Reviewer's report

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

**Version:** 1  **Date:** 13 December 2012

**Reviewer:** Marta STEPHEN Pineda

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Summary
Mismatch repair (MMR)-deficiency allows the identification of a subset of colorectal tumors associated with good prognosis, reduced response to 5-FU-based chemotherapy, and a higher incidence of Lynch syndrome. Several predictive models aimed at identifying MMR-deficient tumors have been reported. Joost et al. report on the validation of the previously published MMR-index prediction model, based on 7 clinicopathologic features (Halvarsson et al., Am J Clin Pathol 2008). The validation series included 474 consecutive colon cancers from 462 patients older than 50 years. In addition, they assess the interobserver variability and compare their results to other MMR-deficiency prediction models. The results confirmed the high predictive value of the model and demonstrated a stable performance and good reproducibility.

**Major Compulsory Revisions**

1. In the manuscript, MMR-deficiency in tumors was defined as the loss of expression of at least 1 of the MMR proteins. Although loss of MMR protein expression usually correlates with microsatellite instability, the term “MSI” should not be used as a synonym of “loss of MMR expression”. We strongly recommend the use of the term “MMR-deficiency” to refer to the loss of MMR protein expression throughout the text.

2. The concepts “sporadic” and “Lynch syndrome tumors” are used confusingly throughout the text. In fact, somatic MLH1 methylation does not explain all the MMR-deficient tumors in which germline MMR mutation has not been identified (Gausachs Eur J Hum Genet 2012, among others). On the other hand, MMR-deficient tumors are considered Lynch syndrome tumors when a germline-causing mutation has been identified. Please, clarify throughout the manuscript. In Background, first paragraph, last sentence: Please, actualize the references.

3. Although MSI/IHC testing is commonly requested without prior selection of tumors diagnosed before age 50, it would be interesting to know about the performance of the MMR-index in Lynch syndrome-suspected tumors. Could the authors know/speculate about this issue?

4. The Abstract should be reorganized: Methods section should not include results.
5. In the Abstract, it is necessary to clarify that the MMR-index has been previously reported, which results confirm the previously obtained, and which of them are novel.

6. Methods, second paragraph: “Tumors with mucinous component that encompassed 10-50% of the area were classified as having a mucinous/signet-ring cell component”. Please, refer to the data that support this affirmation.

7. Methods, fourth paragraph: MMR protein expression is classified as “retained” or “lost”. However, weak and non-evaluable staining results are not rare. How are they considered?

8. Related to the predictive values for MMR-deficiency of the morphologic features (Results, second paragraph and Discussion, first paragraph), please discuss these results in comparison with the previously reported in Halvarsson et al., Am J Clin Pathol 2008.

9. Discussion, third paragraph: Fourteen tumors showed loss of MSH6 expression (5 MSH2/MSH6-, 4 MSH6- and 5 MLH1/PMS2/MSH6-). These data are linked to the characteristics of tumors identified in MSH6 germline mutation carriers. However, the IHC patterns are not suggestive of germline MSH6 mutations in all the cases. Please clarify.

10. Discussion, third paragraph: The statements: “We identified IHC loss (of MSH2 and/or MSH6) suggestive of Lynch syndrome in 3% of the tumors. […] If an equal contribution from MLH1 and MSH2 is assumed in Lynch syndrome, our data suggest that at least 5% of colon cancers that develop after age represent Lynch syndrome” are somewhat vague. I would recommend that authors take into account the prevalence of Lynch syndrome among colorectal cancers (2-5%) and the early age of onset of the syndrome in their assumptions.

11. Conclusions: Concerning that methylation has not been analyzed in the reported series, why authors consider that the MMR-index provides a validated tool to identify the MMR-deficient colon cancers with a particular focus on sporadic MLH1-methylated tumors? It is recommended to perform the analysis of MLH1 methylation in the MLH1-deficient tumors.

12. Conclusions: Please clarify which factors are included in the routine diagnostic work-up.

Minor essential revisions

1. Abstract, fourth paragraph: clarify the meaning of “additional” in the first sentence.

2. Please, provide in Methods the number of patients with synchronous/metachronous colon cancers.

3. Growth pattern was classified as “expanding” or “infiltrating” in the text, but as “pushing” or “infiltrating” in the Table 2. Please, homogenize.

4. Slides were re-evaluated by 2 independent investigators: all of the slides (as stated in Methods, third paragraph) or a subset of 200 tumors (Methods, second
paragraph)? Please clarify.

5. Results, first paragraph: Please refer to Table 2.

6. Clarify the number of patients/tumors from whom complete information was available: 438/462 patients with complete data (Results, second paragraph); 25 cases (tumors or patients?) with missing information (Methods, sixth paragraph).

7. Results, third paragraph: Data concerning performance of the predictive models should be included in Table 4.

8. In order to improve the interpretation of Tables 3 and 4, they could be merged in a single table.

9. Discussion, third paragraph: “We included the 95% of colon cancers…” What does it mean?

Minor issues not for publication

1. Background, second paragraph “MSI tumors are characterized by distinct clinical and morphologic features…”, should be changed to “MMR-deficient tumors are characterized by distinct clinical features…” (Omit morphologic).

2. Table 1: Method applied for determination of “MSI status” should be corrected.

3. Table 2: It is confusing to show the range of ages as well as the % between parentheses.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.