RESEARCH ARTICLE

Editorial Process: Submission:06/06/2024 Acceptance:10/11/2024

Simplifying Mismatch Repair Deficiency Screening in Endometrial Adenocarcinoma: Immunohistochemistry with Two-Antibody Panel (PMS2 and MSH6)

Pinyada Panyavaranant[1], Natkrita Pohthipornthawat[2], Tarinee Manchana[1]*

Abstract

Background: Mismatch repair deficiency (dMMR) is a well-established characteristic of endometrial adenocarcinoma and is crucial in screening for Lynch syndrome, guiding adjuvant treatment decisions, and identifying candidates for immune checkpoint inhibitors. The traditional approach to dMMR screening involves a four-antibody panel, but a simplified two-antibody method utilizing PMS2 and MSH6 has shown promise. This study aims to compare the diagnostic performance of the simplified two-antibody method with the traditional four-antibody panel in endometrial cancer samples. **Methods:** We conducted a retrospective cohort study on endometrial carcinoma cases diagnosed between 2013 and 2022. We compared the diagnostic performance of the two-antibody panel with the traditional four-antibody panel in detecting dMMR. Clinical data and immunohistochemistry results were collected, and agreement between the two methods was evaluated using Cohen's kappa coefficient. **Results:** 304 endometrial cancer cases were included, with 27% demonstrating loss of at least one MMR protein using the four-antibody panel. The two-antibody method detected MMR deficiency in 26.6% of cases, with a high agreement rate of 98.8% between the two methods. Only one case showed discordant results, prompting further investigation. **Conclusion:** The simplified two-antibody MMR IHC screening approach using PMS2 and MSH6 showed high concordance with the traditional four-antibody panel. This suggests its potential as an alternative method for reflex MMR status testing in endometrial adenocarcinoma. The implementation of this approach could streamline the diagnostic process, reduce costs, and improve the detection of Lynch syndrome in affected individuals and their families. Further studies with larger cohorts and long-term follow-up are needed to validate these findings and assess the clinical implications of this approach in routine practice.

Keywords: Endometrial adenocarcinoma- Immunohistochemistry- Lynch syndrome- Mismatch repair deficiency

Asian Pac J Cancer Prev, **25 (10)**, 3667-3671

Introduction

Mismatch repair deficiency (dMMR) is a well-established characteristic of endometrial adenocarcinoma that plays a crucial role in Lynch syndrome screening, guiding adjuvant treatment decisions, and identifying potential candidates for immune checkpoint inhibitors [1, 2]. The MMR system plays a crucial role in maintaining genomic stability by rectifying DNA replication errors and preventing the accumulation of mutations. Inherited or acquired defects in MMR genes can lead to dMMR, which is a hallmark of Lynch syndrome, an autosomal dominant cancer predisposition syndrome that increases the risk of various cancers, including endometrial cancer [3]. Approximately 3% of all endometrial cancer cases are linked to Lynch syndrome, and up to 60% of dMMR endometrial cancers are associated with this syndrome [4]. A recent study in Thai endometrial cancer patients reported a detection rate of dMMR in 34.9% of cases, suggesting the consideration of MMR immunohistochemistry in all patients, regardless of personal or family history of Lynch syndrome-related cancers [5, 3].

The current guideline for dMMR screening in endometrial cancer involves utilizing a four-antibody panel to assess the expression of MMR proteins (MLH1, MSH2, MSH6, and PMS2) through immunohistochemistry (IHC) [6]. This four-antibody panel is recommended by several professional societies and widely recognized as the standard approach for dMMR screening in endometrial cancer [7, 6, 8]. Recent analyses have yielded compelling evidence that substantiates the cost-effectiveness of employing the MMR IHC approach for germline testing [9, 10]. Moreover, it is important to note that MMR testing is not limited to endometrial cancer alone. It also holds significant value for patients diagnosed with colorectal

*[1] Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. [2]Department of Obstetrics and Gynecology, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. *For Correspondence: tarinee.m@chula.ac.th*

cancer and other types of carcinomas that are associated with Lynch syndrome, including ovarian, stomach, and urothelial carcinomas, along with their respective families [11].

The accurate repair of DNA relies on the essential role of mismatch repair (MMR) proteins, which function as heterodimer complexes. Specifically, *MLH1* forms a stable heterodimer with *PMS2*, while *MSH2* pairs with *MSH6*. In cases where there is a loss of protein function in *PMS2* or *MSH6*, *MLH1* and *MSH2* can form heterodimers with alternative proteins. Consequently, when evaluating MMR protein expression using IHC, negative staining is expected for both MMR proteins within the affected heterodimer [12]. Recent reports have suggested a simplified strategy for dMMR screening in endometrial adenocarcinoma, which involves the use of only two antibodies, PMS2 and MSH6 [13-15].

