Response to Editor and Reviewers

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Dear Editor,

We are pleased to receive the reviewer's comments and suggestions on our manuscript entitled "Is Immunohistochemistry Misleading in Microsatellite Instability: A Case Series" We have reviewed the manuscript and made revisions as per your suggestions. Additionally, we have corrected grammar and syntax errors. References, tables, and other manuscript sections have been revised and formatted according to journal style. Thank you for your comments and time.

Round 1

Response to Reviewer 1:

We appreciate the reviewer's valuable comments, suggestions, and time dedicated in reviewing the manuscript. Please find our responses and corresponding revisions made to the manuscript shown below:

1. Interesting study. The authors report a cholangiocellular carcinoma case revealing proficient MMR by IHC but MSI-H by liquid NGS, a cervix cancer case that was dMMR by IHC, MSS by PCR but MSI-H by NGS and an endometrium cancer case found to be pMMR by IHC but MSI-H by NGS. I recommend the authos to provide some relevant figures for these three cases.

Response: The figures of the cervix cancer case is provided; however, the other two cases were international patients and referred to us with their pathology reports. We just recommended NGS tests, and they shared the results. As a result, it is not possible to add their IHC results. But NGS results are provided.

Response to Reviewer 2:

We appreciate the reviewer's pertinent comments and suggestions. Thank you for your time. Please find our responses, revisions, and corrections according to your suggestions shown below:

The study deals with an interesting report of three cases of discordant MSI (Microsatellite Instability) results detected using different methods, and I have some comments to make. Overall, the manuscript raises an interesting topic to be published but needs a major revision to have sufficient quality to be published in this journal.

Comments:

1. I suggest the authors describe the clinical cases in paragraphs continuously without the subsections (Chief complaints, History of present illness, History of past illness, etc). Moreover, I suggest they exclude part of the report that is not essential to the understanding of the cases, such as routine blood and urine analyses or the complete physical examination.

Response: The subsections were written because of the journals formatting. Necessary changes are made accordingly. However, since the format is different during submission, we would like editors to take the uploaded version of the manuscript into consideration.

2. The meaning of the title is based on only 3 cases, so none of the conclusions should be written at that point. Therefore, I suggest the authors modify the title to be more descriptive of what they found. Example: "Discrepancy among MSI detection methodologies in non-colorectal cancer – report of three cases"

Response: The title is changed as suggested.

3. Which parameters of immunohistochemistry (IHC) do the author used to classify it as indeterminate? It should be better described in the manuscript.

Response : MSI is classified as indeterminate in the case of loss of only one heterodimer unit. It is also described in the manuscript.

4. Was the histological analysis performed by more than one pathologist? In order to check if they agree with themselves?

Response: The histological analyses were made by more than one pathologist as the patients were international.

5. What led some cases to perform NGS and not PCR and vice versa? It should be better detailed in the case presentation or the discussion section.

Response: Since the patients were international (referring for consultation but followed by their local doctors), we did not have the pathology blocks so for case 1 and 3, we could not perform PCR but the patients sent their specimens for NGS as

recommended and shared the results. In addition, since NGS gives more detailed molecular analysis we preferred it not to loose time with IHC and PCR. In case 2, all methods were used for confirmation of MSI status.

6. In case 2, which type of biological sample was collected to perform the PCR? If collected from a different location than the original tumor, could it have resulted differently? Along the same line, the sample collection for performing IHC and NGS of case 3 involved samples from the metastases; would that be the cause of the discrepancy between the results? Why it was not collected from the surgical specimen from the primary tumor? All these points mentioned should be included in the discussion as limitations of the study.

Response : In case 2, all the analysis were done from the same sample, the pelvic mass. We believe it is sufficient to perform all analysis from the same specimen to reach a conclusion in terms of discrepancy. In case 3, sample collection was made from the metastases as it had been 10 years since the diagnosis. New biopsy was mandatory to confirm the diagnosis of metastases and it was easily accessible for NGS. The mentioned limitations are included in the discussion section.

7. I missed figures that may show the histological findings or even the results of NGS and PCR.

Response : The available results are added as figures in the manuscript.

Round 2

The authors revised the manuscript according to the reviewer´s comments. They accepted/discussed all the comments and the manuscript has improved after revision. I uploaded a version of the manuscript with writing suggestions for the authors. I have no additional comments.