results.

19. I am confused by the method used to derive hazard ratios from the KM curve. The additional file says that the method by Tierney et al gives a summary hazard ratio. But then the text says the method was applied it to get a relative risk. If the method gives a hazard ratio it should not be combined with relative risks. Rewrite the text in the additional file to clarify how a relative risk was derived. If you actually derived hazard ratios, they should be called hazard ratios and should not be combined with true relative risks.

Response: For the method by Tierney et al, the hazard ratios and variance for the whole curve was derived by combining hazard ratio estimates calculated for each time interval across the follow-up period. We have now clarified that this method derives hazard ratios and not relative risks in the data extraction section on page 8.

20. Can you confirm that hazard ratios and relative risks have not been combined?

Response: In this manuscript, hazard ratios and risk ratios have not been combined in our analysis.
21. If you are presenting relative risks, rephrase ‘time to disease progression’ to ‘disease progressed’; or if you present hazard ratios keep ‘time to disease progression’. This will help avoid confusion.

Response: We have presented hazard ratios for the “time to disease progression” outcome. As the forest plots do not give any indication of a time scale, we have stated in the title of each forest plot which effect estimates (HR or RR) were combined in the plot.

22. Also, I do not understand the assumption that was made to derive the hazard ratio i.e. the proportion of positive versus negative patients was the same in the 75 patients as in the 100 patients. Can you clarify?

Response: In the study by Tsai et al, only the proportion of positive versus negative patients was provided for the total 100 patients. However, the mortality analysis was carried out in only 75 patients (in which the proportion of positive versus negative patients was not provided). We made the assumption that the proportion of positive versus negative patients in the 75 patients is similar to that in the 100 patients. This assumption is stated clearly in the text (Additional file 1 Page 4).

23. Can the discussion be reduced in length? What are the main limitations?

Response: We cannot reduce the discussion because this would risk perturbing responses to the 3 other reviewers of our manuscript who are now satisfied that we have addressed in full all their comments and who considered the manuscript acceptable in its previous revised form. We have therefore focused this revision on the statistical questions raised.

Minor essential comments:

24. In the data sources section, describe whether organisations were contacted to obtain unpublished results; whether authors of conference abstracts and on-going studies were contacted for the published or unpublished report.

Response: We have added the following to the ‘Data sources section’ on page 6: “We did not contact any organisations to obtain unpublished results and we did not identify any on-going studies from our systematic review. Authors of conference abstracts were contacted for the published report.”

25. Search terms are in file 2 not 1 as mentioned in the text?

Response: We have made the corrections on page 6.

26. Additional table 1. ‘M’ has two meanings in the footnotes.

Response: We have made the changes to Additional File 2. “M” is now the abbreviation for the presence of metastasis in Additional table 2.

Minor discretionary comments:

27. Change ‘systematic evaluation’ to ‘systematic review’ in the last paragraph of the background.

Response: We have made the change in the last paragraph of the Background on page 5.