

Dear Editor and Reviewers:

Thank you for reviewing and providing helpful comments on our manuscript entitled "Breast cancer and rectal cancer associated with Lynch syndrome: A case report and literature review" (ID: 92735).

We have studied the comments carefully and have made revision to the original article seriously. Additionally, we uploaded a clean version of the manuscript as requested and revised portion are marked in red in the revised manuscript. The answers to the comments made by the reviewers and the changes made to the original manuscript are listed as follows.

**Reviewers' comments:**

**Reviewer #1:**

In this case report, the authors present a case of a 49-year-old woman who developed two cancers, first of breast at the age of 39 years and second of colorectal region at 49 years. Both were treated surgically. There is no other treatment or follow-up provided in this case report. The patient has been diagnosed as a case of Lynch syndrome (LS) based on the development of two malignant tumors. While potentially interesting, there are several major points in the case report. A major revision is needed.

**Major points:**

1. The case report itself seems incomplete. There is no family history. No information on treatment apart from surgery.

**Answer:**

We deeply appreciate the reviewer for the careful reading and extensive recommendations made to our manuscript that has markedly enhanced the integrity of our work. In the revised manuscript section "4.1 Molecular Mechanism and Classification of LS," we have included additional details regarding the patient's family history and postoperative treatment information. (see page 7, line 155 - line 157 in the revised manuscript).

2. No follow-up data is given.

**Answer:**

We sincerely thank the reviewer for highlighting the absence of follow-up data in our manuscript. We have now included the patient's subsequent treatment details in the revised manuscript. Following the initial postoperative treatment, no additional significant data were observed that require further reporting. Thank you very much for the constructive comments. (see page 6, line 138 - line 142 in the revised manuscript).

3. The pathological results are incomplete. Pathologic staging is not given.

**Answer:**

We appreciate the reviewer for highlighting the gaps in the pathological results and the missing pathological staging. The pathological stage of the breast cancer, "Stage IIa infiltrating ductal carcinoma of the right breast," has been thoroughly described in the revised manuscript under section "2.1 Case Data of BC". The pathological staging of the rectal tumor is moderately differentiated adenocarcinoma (T3), detailed in section 2.2 Case Data of rectal cancer in the revised manuscript. Thank you for your valuable feedback. (see page 3, line 70 and line 73 in the revised manuscript).

4. No gene mutations were tested and IHC results did not show a deficiency of MMR genes, which is not in favor of a diagnosis of LS.

**Answer:**

We sincerely thank the reviewer for kindly pointing out this issue. We sincerely apologize, but due to issues at our company, the original sequencing results have unfortunately been lost. Therefore, we can only provide the remaining data under the identifier 23B03859180\_DX1616\_1698971993321 (supplementary 1).

According to this data, MLH1 shows a low-frequency mutation (0.00002) in the Genome Aggregation Database (GnomAD), and this mutation is classified as a missense variant. Given this information, the mutation may warrant further investigation for its potential pathogenicity.

Regarding your comment that no gene mutations were detected and the

immunohistochemistry (IHC) results did not show a deficiency of MMR genes, which does not support a diagnosis of Lynch Syndrome (LS), we acknowledge this finding. However, based on the patient's typical symptoms, medical history, and family history <sup>[1,2]</sup>(which have been included in the revised manuscript), we believe there is sufficient reason to consider this patient as meeting the diagnostic criteria for LS.

Thank you for your understanding and continued support.

1.Lotsari JE, Gylling A, Abdel-Rahman WM, Nieminen TT, Aittomäki K, Friman M, Pitkänen R, Aarnio M, Järvinen HJ, Mecklin JP, Kuopio T, Peltomäki P. Breast carcinoma and Lynch syndrome: molecular analysis of tumors arising in mutation carriers, non-carriers, and sporadic cases. *Breast Cancer Res* 2012; 14: R90 [PMID: 22691310 DOI: 10.1186/bcr3205]

2..Grolleman JE, Díaz-Gay M, Franch-Expósito S, Castellví-Bel S, de Voer RM. Somatic mutational signatures in polyposis and colorectal cancer. *Mol Aspects Med.* 2019 Oct;69:62-72. doi: 10.1016/j.mam.2019.05.002. Epub 2019 May 23. PMID: 31108140.