This study aims to compare the diagnostic performance of a simplified dMMR screening strategy utilizing only PMS2 and MSH6 IHC, as opposed to the traditional four-antibody panel, in a cohort of endometrial cancer samples. We hypothesize that PMS2 and MSH6 IHC can effectively replace the four-antibody panel without compromising the accuracy of dMMR detection. The results of this study may have significant implications for the clinical management of endometrial adenocarcinoma patients by simplifying the screening process, reducing testing costs, and improving the detection of Lynch syndrome in affected individuals and their families.

Materials and Methods

This retrospective cohort study included endometrial carcinoma patients diagnosed between 2013 and 2022 at King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Patients diagnosed with endometrial cancer and treated during the specified period were identified using the ICD10 code C54 for endometrial cancer from electronic medical records. The inclusion criteria for this study were patients with histologically confirmed endometrial carcinoma who received treatment at the hospital between 2013 and 2022 and underwent dMMR screening using the four-antibody panel. Patients with prior chemotherapy and radiation therapy, incomplete medical records, or unclear/inexplicable MMR staining results were excluded from the study.

The data collection process involved retrieving clinical data from medical records, including age at diagnosis, menopausal status, histologic subtype, and cancer staging according to the 2018 International Federation of Gynecology and Obstetrics (FIGO) uterine cancer staging system. Family history of cancers and Lynch-related cancers were also collected. The Revised Bethesda guidelines were evaluated in each patient.

Formalin-fixed, paraffin-embedded tissue sections from hysterectomy specimens were utilized for IHC analysis. Hematoxylin and eosin-stained slides from each patient were reviewed to confirm the diagnosis and select representative tumor areas for IHC analysis. Specialized pathologists reviewed the IHC results for MLH1, MSH2, MSH6, and PMS2 antibodies. In cases where discrepancies arose in the results, consensus review was conducted to resolve them.

The assessment of normal expression of MMR proteins entailed the examination of nuclear staining within tumor cells as a reliable indicator. To establish a positive internal control, nuclear staining within infiltrating lymphocytes and/or normal stromal cells was employed. MMR deficiency or the loss of expression signified the absence of detectable levels in at least one of the four MMR proteins. Specifically, in the context of the two-antibody panel, dMMR precisely indicated the loss of expression in either PMS2 or MSH6. Conversely, in the four-antibody panel, dMMR corresponded to the loss of expression in one or more of the four proteins.

Patients with dMMR underwent genetic counseling with a geneticist. Following genetic counseling, germline testing was conducted by extracting DNA from saliva or peripheral blood samples upon agreement. If a germline mutation was detected, comprehensive cancer surveillance was provided to the entire family.

Statistical analysis was performed using SPSS version 22.0 (IBM Corporation, Armonk, New York, USA). Quantitative data were analyzed and presented as mean ± standard deviation (SD), while qualitative data were reported as frequencies and percentages. The agreement between the two MMR staining methods was assessed using Cohen's kappa coefficient. The interpretation of kappa values is as follows: values ≤ 0 indicate no agreement, 0.01–0.20 represent none to slight agreement, 0.21–0.40 indicate fair agreement, 0.41 – 0.60 suggest moderate agreement, 0.61 – 0.80 imply substantial agreement, and 0.81 – 1.00 indicate almost perfect agreement [16].

Results

Between January 2013 and December 2022, a total of 304 endometrial cancer patients included in this study. The participants had a mean age of 58.1 ± 11.9 years (ranging from 20 to 85 years). Among them, 24 patients (7.9%) were below 40 years of age, and 61 patients (20.1%) were between 40 and 50 years old. 214 patients (70.4%) were menopause at the time of surgery. Additionally, 82 patients (27%) had a body mass index (BMI) exceeding 30 kg/m^2, with a mean BMI of 27.2 kg/m^2 for the overall study population. Regarding family history, 52 patients (17.1%) had at least one family member with any type of cancer, while 44 patients (14.5%) had at least one family member affected by Lynch-related cancers. Based on the Bethesda guidelines, 77 patients (25.3%) met the criteria for further evaluation.

