

## **Point-by-point responses to the board of editors and reviewers**

### **From the BOARD OF EDITORS:**

#### **Reviewer #1**

This paper compared two- and four-antibody panels of MSI testing in CRC. Usually, MSI testing is performed using NCI-5 Bethesda panel. The authors found a two-antibody panel of PMS2/MSH6 might be the best choice in terms of cost-effectiveness and accuracy. The costs of the MSI test and two-antibody panels of IHC were \$200 and \$80.

**1. However, as a cost-effective screening tool, does the positive /negative patients still need four-antibody panels or NCI-5 Bethesda panel? Then the overall cost will be different?**

Author response: Thank you for the valuable comments. The two-antibody panel using PMS2/MSH6 presented an identical specificity of 99.6% compared to the four-antibody panel. Relating to the reviewer's comment, sensitivity of two-antibody panel was slightly lower (92.2%) compared to the four-antibody panel (97.4%). To assess patients more accurately, an additional four-antibody panel or NCI-5 Bethesda panel could be performed in patients tested negative from two-antibody panel (3295 patients, 94.5%). However, this would result in additional expenses and will be even more costly than initially testing patients using the four-antibody panel.

We believe using the two-antibody panel (PMS2/MSH6) is a cost-effective screening tool with acceptable sensitivity and specificity, but inevitably less accurate compared to the much

expensive four-antibody panel. Therefore, the two-antibody panel should be used according to the different circumstances medicine is practiced.

**2. If not, the authors did not demonstrate / discuss about the prognostic value and predicting response to chemotherapy of this two-antibody panel.**

Author response: As the reviewer pointed out, patients tested with the two-antibody panel could have different prognosis due to the different treatment they may receive compared to the four-antibody panel. We predict the difference not to be significant as the predictive value of the two-antibody panel and four-antibody panel are comparable. However, we did not evaluate the clinical outcomes (recurrence-free survival, overall survival) according to each screening methods. Further research to analyze patient prognosis regarding chemotherapy and type of antibody panels must be performed. We have included these limitations in the discussion section. Page14. Line11.

**Reviewer #2**

**No comments.**

**Reviewer #3**

**The microsatellite instability (MSI) or immunohistochemistry (ICH) are two tests used in DNA mismatch repairing (MMR) screening in colorectal cancer. The authors in this original paper compare sensitivity, specificity, and cost-effectiveness of two-four antibody immunohistochemical panel considering the MSI test as a reference point. The results of the study are encouraging, showing that the sensitivity of a two Ab-panel is only slightly lower than that of Four ab-panel for half the price.**

**1. Although methods, statistical analysis and data research seem to be well structured, the central argument of the paper could be off-topic with respect to the objectives of the journal. The title should be less generic and at least partially hint at the conclusion of the analysis.**

Author response: Thank you for the valuable comments. As the reviewer recommended, we changed our title so that it could be more specific and reflect our conclusion.

Page1. Line6. “Cost-effective screening using a two-antibody panel for detecting mismatch repair deficiency in sporadic colorectal cancer”.

**2. In the introduction the authors assert that “several studies have compared four- and two-antibody panels in term of accuracy and cost-effectiveness” but the reference is only one.**

Author response: We have searched and included references presenting accuracy and cost-effectiveness of antibody panels.

- 7 Shia J, Tang LH, Vakiani E, Guillem JG, Stadler ZK, Soslow RA, Katabi N, Weiser MR, Paty PB, Temple LK, Nash GM, Wong WD, Offit K, Klimstra DS. Immunohistochemistry as first-line screening for detecting colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: a 2-antibody panel may be as predictive as a 4-antibody panel. *Am J Surg Pathol* 2009; 33: 1639-1645 [PMID: 19701074 DOI: 10.1097/PAS.0b013e3181b15aa2]
- 8 Gould-Suarez M, El-Serag HB, Musher B, Franco LM, Chen GJ. Cost-effectiveness and diagnostic effectiveness analyses of multiple algorithms for the diagnosis of Lynch syndrome. *Dig Dis Sci* 2014; 59: 2913-2926 [PMID: 24957400 DOI: 10.1007/s10620-014-3248-6]
- 9 Snowsill TM, Ryan NAJ, Crosbie EJ, Frayling IM, Evans DG, Hyde CJ. Cost-effectiveness analysis of reflex testing for Lynch syndrome in women with endometrial cancer in the UK setting. *PLoS One* 2019; 14: e0221419 [PMID: 31469860 DOI: 10.1371/journal.pone.0221419]

**3. Some typos in the text are present (splenic flexion, splenic flexure??)**

Author response: We are sorry for the confusion arising from simple error. We corrected “splenic flexion” to “splenic flexure”.

