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ORIGINAL RESEARCH

Assessment of Immune Status in Patients with Mismatch Repair Deficiency Endometrial Cancer

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Objective: This study introduced a novel subtype classification method for endometrial cancer (EC) with mismatch repair deficiency (MMRd) by employing immune status and prognosis as the foundational criteria. The goal was to enhance treatment guidance through precise subtype delineation.

Methods: Study Cohort: This study encompassed a cohort of 119 patients diagnosed with MMRd-EC between 2015 and 2022. Analyses using *t*-tests and Mann–Whitney *U*-tests were performed to assess prognostic markers and peripheral blood immune cell profiles in patients with MutS deficiency (MutS-d) versus those with MutL deficiency (MutL-d). Logistic regression analysis was used to identify independent risk factors. Bioinformatics Analysis: An online database was used to assess the prognostic implications, immune cell infiltration, and immune checkpoint involvement associated with the deficiency of MutS versus MutL in EC.

Results: Patients with MutL-d exhibited heightened risk factors, including elevated cancer grade and increased myometrial invasion, leading to poorer prognosis and shorter overall survival and progression-free survival. Regarding systemic immune status, patients with MutL-d demonstrated decreased peripheral blood lymphocyte percentage, lymphocyte count, and CD8+ T cell percentage. For local immunity, the infiltration of natural killer cells, CD8+ T cells, and cytotoxic T lymphocytes in the tumor tissue was reduced in patients with MutL-d. Additionally, patients with MutL-d exhibited lower expression of immune checkpoint markers. The composition of immune subtypes and survival outcomes also indicate that patients with MutL-d have a poorer immune status and prognosis than the patients with MutS-d.

Conclusion: Patients with MMRd-EC can be subclassified according to MutS or MutL deficiency. Patients with MutS-d exhibited better immune status, prognosis, and immunotherapy benefits than those with MutL-d. These results can help guide patients to a more precise treatment.

Keywords: endometrial cancer, mismatch repair-deficiency, immune, molecular classification, MutL, MutS

Introduction

Endometrial cancer (EC) is a prevalent malignant epithelial tumor, and its incidence is increasing every year.[1] The heterogeneity of EC is evident from the diverse genetic and molecular profiles of cancer cells. Traditional classification methods often lack the ability to adequately address tumor heterogeneity, thereby, limiting their clinical applicability. Molecular typing of EC via classifications, such as ProMisE and TCGA, plays a vital role in prognosis prediction and facilitation of precision treatments.[2,3]

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Mismatch repair deficiency (MMRd)-EC account for 29% of all EC cases. Therefore, patients with MMRd-EC may benefit from immunotherapy.[4,5] However, real-world data suggest that the response to immune checkpoint blockade is inconsistent.[6] The heterogeneity observed in MMRd-EC necessitates the creation of more refined subgroups to effectively guide clinical treatment.

Mismatch repair genes comprise two distinct families: MutS family members, MSH2 and MSH6 that recognize and pinpoint mismatched bases, whereas MutL family members, MLH1 and PMS2, facilitate the hydrolysis of mismatched regions.[7] Deletions in different mismatch repair families may result in different clinical outcomes.[8] However, the relationship between specific deficiencies in various mismatch repair family members and their association with EC remains unclear.

Our study aimed to elucidate the prognosis and immunological characteristics inherent to MutS-d and MutL-d with the ultimate goal of establishing an innovative method for subgrouping MMRd-EC. By delineating these subgroups, we can refine the precise treatment strategies to tailor them according to the needs of the patients diagnosed with MMRd-EC.

Methods

Patient Selection

Cohort data: This retrospective study comprised 1240 patients who underwent initial hysterectomy for EC with immunohistochemical pathology at Fujian Provincial Maternity and Children's Hospital between January 2015 and January 2022. Exclusion criteria were as follows: (1) incomplete clinical and pathological data (n = 122); (2) absence of molecular typing marker tests (n = 558); (3) absence of MMRd diagnosis (n = 397); and (4) the presence of other types of tumors or precarcinomas (n = 10), hematological and immune system disorders (n = 4), acute inflammation (n = 3), or non-endometrioid adenocarcinomas (n = 6) within the subset of patients diagnosed with MMRd (n = 163). After applying these criteria, a final sample of 119 patients was included. This retrospective study was approved by the Ethics Committee of Fujian Maternal and Child Health Hospital. Bioinformatics analysis: MMRd-EC patient data were obtained from TCGA database and various online sources (http://bioinfo.life.hust.edu.cn/GSCA, http://cis.hku.hk/TISIDB/index.php).

Data Collection

Demographic and clinical indicators were retrieved from the healthcare information system of the Fujian Maternal and Child Health Hospital. Pathological information included the International Federation of Gynecology and Obstetrics stage, histologic type, tumor grade, deep myometrial invasion (DMI), lymphovascular space invasion (LVSI), lymph node metastasis (LNM), and expression levels of estrogen receptor (ER), progesterone receptor (PR), Ki67, Vimentin, P16, MLH1, PMS2, MSH2, and MSH6. Peripheral hematology parameters including white blood cell count, percentage of neutrophils (Neu%), percentage of lymphocytes (Lym%), percentage of monocytes (Mon%), neutrophil count (Neu), lymphocyte count (Lym), percentage of monocyte (Mon%), percentage of B cells (B cell %), percentage of T cells (T cell %), percentage of NK cells (NK cell %), percentage of helper T cells ($CD4^+$ T cell %), percentage of suppressor T cells ($CD8^+$ T cell %), T-helper/T-suppressor ratio (TH/TS, CD4/CD8), and cancer antigen 125 (CA125). Blood tests were performed one week before surgery. Bioinformatics data including survival curves, immune cell infiltration, immune checkpoints, and immune subtypes were analyzed using TCGA, TISIDB, and GSCA online databases.

