Author's response to reviews

Title: Efficient and reproducible identification of mismatch repair-defective colon cancer: validation of the MMR index and comparison with other predictive models

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Version: 2 Date: 25 February 2013

Author's response to reviews: see over
Dear Editor,

Thank you for the constructive review of our manuscript "Efficient and reproducible identification of mismatch repair-deficient colon cancer".

We have, in accordance with the reviewers' recommendations, made the following changes.

**Reviewer MSP:**

**Major Compulsory Revisions:**

1. "MMR deficiency" has now been used in the title and throughout the manuscript.

2. The causes of MMR deficiency have been clarified in the introduction and references have been updated and in this regard a reference to Parsons et al., 2012 has been added.

3. A discussion around the application of the MMR index for the identification of Lynch syndrome tumors has been added.

4. The abstract has been reorganized accordingly.

5. This point has been considered in the revised manuscript.

6. The definitions have been clarified and a reference has been added. The definition of tumors with mucinous component was the same as that applied in the development of the MMR index.

7. From our experience with Lynch Syndrome diagnostics and our clinical experience in general, we recognize that there is a subset of tumors with weak or reduced staining. Such tumors were, however, not identified in the present series. We believe this finding relates to the fact that MLH1 hypermethylation results in total functional inactivation, whereas Lynch Syndrome mutations, e.g. missense mutations, might be associated with partially retained protein/epitope function.

8. The results have been discussed in relation to the previous publication by Halvarsson et al 2008.

9. This comment referred to concordant losses of MSH6 and MLH1/PMS2 in 5 tumors, which most likely represents a MSH6 tumor with somatic MLH1 inactivation. 5 tumors showed combined loss of MSH2/6 – but the distribution of either MSH2 or MSH6 remains unclear. This point has been clarified in the revised manuscript.

10. Regarding the contribution from Lynch syndrome, our data suggest that 3-5% of colon cancer diagnosed after age 50 may be linked to Lynch syndrome. Though mutation data are needed to support this statement, MSH6 mutations have likely escaped detection due to reduced penetrance and failure to test older patients. This observation is supported by several publications, to which references have been added. Additionally, Lynch syndrome cases caused by MLH1 mutations may be missed. This may represent a
conservative estimated, but the main point refers to the undetected contribution from MSH6 defects.

11. We agree with the reviewer that MLH1 methylation analysis would have been useful. It was unfortunately not possible to perform since we only had access to sectioned slides and not the tumor block, which would be needed for DNA-extraction.

12. The factors included in routine analysis have been specified.

**Minor essential revisions:**
All changes have been performed. This includes:
- The meaning of “additional”, the number of patients with synchronous/metachronous tumors and the use of “expanding” throughout the manuscript. Also, the distribution of slides has been clarified and a reference to Table 2 has been added (line 4).
- “25 cases” has been changed: “25” is a clerical error and has been changed to “24”. “Cases” has been changed to “patients”.
- Table 3 and 4 are kept separate to ease reading. We do, however, suggest that table 3 is converted into a supplementary table [Additional file 1].
- Relevant minor changes suggested have been included.

**Reviewer PMW:**
**Discretionary revisions:**
The manuscript has undergone language revision.

**Minor essential revisions:**
The definitions of tumors with mucinous components (10-15% mucinous areas) and strictly mucinous tumors (areas >50%) have been clarified.

We did indeed also take MLH1 mutations into account in the calculation of total Lynch syndrome rate of 3-5%. This may represent a conservative estimated, but the main point refers to the undetected contribution from MSH6 defects.

Given these alterations we hope that the manuscript may now be acceptable for publication.

On behalf of the authors

Patrick Joost, MD