**Minor points:**

5. What was the size of the breast cancer and what was its pathological stage?

**Answer:**

We genuinely appreciate the reviewer for pointing out the concerns regarding the completeness of information on the size and pathological stage of the breast cancer. The size of the breast cancer is 2.1 x 1.2 cm, and it is pathologically staged as "Stage IIa infiltrating ductal carcinoma of the right breast". These details have been thoroughly described in section 2.1 Case Data of BC within the revised manuscript. Thank you once again for your insightful feedback. (see page 3, line 70 and line 73 in the revised manuscript).

6. What are local fibrous breast lesions? Use standard terminology.

**Answer:**

We sincerely thank the reviewer for this important comment. Localized fibrocystic change in the breast refers to fibrosis occurring in part of the breast lesion. We appreciate the reviewer for highlighting our issue. In the revised manuscript, we have revised this to "with localized fibrocystic changes observed in the surrounding breast tissue". (see page 3, line 76- line 77 in the revised manuscript).

7. Some terms are confusing, eg. Basal fascia or outer fibrous tissue membrane.

**Answer:**

We appreciate the reviewer for bringing this issue to our attention. In the revised manuscript, we have carefully reconsidered and adjusted these terms, omitting "standard terminology." (see page 3, line 76- line 79 in the revised manuscript).

8. PR is wrongly written as FR.

**Answer:**

We appreciate the reviewer for noting this issue. The error has been corrected in the revised manuscript. Thank you very much! (see page 3, line 83 in the revised manuscript).

9. What are TUPO II and TDPO II? Are these same or different? Please specify.

**Answer:**

We greatly appreciate the reviewer for thoroughly reviewing our work and pointing out this issue. TUPO II and TDPO II should both be described as topoisomerase II (TOPO II). We have made the necessary corrections in the revised manuscript. Thank you very much for your guidance. (see page 3, line 84 in the revised manuscript).

10. On IHC, all four MMR genes were positive, then how a diagnosis of LS was made?

**Answer:**

We appreciate the reviewer for indicating the problems. Lynch syndrome (LS), also known as hereditary non-polyposis colorectal cancer (HNPCC), is a common hereditary cancer syndrome primarily caused by mutations in DNA mismatch repair (MMR) system genes, particularly MLH1, MSH2, MSH6, and PMS2<sup>[1]</sup>. For patients suspected of having Lynch syndrome with colorectal cancer, diagnosis involves several diagnostic steps<sup>[2,3]</sup>.

1) Immunohistochemical staining of tumor tissue to assess the expression of MLH1, MSH2, MSH6, and PMS2 proteins for mismatch repair deficiency.

2) Microsatellite instability (MSI) testing of tumor tissue and normal tissue to detect microsatellite instability.

3) Genetic testing of blood samples to identify pathogenic germline mutations in mismatch repair genes, confirming Lynch syndrome.

4) Genetic testing of first-degree relatives (parents, children, siblings) to assess familial risk of Lynch syndrome.

Additionally, other examinations such as gastroscopy, colonoscopy, CA125, pelvic ultrasound, and endometrial biopsy are necessary to exclude or confirm gastric cancer, colorectal cancer, or endometrial cancer.

In this case, the patient shows positive results for all four MMR genes on MHC testing, with genetic testing revealing mutations and a significant family history with typical symptoms. Thank you very much!

1. Provenzale D, Gupta S, Ahnen DJ, Bray T, Cannon JA, Cooper G, David DS, Early DS, Erwin D, Ford JM, Giardiello FM, Grady W, Halverson AL, Hamilton SR, Hampel H, Ismail MK, Klapman JB, Larson DW, Lazenby AJ, Lynch PM, Mayer RJ, Ness RM, Regnbogen SE, Samadder NJ, Shike M, Steinbach G, Weinberg D, Dwyer M, Darlow S. Genetic/Familial High-Risk Assessment: Colorectal Version 1.2016, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*. 2016 Aug;14(8):1010-30. doi: 10.6004/jnccn.2016.0108. PMID: 27496117.

<https://doi.org/10.6004/jnccn.2016.0108>

2. Grolleman JE, Díaz-Gay M, Franch-Expósito S, Castellví-Bel S, de Voer RM. Somatic mutational signatures in polyposis and colorectal cancer. *Mol Aspects Med*. 2019 Oct;69:62-72. doi: 10.1016/j.mam.2019.05.002. Epub 2019 May 23. PMID: 31108140.

<https://doi.org/10.1016/j.mam.2019.05.002>

3. Alquati C, Prossomariti A, Piazzini G, Buttitta F, Bazzoli F, Laghi L, Ricciardiello L. Discovering the Mutational Profile of Early Colorectal Lesions: A Translational Impact. *Cancers (Basel)*. 2021 Apr 25;13(9):2081. doi: 10.3390/cancers13092081. PMID: 33923068; PMCID: PMC8123354.