MMRd and Subgroup Diagnosis

The ProMisE classification was performed as described in the original article.[2] Immunohistochemistry was conducted to identify MMR proteins (MLH1, MLH2, MSH6, and PMS2) in the EC tissues. Samples were classified into MutS (MSH2/MSH6) deficiency and MutL (MLH1/PMS2) deficiency groups according to protein expression status.[7] Patients with protein deficiencies in both the MutS and MutL families were excluded.

Statistical Analysis

Statistical analyses were conducted using SPSS (version 22.0; IBM Corp., Armonk, NY, USA) and R version 4.0.2. The data were visualized using the R package ggplot2. Continuous variables were analyzed using the Student's *t*-test or Mann–Whitney *U*-test. Categorical variables are presented as frequencies and percentages. Receiver operating

characteristic (ROC) curves were generated to determine the optimal cutoff values for the continuous variables. Categorical variables were analyzed using the chi-squared test or Fisher's exact test. Multiple logistic regression analysis was conducted to explore the binary associations. $p<0.05$ was considered to be statistically significant.

Results

Patients with MutL-d-EC Have More Risk Factors for Poor Prognosis and a Worse Survival Prognosis Than Those with MutS-d-EC

This retrospective study included 1240 patients with EC. After screening, 119 patients with MMRd-EC were included (Figure 1). We classified them into MutL-d (n=83) and MutS-d (n=36) groups based on their deficiencies in mismatch repair. The patients with MutL-d had higher cancer grade (p=0.006) and more DMI (p=0.010), indicating a higher prevalence of poor prognostic risk factors (Table 1). As no instances of death or disease progression were reported during the follow-up period for the included patients, we used the TCGA database to analyze the impact of deficiency on survival. In patients with EC, those carrying MutL-d were significantly associated with shorter overall survival (OS) (p=0.041) and progression-free survival (PFS) (p=0.01) compared with those carrying MutS-d (Figure 2).

Characteristics of Systemic Immune Status of MutL-d and MutS-d Subgroups

The status of peripheral blood immune cells represents the systemic immune status. Analysis of the selected patients' peripheral blood immune cells showed a significantly lower lymphocyte percentage (p=0.041), lymphocyte count (p=0.005), and monocyte count (p=0.039) in the MutL-d group (Figure 3A and B). Subsequently, differential indicators of the selected patients were included in a multifactorial analysis. ROC curves were generated to determine the optimal

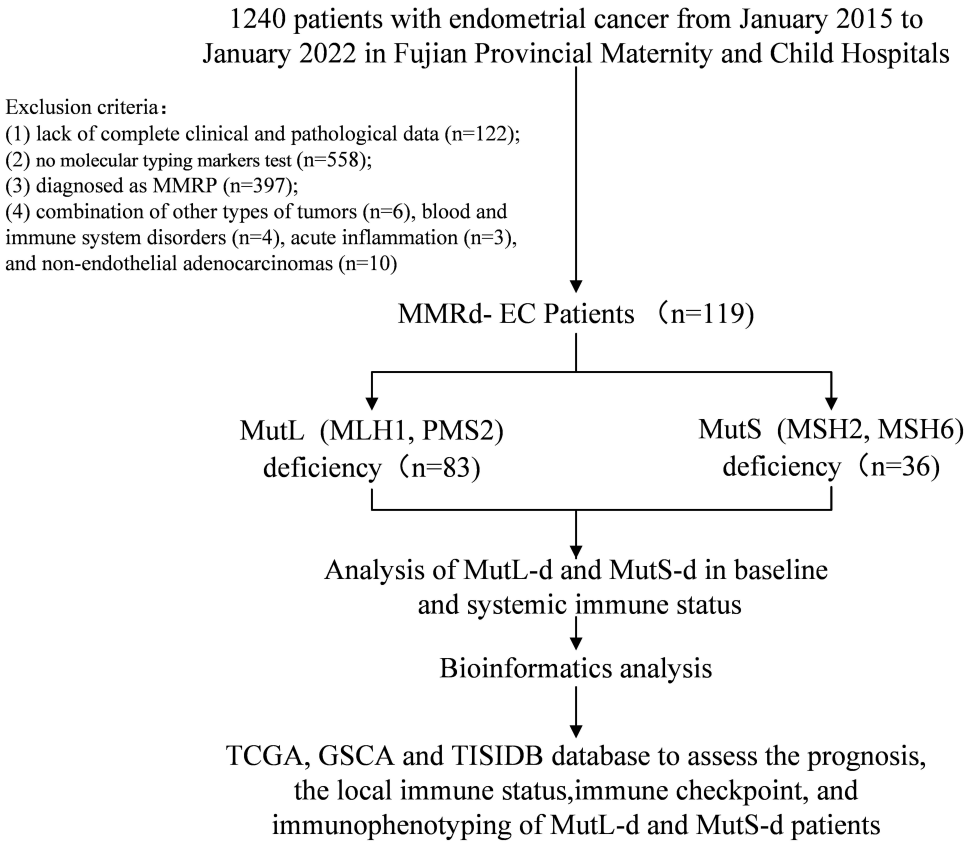


Figure 1 Study cohort flowchart.
Abbreviations: EC, Endometrial cancer; MMRd, Mismatch repair deficiency; MutS-d, MutS deficiency; MutL-d, MutL deficiency; TCGA, The Cancer Genome Atlas; GSCA, Gene Set Cancer Analysis.