Of the total patients, 232 patients (76.3%) were categorized as stage I, 14 patients (4.6%) as stage II, 48 patients (15.8%) as stage III, and 10 patients (3.3%) as stage IV. In terms of histology, 278 patients (91.4%) were classified as endometrioid, 10 patients (3.3%) as mixed adenocarcinoma, 9 patients (3%) as carcinosarcoma, and 7 patients (2.3%) as papillary serous carcinoma. Regarding tumor grade, 153 patients (50.3%) were grade 1, 72 patients (23.7%) were grade 2, and 79 patients (26%) were grade 3 (Table 1).

Table 1. Demographic and Pathological Data

	N = 304
Age (years), mean ± SD	58.1 ± 11.9
Age < 40 years, n (%)	24 (7.9)
Age < 50 years, n (%)	61 (20.1)
Menopause, n (%)	214 (70.4)
BMI (kg/m^2), mean ± SD	27.2 ± 6.6
	(Range 16.4 - 58.4)
Obesity (BMI ≥ 30kg/m^2), n (%)	82 (27)
Family history of cancers, n (%)	52 (17.1)
Family history of Lynch-related cancers, n (%)	44 (14.5)
Met the criteria in Bethesda guidelines, n (%)	77 (25.3)
Stage, n (%)	
I	232 (76.3)
II	14 (4.6)
III	48 (15.8)
IV	10 (3.3)
Histology, n (%)	
Endometrioid	278 (91.4)
Mixed adenocarcinoma	10 (3.3)
Carcinosarcoma	9 (3)
Papillary serous carcinoma	7 (2.3)
Tumor grade, n (%)	
Grade 1	153 (50.3)
Grade 2	72 (23.7)
Grade 3	79 (26)

Among the patients, 82 (27%) demonstrated the loss of expression in at least one MMR protein using the four-antibody panel. Specifically, out of these 82 patients, 54 showed the loss of MLH1 and PMS2 expression, 16 showed the loss of MSH2 and MSH6 expression, 6 showed the loss of only MSH6 expression, 5 showed the loss of only PMS2 expression, and one patient showed the loss of only MSH2 expression. (Table 2) Notably, the patient who showed the sole loss of MSH2 expression underwent germline testing, and the results were intriguing as they revealed no MMR gene mutation.

Using the two-antibody panel, dMMR was detected in 81 patients (26.6%), with only one patient showing the loss of any MMR protein using the four-antibody panel that could not be detected using the two-antibody panel (Table 3). Overall, the results from the two-antibody panel agreed with the four-antibody panel in 98.8% (81/82) of patients. The agreement between the two-antibody panel and four-antibody panel was measured using Kappa correlation, yielding a value of 0.992 (SD = 0.008, p-value < 0.001).

Out of the 59 patients that had loss of PMS2 expression, 54 patients also showed loss of MLH1 expression. Similarly, all 54 patients with loss of MLH1 expression exhibited loss of PMS2 expression. In patients who had loss of MSH6 expression (n = 22), 16 of them also displayed loss of MSH2 expression.

Discussion

The importance of dMMR in endometrial adenocarcinoma cannot be overstated. dMMR is a well-established characteristic of this cancer type and is associated with a favorable prognosis and responsiveness to immune checkpoint inhibitors [17]. In our study, we observed that approximately 27% of the endometrial cancer patients demonstrated loss of expression in at least one MMR protein using the four-antibody panel. This finding is consistent with previous reports highlighting the prevalence of dMMR in endometrial cancer. Previous study in the Thai endometrial cancer patients in 2021 reported that 34.9% of surgical specimens had one or more MMR deficiencies [5]. In 2022, a study from Iran found that 23% of patients were MMR-deficient identified through IHC screening [18]. Notably, the majority of

Table 2. MMR IHC Staining Patterns Using Four-Antibody Panel and Two-Antibody Panel

MMR IHC staining patterns, N (%)	Four-antibody panel	Two-antibody panel
Intact MMR immunohistochemistry staining, N (%)	222 (73)	223 (73.4)
Loss of any on of MMR IHC staining, N (%)	82 (27)	81 (26.6)
Loss of MLH1 and PMS2	54 (17.8)	-
Loss of MSH2 and MSH6	16 (5.3)	-
Loss only MSH6	6 (2)	22 (7.2)
Loss only PMS2	5 (1.6)	59 (19.4)
Loss only MSH2	1 (0.3)	-

Table 3. Comparison of IHC Staining Results between the Four-Antibody Panel and the Two-Antibody Panel.

		Two-antibody panel		
		Loss IHC staining	Intact IHC staining	Total
Four-antibody panel	Loss IHC staining	81	1	82
	Intact IHC staining	0	222	222
	Total	81	223	304

dMMR patients in our cohort showed loss of MLH1 and PMS2 expression, underscoring the importance of these proteins in the development of dMMR.