<https://doi.org/10.3390/cancers13092081>

11. The description of loose stools and their appearance is confusing and not clear.

**Answer:**

We sincerely thank the reviewer for kindly pointing out this issue. In the revised manuscript, we have revised this to "The patient is a 49-year-old female who, since September 2023, has experienced unformed, loose stools without an apparent cause. These stools are often accompanied by small amounts of blood, mostly adherent to the surface, and occasionally mixed with mucus. She does not report tenesmus or diarrhea, and has a bowel movement frequency of 12-13 times per day." (see page 3, line 88 - line 94 in the revised manuscript).

12. Peripheral lymph nodes should be changed to local or regional or pelvic lymph nodes.

**Answer:**

We sincerely thank the reviewer for kindly pointing out this issue. In the revised manuscript, we have revised this to "pelvic lymph nodes". Thank you once again for your instructive comment. (see page 3, line 96 - line 97 in the revised manuscript).

13. Figure legends of Figures 1 and 2 are deficient. Please describe in some detail what is shown and point it out with arrows etc.

**Answer:**

We sincerely thank the reviewer for this important comment. In the revised manuscript, we have revised the Figure legends of Figures 1 and 2.

Fig.1 Molybdenum target mammography showed local fibroid breast lesions around the right breast

A. Right Breast Axis (Breast Cancer - Right Breast Upper Outer Quadrant); B. Right Breast Oblique (Breast Cancer - Right Upper Outer Quadrant). The red arrow points to the tumor. (see page 5, line 117 - line 121 in the revised manuscript).

Fig.2 Pelvic magnetic resonance (MR) shows sigmoid colon mass

A. Pelvic Computed tomography (CT) - Coronal Position; B. Pelvic CT – Sagittal. The red arrow points to the tumor. (see page 6, line 124- line 128 in the revised manuscript).

14. What approach was used for radical resection of rectal cancer?

**Answer:**

We appreciate the reviewer for indicating the problems. Rectal cancer radical resection typically involves surgical methods such as Low Anterior Resection (LAR), Abdominoperineal Resection (APR), Ultra-low Anterior Resection, Intersphincteric Resection (ISR), Laparoscopic Surgery, and Robotic-Assisted Surgery. In this case, the patient underwent laparoscopically assisted radical resection of the rectal tumor under general anesthesia.

15. Was it rectal or sigmoid colon cancer?

**Answer:**

We sincerely thank the reviewer for kindly pointing out this issue. We have revised the manuscript accordingly to specify "rectal cancer". Thank you very much!

16. It is repeatedly mentioned that this presentation with breast cancer or CRC was typical or prototypical of LS. How, when no family history is provided.

**Answer:**

We thank the reviewer for the valuable comments. We recognize LS as the most prevalent hereditary colorectal cancer (CRC) syndrome, yet it is also associated with other types of cancers, including breast cancer. Therefore, we have augmented the description of the patient's family history in the revised manuscript section 4.1 "Molecular Mechanism and Classification of LS". Thank you once again for your instructive comment. (see page 7, line 155 - line 157 in the revised manuscript).

17. TILs are not mentioned in the pathology of CRC. It is given in discussion only.

**Answer:**

We sincerely thank the reviewer for indicating the concerns in our paper. TLR stands for "Toll-like Receptor"<sup>[1]</sup>. They are receptors in the innate immune system capable of recognizing pathogen-associated molecular patterns (PAMPs) and play a crucial role in immune responses<sup>[1,2]</sup>. While TLRs are extensively studied for their roles in immune responses and inflammation, their specific pathological significance in CRC is typically not included in standard pathology reports<sup>[1,2]</sup>. Therefore, we only provided a description in the discussion. We greatly appreciate the reviewers for pointing out our issues.

1. Sheikh A, Taube J, Greathouse KL. Contribution of the microbiota and their secretory products to inflammation and colorectal cancer pathogenesis: the role of toll-like receptors. *Carcinogenesis*. 2021 Oct 5;42(9):1133-1142. doi: 10.1093/carcin/bgab060. PMID: 34218275

2. Sheikh A, Taube J, Greathouse KL. Contribution of the microbiota and their secretory products to inflammation and colorectal cancer pathogenesis: the role of toll-like receptors. *Carcinogenesis*. 2021

Oct 5;42(9):1133-1142. doi: 10.1093/carcin/bgab060. PMID: 34218275.