Table 1 Baseline Characteristics of Patients with MutL-d and MutS-d EC

Characteristics		MutL-d	MutS-d	P value
N		83	36	
Age, median (IQR)		56 (53, 60)	53 (50, 59.75)	0.188
Menopause, n (%)	YES	57 (47.9%)	19 (16%)	0.097
	NO	26 (21.8%)	17 (14.3%)	
Diabetes n (%)	YES	16 (13.4%)	6 (5%)	0.832
	NO	67 (56.3%)	30 (25.2%)	
Hypertension, n (%)	YES	31 (26.1%)	8 (6.7%)	0.106
	NO	52 (43.7%)	28 (23.5%)	
FIGO	I~II	79 (66.4%)	35 (29.4%)	0.990
	III~IV	4 (3.4%)	1 (0.8%)	
Grade	G1~G2	60 (50.4%)	34 (28.6%)	**0.006**
	G3	23 (19.3%)	2 (1.7%)	
Myometrial invasion, n (%)	YES	34 (28.6%))	3 (2.5%)	**0.010**
	NO	49 (41.2%)	33 (27.7%)	
Cervical involvement, n (%)	YES	11 (9.2%)	2 (1.7%)	0.359
	NO	72 (60.5%)	34 (28.6%)	
LVSI, n (%)	YES	15 (12.6%)	3 (2.5%)	0.173
	NO	68 (57.1%)	33 (27.7%)	
ER, n (%)	-~+	27 (22.7%)	13 (10.9%)	0.704
	++~+++	56 (47.1%)	23 (19.3%)	
PR, n (%)	-~+	38 (31.9%)	18 (15.1%)	0.672
	++~+++	45 (37.8%)	18 (15.1%)	
Vimentin, n (%)	-~+	64 (53.8%)	29 (24.4%)	0.676
	++~+++	19 (16%)	7 (5.9%)	
P16, n (%)	-~+	71 (59.7%)	33 (27.7%)	0.533
	++~+++	12 (10.1%)	3 (2.5%)	
KI67, n (%)	-~+	58 (48.7%)	24 (20.2%)	0.728
	++~+++	25 (21%)	12 (10.1%)	
CA125, median (IQR)		20.9 (12.45, 44.45)	16.8 (11.65, 28.7)	0.246

Notes: 1. p<0.05 was considered to be statistically significant, bolded in the table 2 **- ~ + / ++~+++**: Immunohistochemical staining assessment, semi-quantitative scoring was assessed which evaluated both the number of positively stained cells and color depth. The percentage of positive cells was scored as 0 (≤ 5%), 1 (6–25%), 2 (26–50%), 3 (51–75%), and 4 (>75%). Color depth of positive cells was graded as 0 (no coloration), 1 (light yellow), 2 (pale brown), and 3 (dark brown). After multiplying the 2 scores, we got a negative result (-) for 0–2 points, weakly positive (+) for 3–4 points, moderately positive (++) for 5–8 points, and strongly positive (+++) for 9–12 points.

Abbreviations: IQR, interquartile range; FIGO, International Federation of Gynecology and Obstetrics; LVSI, Lymphovascular space invasion; ER, Estrogen receptor; PR, progesterone receptor; MutS-d, MutS deficiency; MutL-d, MutL deficiency.

cutoff values for continuous variables (Figure 3C–E). Logistic regression analysis confirmed that low lymphocyte count and lymphocyte percentage, and increased myometrial infiltration were independent risk factors for MutL-d (all p<0.05) (Figure 3F). Considering the lymphocyte differences between the two groups, we analyzed the peripheral blood lymphocyte subtypes of the patients in detail. Patients with MutL-d had lower levels of $CD8^+$ T cells (%) (p=0.022), whereas the $CD4^+$ T cells (%) (p=0.005) and TH/TS (p=0.005) were higher than those in patients with MutS-d (Figure 4A). ROC curve analysis and logistic regression confirmed that a low percentage of $CD8^+$ T cells (%) was an independent risk factor for MutL-d (Figure 4B–E).

Features of Local Immune Infiltration of MutL-d and MutS-d Subgroups

Immune cell infiltration was assessed using the TCGA database. The numbers of NK cells (p<0.05) and $CD8^+$ T cells (p<0.05) were lower in patients with MutL-d-EC than in those with MutS-d (Figure 5A). Subsequently, we analyzed the $CD4^+$ and $CD8^+$ T cell subtypes. Patients with MutL-d-EC had lower levels of CTLs and higher levels of naïve-like $CD8^+$ T cells (Figure 5B). To

