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Retrospective Study

Circulating cytokeratin-positive cells and tumor budding in colorectal cancer

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Reviewed by 03003594

SUMMARY This is an interesting manuscript which appears to add to the existing body of literature around this subject. The design is clear, however the sample size may be too small, with no description of a sample size/power calculation.

We added a sample size calculation on P4/5:

We assumed a strong correlation between the detection of circulating CK+ cells and the occurrence of distant metastases with lethal outcome. An absolute difference concerning lethal outcome of 50% with a power of 0.8 and with Alpha = 0.05 resulted in a calculated sample size of 19 cases in each group (proportions sample size test).

Study strengths and limitations, including the small size of the study, were appropriately acknowledged by the authors in the discussion, who noted that it is underpowered to detect effects smaller than expected. MATERIALS AND METHODS There is a discrepancy regarding patient consent - in the materials and methods section it says that "informed and written consent was obtained from all patients" yet in the data sharing statement it says that patient consent was not obtained.

Answer: The patients gave consent to the study. However, the consent did not include data sharing!

The authors state that CK+ cells/clusters were counted manually in the blood samples. Was this count performed by a single person or by multiple people? Was there any chance for inter-observer variation here?

We added a statement to clarify this on P5/6:

For that all slides were screened by a very experienced technician. All positive cases were confirmed by a hemato-oncologist (DO). Data concerning interobserver agreement between these two investigators are not available.

The authors state that the surgical technique did not influence the occurrence of CK+ cells/clusters – was there any potential for inter-observer variation with this technique, e.g. multiple surgeons; surgery performed at more than one site etc.

Answer: We did not consider analyses concerning different surgeons because of the small sample size that would result from sub-group selections.

STATISTICS The statistical methods used seem appropriate and clear. GRAMMAR/SPELLING Page 5, line 2 – “Additional data were acquired clinical and laboratory information systems.” Should this include the word ‘from’?

We corrected this on P5!

Page 8, line 3 – it says “(Table XXX)” which I think should say “(Table 1)”

We corrected this on P8!

Page 20, Table 2 – in the bottom line it should say “no separate evaluation for blood samples”

We corrected this on P20!

DISCUSSION If there had been evidence that CK+ cells in the mesenteric blood of colorectal cancer specimens were a demonstrable prognostic factor, would this lead to an avoidance of “delay and additional risk during the operation”? Perhaps this statement could be further clarified.

Answer: It was our idea that drawing blood from the specimen would avoid delay and additional risk during the operation. We tried to clarify that by editing this statement on P9:

... and could serve as an easy to determine prognostic *factor. Drawing the blood after resection would avoid* delay and additional risk during the operation....

Reviewed by 03551824

This study investigated the clinical significance of CTCs in mesenteric vein of surgical specimen using immunohistochemistry. The author concluded that CTCs have no prognostic significance. Although this conclusion seems resonable, this study included only 56 patients, which may be insufficient for leading the conclusion. In this study, tumor budding and nodal metastasis were not significant prognostic factor in univariate analysis. These parameters are well-established, and significant factor in many studies. I am affraid that these indicate insufficient number of patients in this study.

Answer: We agree with the reviewer that our study may be underpowered to detect small effect. Nevertheless, one can draw the conclusion that the assumed effects are much smaller than expected by us (and probably many others) and other directions of investigations may be more promising. Adding more samples now would be not appropriate because of dramatically different follow-up times.

Reviewed by 03270786

Dear Authors, I would like to congratulate you well designed and executed research. I my personal opinion putting attention to tumour budding and bloodstream delivering is important for creation new attempt to a categorisation CRC. Although, molecular profiling of CRC becomes a standard, the changes in grading cancer should be made soon. Enrichment of landscape of predictive/prognostic factors is a need in Age of personalised therapy. I have found a few misprints - Abstract. There is used CK2 (correctly should be CK18 (Clone CK2),

We corrected this through out!

Page 8 - Table XXX,

We corrected this on P8!

Graphs description double 'negative' and in B 'negativ' 'positiv' All this is not important and resulted from inattention.

We corrected the legends of Graph 3.