**4. The study is well conceived and with a practical application of saving. However, its validity is greatly influenced by too conspicuous variability between the different local health systems policies and by the price variability of immunohistochemistry tests in the**

**various countries. A multicenter analysis involving in different countries would be more appropriate for the study in question.**

Author response: We agree with the reviewer and this limitation of our study has been stated in the discussion section. As the reviewer commented, a prospective multicenter multinational study is necessary to accurately compare the immunohistochemistry tests. This could further provide oncologic outcomes in patients treated according to different antibody panels.

We searched for costs of immunohistochemistry test and MSI in different countries. In South Korea, The costs of the MSI test and two-antibody panels of IHC were \$200 and \$80, while in China the costs of the MSI test and two-antibody panels of IHC were 1500 RMB yuan ( $\approx$ \$234) and 500 RMB yuan ( $\approx$ \$78).<sup>[1]</sup> In the United Kingdom, the costs of the MSI test and two-antibody panels of IHC were £202( $\approx$ \$285) and £105( $\approx$ \$148)<sup>[2]</sup> and the average costs of the MSI test and two-antibody panels of IHC were \$450and\$250 in the United States of America.<sup>[3]</sup>This information regarding costs of tests were not compared at the same time period but we could indirectly see that the two-antibody panels of IHC was approximately half the price of MSI tests in represented countries.

Page12. Line12.

#### **Reviewer #4**

**I would like to thank the opportunity to review the present paper. This study has an important cohort from a single centre. The information presented is of great importance for centres where the cost of diagnostic procedures could have an impact in**

**health care systems or in countries where different systems may coexist. I have some questions:**

**1. The authors state that the objective of the paper is to evaluate the accuracy and cost benefit of the two-antibody panel of IHC for detecting MMR deficiency. I have read the cost of each individual test but not the clinical benefit obtained from this approach compared to the MSI test on the cohort studied. Also, the evaluation of sensitivity and specificity is not an evaluation of accuracy.**

Author response: Thank you for the detailed review which can improve our study. As the reviewer pointed out, our present study only compared cost and accuracy of different tests. To analyze clinical benefits, we would need to collect survival data (disease-free survival or overall survival) which was not the primary outcome of our study. Once we have shown that the two-antibody panel is a comparable modality for detecting mismatch repair deficiency in sporadic colorectal cancer, a follow-up study should be performed for comparing survival outcome. If the clinical outcome is also comparable between two- and four-antibody panels, it would provide a stronger evidence for applying the two-antibody panels as it is more cost-effective.

As to the evaluation of accuracy, we consulted our medical statistician (Park SY MD. PhD.). According to classic textbook of statistics, there are four measurements for measuring accuracy which are false positive fraction (FPF), true positive fraction (TPF), positive predictive value (PPV), and negative predictive value (NPV). Because  $FPF=1-\text{specificity}$  and

TPF=sensitivity, generally specificity and sensitivity can be referred for measures of accuracy.<sup>[4]</sup>

If the reviewer is meaning the classification accuracy (=correct classification rate), numerous articles demonstrated that this is a poor, misleading metric to evaluate the performance of a diagnostic test (or binary classifier) because it is heavily affected by disease prevalence and does not distinguish two types of error (false positive vs. false negative).

**2. The definition of rectum should be revisited: "...from the rectosigmoid junction to the rectum"**

Author response: It is generally accepted that rectum starts from the rectosigmoid junction and we included anatomical characteristic of the rectum compared to colon for a clearer delivery.

Page7. Line13. *"and "rectum" from the rectosigmoid junction to the rectum in which the confluence of the taeniae coli and presence of epiploica appendices are absent."*<sup>[5]</sup>

**3. I would recommend to change the term "histology" to a more accurate as "Differentiation grade"**

Author response: We changed the term “histology” to “differentiation grade” according to the reviewer’s comment.

- 1 Chao X, Li L, Wu M, Ma S, Tan X, Zhong S, Bi Y, Lang JJCC. Comparison of screening strategies for Lynch syndrome in patients with newly diagnosed endometrial cancer: a prospective cohort study in China 2019; **39**: 1-12
- 2 Snowsill T, Coelho H, Huxley N, Jones-Hughes T, Briscoe S, Frayling IM, Hyde C. Molecular testing for Lynch syndrome in people with colorectal cancer: systematic reviews and economic evaluation 2017;
- 3 Gould-Suarez M, El-Serag HB, Musher B, Franco LM, Chen GJJd, sciences. Cost-effectiveness and diagnostic effectiveness analyses of multiple algorithms for the diagnosis of Lynch syndrome 2014; **59**: 2913-2926
- 4 Pepe MS. The statistical evaluation of medical tests for classification and prediction: Medicine, 2003
- 5 Beck DE, Roberts PL, Saclarides TJ, Senagore AJ, Stamos MJ, Wexner SD. The ASCRS textbook of colon and rectal surgery: Springer Science & Business Media, 2011