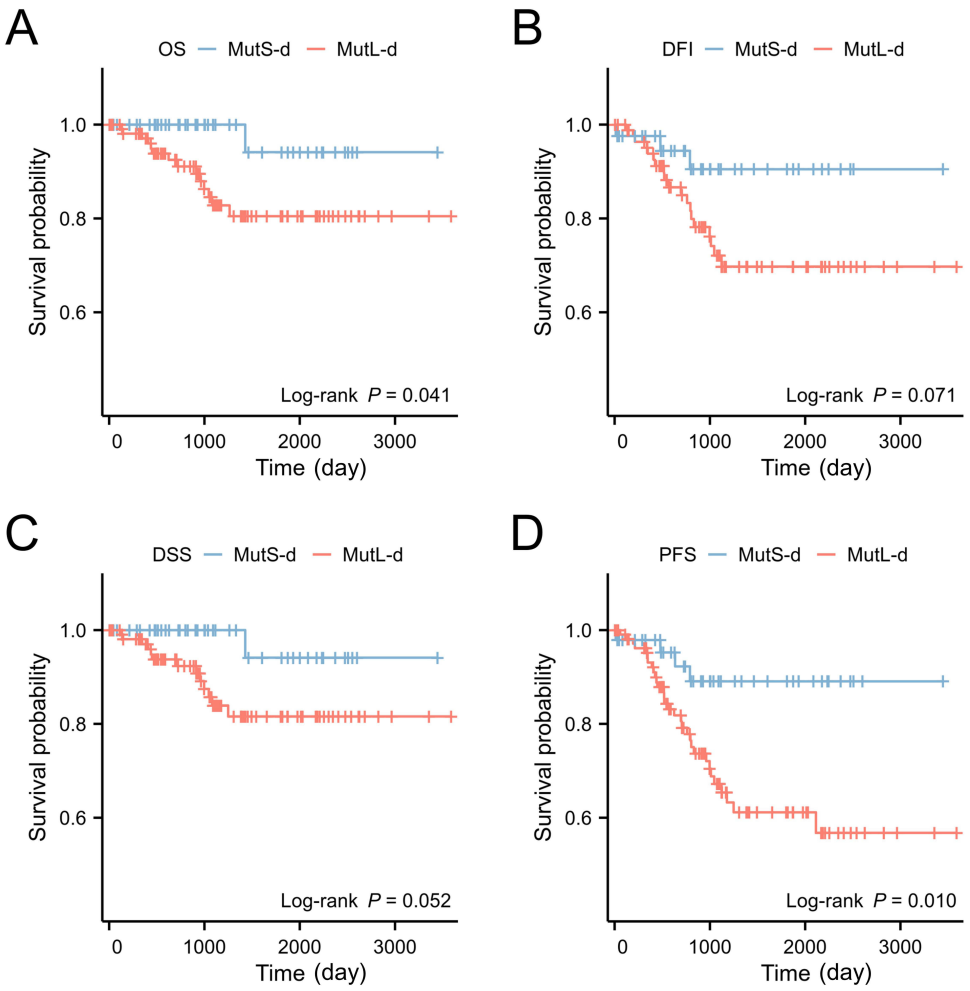


Figure 2 Prognosis of MutS-d and MutL-d. (**A**) OS was shorter in the MutL-d group, (**B**) DFI in the MutL-d and MutS-d groups, (**C**) DSS in the MutL-d and MutS-d groups, (**D**) PFS was shorter in the MutL-d group.
Note: p<0.05 indicates significant differences.
Abbreviations: MutS-d, MutS deficiency; MutL-d, MutL deficiency; OS, overall survival; DSS, disease-specific survival; DFI, disease free interval; PFS, progression-free survival.

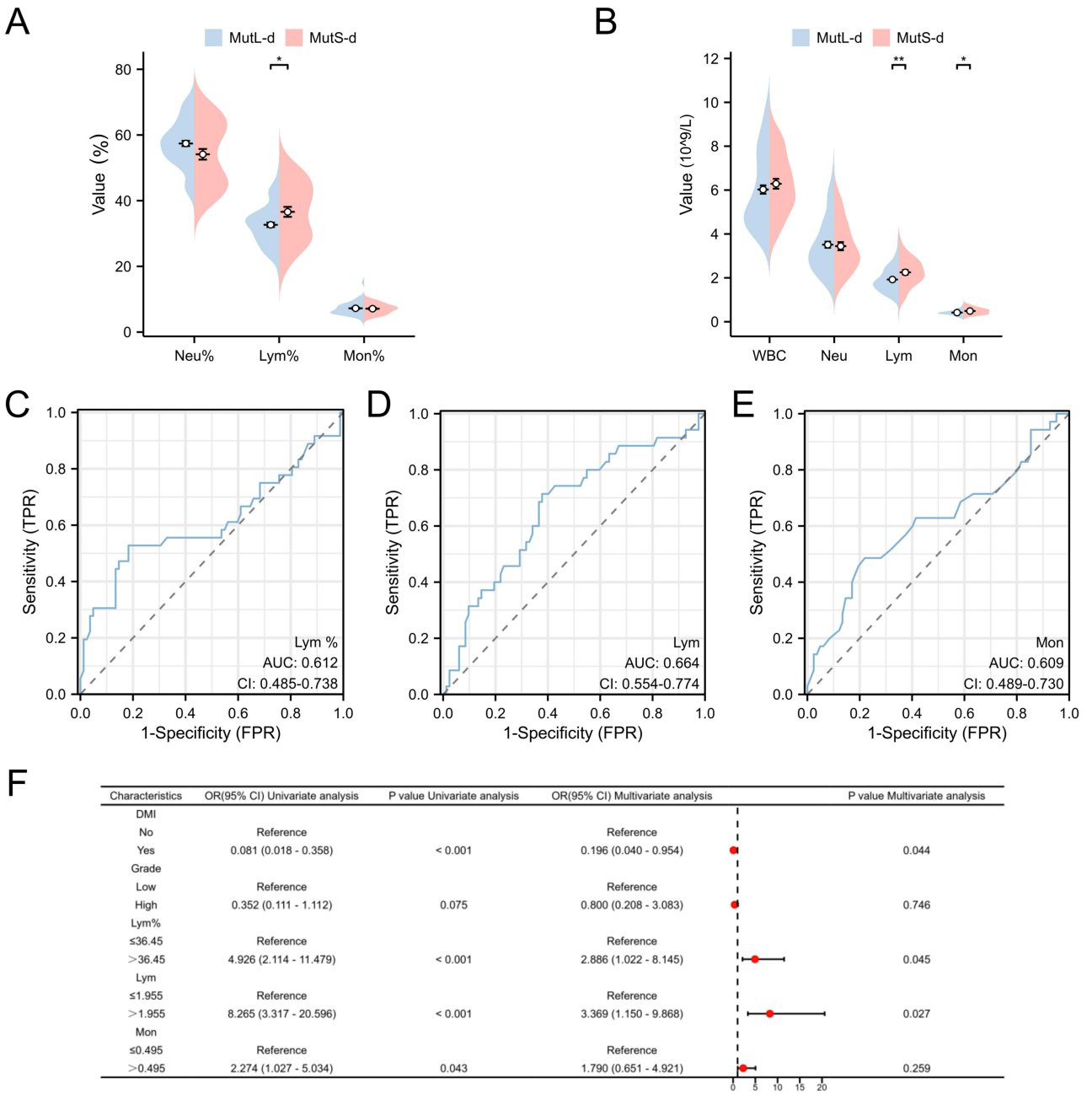
investigate the factors contributing to these differences in immune infiltration, we analyzed the impact of single-gene deletions on immune infiltration. Correlation analysis conducted using the TISIDB database revealed a stronger negative correlation between MSH2 and MSH6 and chemokines and chemokine receptors, including CCL14, CCL17, CCL22, CCL24, CXCL2, CXCL14, CXCL17, XCL2, CCR7, CXCR1, CXCR2, CXCR3, and CXCR5. Therefore, when MSH2 and MSH6 (MutS) are deleted, the expression levels of chemokines and their receptors increase. This recruits more immune cells. (Figure 5C and D)

Profiles of Immune Checkpoint of MutL-d and MutS-d Subgroups

We assessed immune checkpoint expression between the MutS-d and MutL-d using the TCGA database, which revealed significantly lower expression levels of several immune checkpoints in the MutL-d group compared to that in the MutS-d group. Statistically significant differences were observed in the expression levels of PD-1, TIM-3, CTLA-4, LAG-3, TIGIT, ICOS, BTLA, and VISTA (Figure 6A). Higher expression levels of immune checkpoints correlate with more favorable treatment outcomes within a certain range.