The traditional approach to dMMR screening in endometrial cancer involves the use of a four-antibody panel comprising MLH1, MSH2, MSH6, and PMS2. This four-antibody panel has been widely adopted as the standard approach for dMMR screening in endometrial cancer based on recommendations from professional societies. However, our study explores the potential of a simplified screening strategy utilizing only PMS2 and MSH6 IHC. This approach is motivated by the frequent co-expression of PMS2 and MSH6 in endometrial tumors and the higher prevalence of MSH6 inactivation in endometrial cancer [19]. By utilizing only two antibodies, this simplified approach aims to streamline the screening process, reduce testing costs, and improve the detection of Lynch syndrome, a cancer predisposition syndrome associated with dMMR.

Our study demonstrates promising concordance between the two-antibody panel and four-antibody panel. By using the two-antibody panel, we detected dMMR in 26.6% of patients, with only one patient showing the loss of MSH2 expression using the four-antibody panel that could not be detected using the two-antibody panel. This patient performed germline testing. Interestingly, the germline testing revealed no MMR gene mutation.

Furthermore, the implementation of the two-antibody panel offers several advantages, including reduced testing costs, streamlined workflow, and improved overall efficiency. Specifically, by adopting the two-antibody approach, we can save approximately 1,420 THB (41.26 USD) per test, significantly reducing the total cost from 2,940 THB (85.43 USD) for the four-antibody panel to 1,520 THB (44.17 USD) for the two-antibody panel. This cost reduction is especially significant, considering the large number of endometrial adenocarcinoma patients that may require dMMR screening.

The high agreement rate of 98.8% between the two methods further supports the reliability and validity of the simplified approach. By simplifying the dMMR screening process in endometrial adenocarcinoma, our study has potential clinical implications. The two-antibody panel not only streamlines the diagnostic process but also enhances accessibility to dMMR screening, particularly for patients without a personal or family history of Lynch syndrome-related cancers. Additionally, the simplified approach has the potential to improve the detection of Lynch syndrome in affected individuals and their families, enabling appropriate genetic counseling and comprehensive cancer surveillance.

Nevertheless, it is important to acknowledge the limitations of our study. The retrospective nature of the study design and the use of data from a single institution may introduce selection bias. Further studies with larger multicenter cohorts are warranted to validate the findings and assess the generalizability of the simplified approach. Additionally, long-term follow-up and evaluation of patient outcomes are necessary to fully understand the clinical impact of adopting the two-antibody panel in dMMR screening.

In conclusion, our study provides evidence supporting the use of a simplified two-antibody panel utilizing PMS2 and MSH6 IHC in endometrial adenocarcinoma. This approach demonstrates a high level of concordance with the traditional four-antibody panel, indicating its potential as an alternative method for reflex MMR status testing. The implementation of this simplified approach has the potential to streamline the diagnostic process, reduce costs, and improve the detection of Lynch syndrome in affected individuals and their families. Further research with larger cohorts is warranted to validate our findings and investigate the concordance of germline testing with the two-antibody panel, thereby assessing the broader clinical implications of this approach in routine practice.

Author Contribution Statement

Pinyada Panyavaranant: Conceived and designed the analysis, collected data, contributed data or analysis tools, performed the analysis, wrote the paper. Natkrita Pohthipornthawat: Collected data, reviewed pathology, contributed data or analysis tools. Tarinee Manchana: Conceived and designed the analysis, provided advice on the concept, wrote the paper.

Acknowledgements

The authors would like to thank the Obstetrics & Gynecology Department, Faculty of Medicine, Chulalongkorn Memorial Hospital, Bangkok, Thailand, for their support and facilities provided for this research.

Institutional Review Board Approval & Ethical Considerations

This study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn Memorial Hospital (IRB number: 594/66) and conducted in compliance with its ethical standards.

Availability of Data

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Registration of Study

This study was not registered in any clinical trial registration.

Additional Information

This research is not part of an approved student thesis.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

1. Meyer LA, Broaddus RR, Lu KH. Endometrial cancer and lynch syndrome: Clinical and pathologic considerations. Cancer Control. 2009;16(1):14-22. https://doi.org/10.1177/107327480901600103.