Profiles of Immunophenotyping Composition of MutL-d and MutS-d Subgroups

Thorsson et al delineated six discrete immune subtypes of tumors: C1 (wound-healing), C2 (IFN-γ dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet), and C6 (TGF-β dominant).[9] We employed data from TCGA



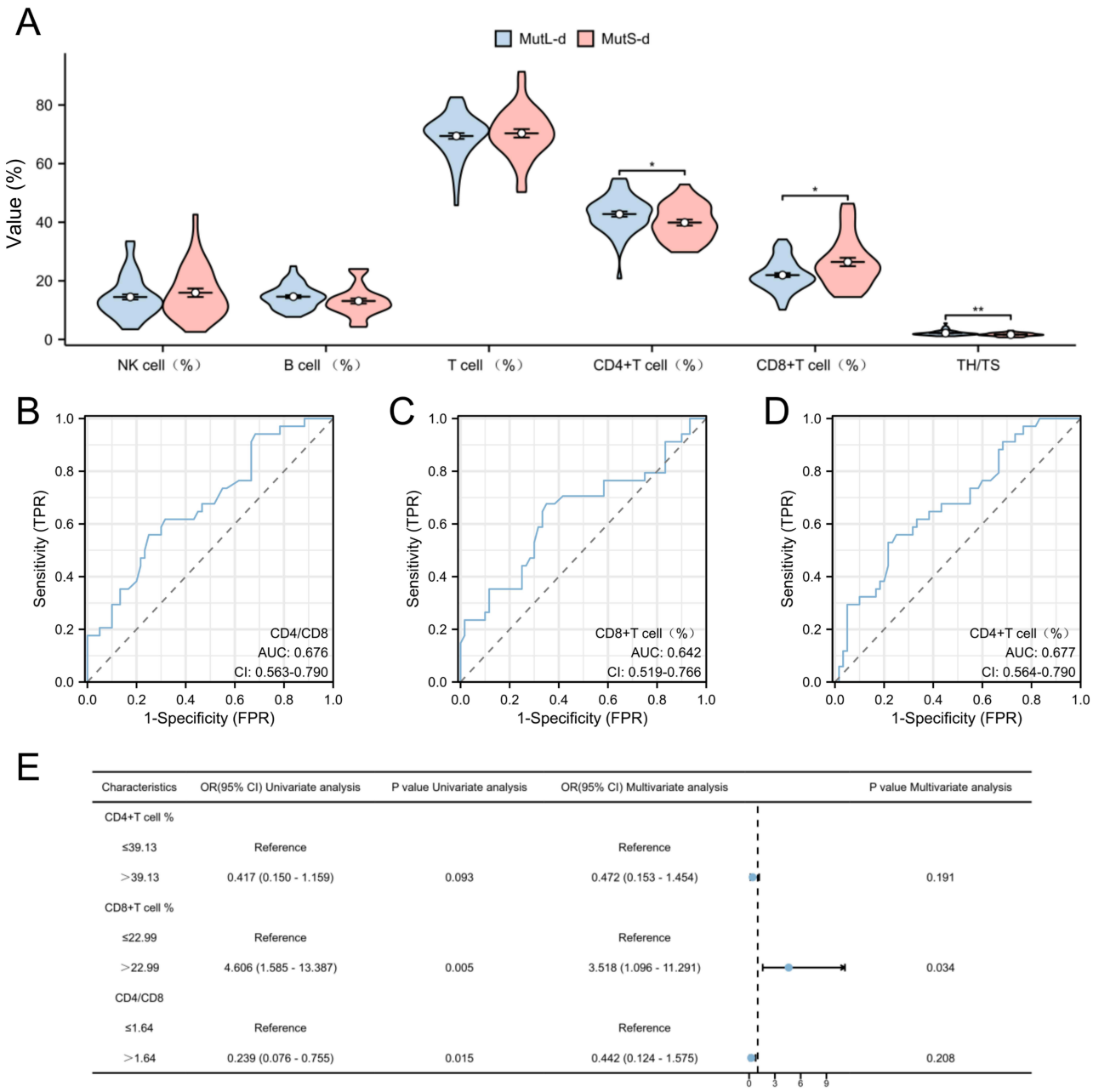
Characteristics	OR(95% CI) Univariate analysis	P value Univariate analysis	OR(95% CI) Multivariate analysis	P value Multivariate analysis
DMI				
No	Reference		Reference	
Yes	0.081 (0.018 - 0.358)	< 0.001	0.196 (0.040 - 0.954)	0.044
Grade				
Low	Reference		Reference	
High	0.352 (0.111 - 1.112)	0.075	0.800 (0.208 - 3.083)	0.746
Lym%				
≤36.45	Reference		Reference	
>36.45	4.926 (2.114 - 11.479)	< 0.001	2.886 (1.022 - 8.145)	0.045
Lym				
≤1.955	Reference		Reference	
>1.955	8.265 (3.317 - 20.596)	< 0.001	3.369 (1.150 - 9.868)	0.027
Mon				
≤0.495	Reference		Reference	
>0.495	2.274 (1.027 - 5.034)	0.043	1.790 (0.651 - 4.921)	0.259

Figure 3 Low Lym%, Low Lym, and further DMI are independent risk factor for MutL-d group. (**A** and **B**) Peripheral blood immune cells in the MutL-d and MutS-d groups; (**C**–**E**) ROC curves were implemented to obtain the cutoff value of Lym%, Lym and Mon between MutL-d and MutS-d (The solid blue line is the ROC curve and the dashed black line is the reference line). The cutoff values for these indicators were identified as cut off (Lym%) =36.45, cut off (Lym)=1.955, and cut off (Mon) =0.495; (**F**) Logistic regression analyses for the MutL-d group, Low Lym%, Low Lym and further DMI were independent risk factors.

Notes: *p < 0.05; **p < 0.01; p<0.05 indicates significant differences.

Abbreviations: WBC, white blood cell count; Neu%, percentage of neutrophils; Lym%, percentage of lymphocytes; Mon%, percentage of monocytes; Neu, neutrophil count; Lym, lymphocyte count; Mon%, monocyte count; CI, Confidence intervals; AUC, Areas under the curve; DMI, Deep myometrial invasion; ROC, Receiver operating characteristic.

and TISIDB databases to analyze the patients' immune subtypes. Among the patients with MutS-d, 18.75% had subtype C1, 37.5% had subtype C2, 37.5% had subtype C3, 4.17% had subtype C4, and 2.08% had subtype C6. In contrast, for patients with MutL-d, the distribution was 48.18% for C1, 42.73% for C2, 5.45% for C3, and 3.64% for C4 (Figure 6B). C3 exhibited exceptional OS, whereas C2 and C1 showed inferior prognoses. Conversely, C4 and C6 are associated with the most unfavorable prognosis.[9] This also indicates that the immune status and prognosis of MutS-d are better than those of MutL-d.



Characteristics	OR(95% CI) Univariate analysis	P value Univariate analysis	OR(95% CI) Multivariate analysis	P value Multivariate analysis
CD4+T cell %				
≤39.13	Reference		Reference	
>39.13	0.417 (0.150 - 1.159)	0.093	0.472 (0.153 - 1.454)	0.191
CD8+T cell %				
≤22.99	Reference		Reference	
>22.99	4.606 (1.585 - 13.387)	0.005	3.518 (1.096 - 11.291)	0.034
CD4/CD8				
≤1.64	Reference		Reference	
>1.64	0.239 (0.076 - 0.755)	0.015	0.442 (0.124 - 1.575)	0.208

Figure 4 Low CD8+ T cell (%) is an independent risk factor for MutL-d group. (**A**) Lymphocyte status in the MutL-d and MutS-d groups; (**B**–**D**) ROC curves were implemented to obtain the cutoff value of CD4/CD8, CD8+ T cell % and CD4+ T cell % between MutL-d and MutS-d (The solid blue line is the ROC curve and the dashed black line is the reference line). The cutoff values for these indicators were identified: cutoff (CD4/CD8) =1.64, cutoff (CD8+ T cell %) =22.99, cutoff (CD4+ T cell %) =39.13; (**E**) Logistic regression analyses for MutL-d group, Low CD8+ T cell (%) is an independent risk factor.

Notes: *p < 0.05; **p < 0.01; p<0.05 indicates significant differences.

Abbreviations: B cell %, percentage of B cells; T cell %, percentage of T cells; NK cell %, percentage of NK cells; CD4+ T cell %, percentage of helper T cells, CD8+ T cell %, percentage of suppressor T cells, TH/TS, T-helper/T-suppressor ratio; CI, Confidence intervals; AUC, Areas under the curve.

Discussion

EC exhibits the highest MMRd occurrence ratio among gynecological tumors.[10] Previous studies have classified MMRd-EC/MSI-H into subtypes including MLH1-hypermethylated EC (EC-met), Lynch syndrome-associated EC (EC-ls), and mismatch repair gene double somatic pathogenic variants (EC-dspv). Patients with EC-met were the oldest, had the highest incidence, and a poorer prognosis than the other two subtypes.[11,12] Pasanen et al divided MMRd-EC into two subgroups: EC with highly methylated MLH1 (EC-met), and EC without highly methylated MLH1 (EC-non-met).