2. Therkildsen C, Jensen LH, Rasmussen M, Bernstein I. An update on immune checkpoint therapy for the treatment of lynch syndrome. Clin Exp Gastroenterol. 2021;14:181-97. https://doi.org/10.2147/CEG.S278054.
3. Bucksch K, Zachariae S, Aretz S, Buttner R, Holinski-Feder E, Holzapfel S, et al. Cancer risks in lynch syndrome, lynch-like syndrome, and familial colorectal cancer type x: A prospective cohort study. BMC Cancer. 2020;20(1):460. https://doi.org/10.1186/s12885-020-06926-x.
4. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Screening for the lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med. 2005;352(18):1851-60. https://doi.org/10.1056/NEJMoa043146.
5. Manchana T, Ariyasriwatana C, Triratanachat S, Phowthongkum P. Lynch syndrome in thai endometrial cancer patients. Asian Pac J Cancer Prev. 2021;22(5):1477-83. https://doi.org/10.31557/APJCP.2021.22.5.1477.
6. Zhao S, Chen L, Zang Y, Liu W, Liu S, Teng F, et al. Endometrial cancer in lynch syndrome. Int J Cancer. 2022;150(1):7-17. https://doi.org/10.1002/ijc.33763.
7. Latham A, Srinivasan P, Kemel Y, Shia J, Bandlamudi C, Mandelker D, et al. Microsatellite instability is associated with the presence of lynch syndrome pan-cancer. J Clin Oncol. 2019;37(4):286-95. https://doi.org/10.1200/JCO.18.00283.
8. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. Esgo/estro/esp guidelines for the management of patients with endometrial carcinoma. Int J Gynecol Cancer. 2021;31(1):12-39. https://doi.org/10.1136/ijgc-2020-002230.
9. Goverde A, Spaander MC, van Doorn HC, Dubbink HJ, van den Ouweland AM, Tops CM, et al. Cost-effectiveness of routine screening for lynch syndrome in endometrial cancer patients up to 70years of age. Gynecol Oncol. 2016;143(3):453-9. https://doi.org/10.1016/j.ygyno.2016.10.008.
10. 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(8):e0221419. https://doi.org/10.1371/journal.pone.0221419.
11. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised bethesda guidelines for hereditary nonpolyposis colorectal cancer (lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96(4):261-8. https://doi.org/10.1093/jnci/djh034.
12. Tamura K, Kaneda M, Futagawa M, Takeshita M, Kim S, Nakama M, et al. Genetic and genomic basis of the mismatch repair system involved in lynch syndrome. Int J Clin Oncol. 2019;24(9):999-1011. https://doi.org/10.1007/s10147-019-01494-y.
13. Stelloo E, Jansen AML, Osse EM, Nout RA, Creutzberg CL, Ruano D, et al. Practical guidance for mismatch repair-deficiency testing in endometrial cancer. Ann Oncol. 2017;28(1):96-102. https://doi.org/10.1093/annonc/mdw542.
14. Hicks SC, Ward RL, Hawkins NJ. Immunohistochemistry for pms2 and msh6 alone can replace a four antibody panel for mismatch repair deficiency screening in colorectal adenocarcinoma. Pathology. 2011;43(1):84-5; author reply 5-6. https://doi.org/10.1097/PAT.0b013e3283410b26.
15. Aiyer KTS, Doeleman T, Ryan NA, Nielsen M, Crosbie EJ, Smit V, et al. Validity of a two-antibody testing algorithm for mismatch repair deficiency testing in cancer; a systematic literature review and meta-analysis. Mod Pathol. 2022;35(12):1775-83. https://doi.org/10.1038/s41379-022-01149-w.
16. McHugh ML. Interrater reliability: The kappa statistic. Biochem Med (Zagreb). 2012;22(3):276-82.
17. Glaire MA, Ryan NA, Ijsselsteijn ME, Kedzierska K, Obolenski S, Ali R, et al. Discordant prognosis of mismatch repair deficiency in colorectal and endometrial cancer reflects variation in antitumour immune response and immune escape. J Pathol. 2022;257(3):340-51. https://doi.org/10.1002/path.5894.
18. Noei Teymoordash S, Arab M, Bahar M, Ebrahimi A, Hosseini MS, Farzaneh F, et al. Screening of lynch syndrome in endometrial cancer in iranian population with mismatch repair protein by immunohistochemistry. Caspian J Intern Med. 2022;13(4):772-9. https://doi.org/10.22088/cjim.13.4.772.
19. Liu YL, Cadoo KA, Maio A, Patel Z, Kemel Y, Salo-Mullen E, et al. Early age of onset and broad cancer spectrum persist in msh6- and pms2-associated lynch syndrome. Genet Med. 2022;24(6):1187-95. https://doi.org/10.1016/j.gim.2022.02.016.