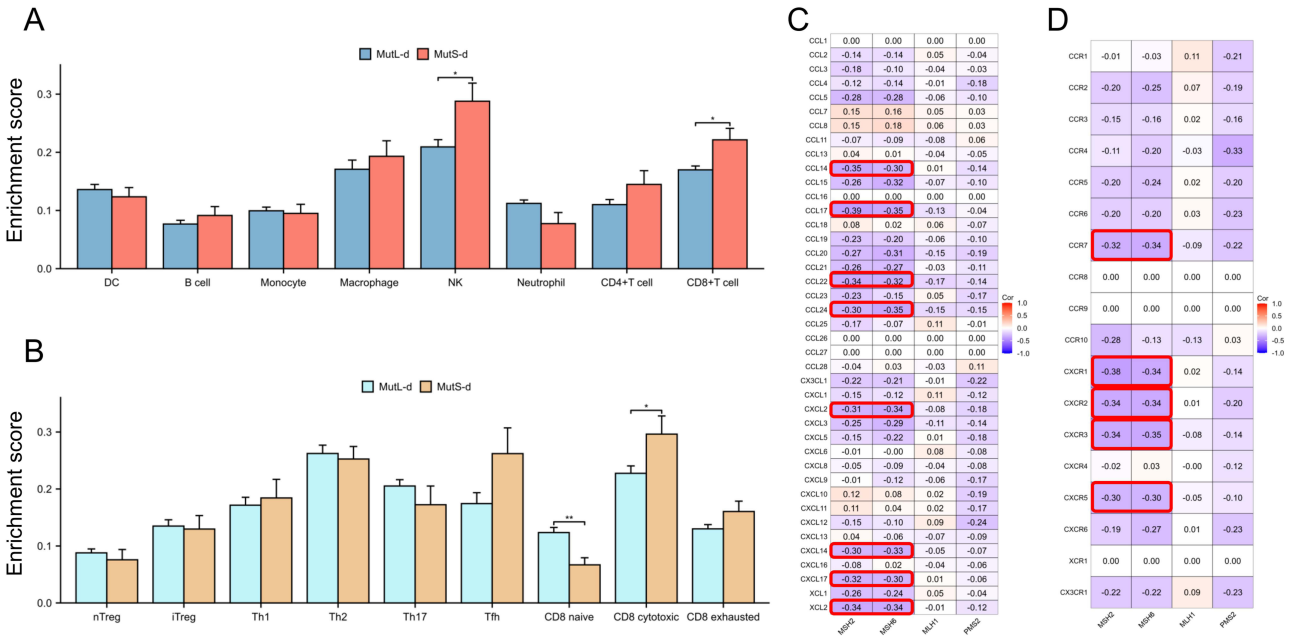


Figure 5 Local immune infiltration of MutS-d and MutL-d. (**A** and **B**) Infiltration of DC, B cells, monocytes, macrophages, NK cells, neutrophils, $CD4^+$T cells, $CD8^+$T cells, nTregs, iTregs, Th1, Th2, Th17, Tfh, CD8 naïve, CD8 cytotoxic, and CD8 exhausted cells between MutS-d and MutL-d; (**C** and **D**) Association of MSH2, MSH6, MLH1, and PMS2 with chemokines and chemokine receptors. Red outline boxes represent chemokines and chemokine receptors with high relevance to MSH2/MSH6. The absolute value of the correlation is greater than or equal to 0.3.

Notes: *p < 0.05; **p < 0.01; p<0.05 indicates significant differences.

Abbreviations: MutS-d, MutS deficiency; MutL-d, MutL deficiency; DC, Dendritic cell; NK, natural killer cell; Treg, regulatory T cells; Th, Helper T cells; Tfh, Follicular helper T cell.

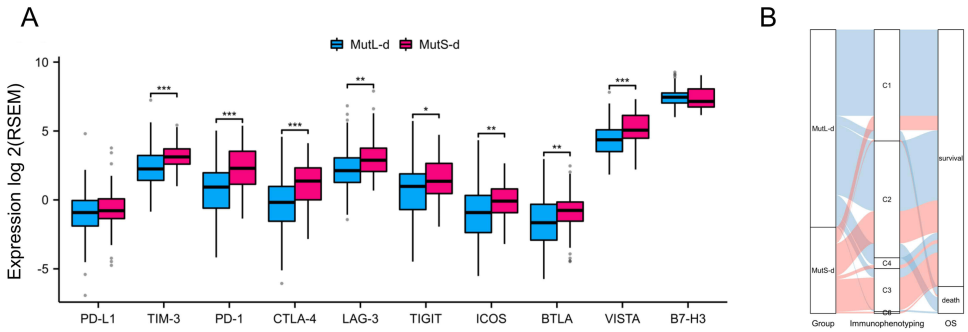


Figure 6 Profiles of immune checkpoint and immunophenotyping composition of MutS-d and MutL-d. (**A**) Expression of the MutS-d and MutL-d immune checkpoints. (**B**) Immunophenotyping and prognosis of MutS-d and MutL-d.

Notes: *p < 0.05; **p < 0.01; ***p < 0.001; p<0.05 indicates significant differences.

Abbreviations: MutS-d, MutS deficiency; MutL-d, MutL deficiency; OS, overall survival; C1, wound-healing type; C2, IFN-γ dominant type; C3, inflammatory type; C4, lymphocyte depleted type, C5, immunologically quiet type; C6, TGF-β dominant type.

Patients with EC-met exhibited an older age of onset along with higher rates of LVSI and LNM than EC-nonmet cases.[13] These classification methods involving methylation detection pose economic and technical challenges that hinder their widespread application. Our analysis proposes a subgrouping of MMRd-ECs based on MutS (MSH2, MSH6) and MutL (MLH1, PMS2) deletions. This classification method relies exclusively on the immunohistochemical staining of relevant proteins, eliminating additional economical and technical complexities and making it more feasible for widespread adoption and utilization. From the above groupings, it was found that patients with MutL-d exhibit heightened risk factors, including elevated cancer grading and increased DMI, leading to a poorer prognosis with shorter OS and PFS. In all patients with colorectal cancer (CRC), the median OS was longer in MutS co-loss (N=153) than in MutL co-loss (N=986) (54.6 months (m) vs 36 months; hazard ratio (HR) = 0.766; p=0.025). In all patients with EC, the median OS

was longer in MutS co-loss (N=104) compared to MutL co-loss (N=1870) (81.5 m vs 48.2 m; HR= 0.535, p<0.001).[14] When subdividing the MMR-d group into MLH1/PMS2 loss and other MMR-d subgroup (including MSH2/MSH6 loss, MSH6 only and PMS2 only loss), MLH1/PMS2 loss was significantly associated with inferior PFS (p=0.008).[15] Doulgeraki et al revealed a correlation between MLH1/PMS2 deletion and the depth of myometrial infiltration, whereas MSH6 protein deletion was notably correlated with lymph node metastasis.[16] These findings are consistent with our observations, suggesting that distinct mismatch repair protein types can serve as prognostic indicators of different tumor subtypes.

Elevated levels of tumor-infiltrating lymphocytes (TILs) are recognized as reliable prognostic markers.[17] Bohaumilizky et al revealed that Lynch syndrome-associated endometrial cancer (EC-ls) showed notably higher counts of CD8+ T cells, increased PD-L1 expression, and a higher incidence of beta-2-microglobulin mutations compared to sporadic MMRd-EC.[18] Similarly, research conducted by Ramchander et al highlighted significantly higher counts of CD3+, CD8+, CD45RO+, and PD-1+ T lymphocytes in EC-ls tumor tissues than in EC-met.[19] Our findings suggest that MutL-d is associated with reduced infiltration of immune cells, including NK cells, CD8+ T cells, and CTLs, in contrast to MutS-d. This indicated that the local immune status of MutL-d was impaired.

Peripheral blood immune cell subpopulations, particularly lymphocyte subsets, play a pivotal role in tumor development and serve as crucial indicators for the assessment of the systemic immune status. An increase in CD8+ T cells is observed in the peripheral blood of patients with early-stage colon cancer and a decrease in advanced disease stages.[20] Remarkably, individuals with MutL-d demonstrated lower peripheral blood lymphocyte counts, lymphocyte percentages, and CD8+ T cell percentages, which is indicative of a compromised immune status. Hence, MutL-d and MutS-d can potentially serve as indicators of systemic and local immune status in tumor subtypes.

MMRd tumors exhibit increased accumulation of somatic mutations, leading to increased expression of antigenic materials. Consequently, MMRd tumors become more susceptible to immune recognition and subsequent immune-mediated responses, which contributes to the observed clinical benefits of immunotherapy in patients with MMRd.[10,21] The mean tumor mutational burden (TMB) in MutS co-loss was 44 mut/Mb versus 40.5 mut/Mb (q<0.059) in CRC and 30 mut/Mb versus 22 mut/Mb (q<0.0001) in EC.[14] Previous studies proposed that among all tumors, loss of co-expression of MSH2/MSH6 was associated with a higher mean TMB (46.83 mut/Mb) than loss of MLH1/PMS2 (25.03 mut/Mb; p<0.0001). This indicates a potentially greater significance of the MutSα protein complex (MSH2/MSH6) in ensuring intact MMR compared to the MutLα complex (MLH1/PMS2).[22] TMB is a predictive biomarker for ICIs. A comprehensive study involving 1662 patients across 10 cancer types treated with ICIs (anti-CTLA-4 or anti-PD-1/PD-L1 drugs) demonstrated a distinct dose-response relationship between TMB and OS after ICI initiation. Patients whose tumors ranked within the top 10% of TMB within their respective histology experienced prolonged OS compared to those in the 10–20% range, who, in turn, exhibited longer survival than the remaining 80%.[23] In ICI-treated patients with CRC, the median OS was longer for MutS co-loss (N=32) than for MutL co-loss (N=184) (not reached (NR) vs 36 months; HR= 0.378, p=0.011). In ICI-treated patients with EC, the median OS in patients with MutS co-loss (N=16) compared to those with MutL co-loss (N=324) was NR vs 42.2 m (HR= 0.845, p=0.711).[14]

Based on the heterogeneity of MMRd-EC, our proposed classification approach offers a streamlined and economically viable alternative technique that effectively stratifies patients with MMRd and informs them of treatment choices. Patients with MutS-d EC have a favorable prognosis and immune phenotype. Hence, immunotherapy is recommended for these patients. However, patients with MutL-d EC have worse prognosis and immune phenotype. Therefore, it is essential to consider implementing more proactive and comprehensive treatment methods for patients with MutL-d EC. This allows for better precision in the treatment of patients with MMRDs. Furthermore, assessment of peripheral blood immune cell status can be used as an indicator for evaluating tumor progression and treatment efficacy in patients with MMRd EC.

Relative to previous studies,[14,15] the strength of this study lies in its ability to harness proprietary and publicly accessible datasets to cross-validate the drawn results. Differences between the patients with MutS-d and MutL-d were clarified by comparing the risk factors for poor prognosis and survival analysis. Differences in the immune statuses of MutS-d and MutL-d were described by comparing peripheral blood immune cells, local immune cell infiltration in tumor tissues, cytokine secretion, expression of immune checkpoints, and immunophenotyping in a comprehensive and

multilevel manner. This study had several limitations. This study, conducted at a single center, featured a homogeneous population, which may have introduced potential epidemiological biases. Therefore, it is necessary to validate these findings in larger multicenter trials. Notably, the diagnosis and management of Lynch syndrome were not addressed in this study. Finally, this study did not include in vivo studies. Next, we validated the differences in survival and immune status between patients with MutS-d and MutL-d by constructing a mouse model of patient-derived tumor xenografts. Further cohort studies on patients with MutS-d and MutL-d are needed to obtain more data on prognosis, immune status assessment, and clinical immunotherapy.

Conclusion

MMRd-EC patients can be subclassified according to their MutS or MutL deficiency. Patients with MutS-d exhibit a better immune status, better prognosis and better immunotherapy benefits compared to MutL-d. From there, this can guide MMRd patients to more precise treatment.

Abbreviations

EC, Endometrial cancer; FIGO, International Federation of Gynecology and Obstetrics; LVSI, Lymphovascular space invasion; LNM, lymph node metastasis; DMI, Deep myometrial invasion; BMI, Body mass index; ORs, Odds ratios; CIs, Confidence intervals; ROC, Receiver operating characteristic; AUC, Areas under the curve; TCGA, The Cancer Genome Atlas; MMRd, Mismatch repair deficiency; MutS-d, MutS deficiency; MutL-d, MutL deficiency; ER, Estrogen receptor; PR, progesterone receptor; CD4/CD8 or TH/TS, T-helper/T-suppressor ratio; OS, overall survival; PFS, progression-free survival; DSS, disease-specific survival; DC, Dendritic cell; NK, natural killer cell; cytotoxic T lymphocytes, CTLs; tumor mutational burden, TMB; immune-checkpoint inhibitors, ICIs; colorectal cancers; WBC, white blood cell count; Neu%, percentage of neutrophils; Lym%, percentage of lymphocytes; Mon%, percentage of monocytes; Neu, neutrophil count; Lym, lymphocyte count; Mon%, monocyte count; B cell %, percentage of B cells; T cell %, percentage of T cells; NK cell %, percentage of NK cells; CD4+ T cell %, percentage of helper T cells, CD8+ T cell %, percentage of suppressor T cells, CA125, cancer antigen 125.

Data Sharing Statement

The datasets analyzed in the current study are available from the corresponding author upon request.

Ethics Approval and Consent to Participate

This study conformed to the Declaration of Helsinki on Human Research Ethics standards and was approved by the Ethics Committee of Fujian Maternal and Child Health Hospital (Approval No. 2022KYLLRO3028). The need for written informed consent was waived by the Ethics Committee of Fujian Maternal and Child Health Hospital because of its retrospective design (Approval No. 2023KYLLRK01088). This study strictly protected the privacy of the patients and maintained the confidentiality of their personal information. Patient-identifiable information is hidden when data are analyzed for reporting purposes.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no conflicts of interest to declare.

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