EJMA

DOI: 10.14744/ejma.2023.74946
EJMA 2024;4(1):22–27

Research Article

The Association of Mismatch-Repair (MMR) Deficiency with Tumor Infiltrating Lymphocytes and Survival in Patients with Ovarian Cancer

Burcu Yapar Taşköylü,[1] Ferda Bir,[2] Atike Gökçen Demiray,[1] Serkan Değirmencioğlu,[1] Gamze Gököz Doğu,[1] Arzu Yaren,[1] Ahmet Ergin,[3] Canan Karan,[1] Burçin Çakan Demirel,[1] Tolga Doğan,[1] Melek Özdemir,[1] Taliha Güçlü Kantar[1]

[1]Department of Medical Oncology, Pamukkale University Faculty of Medicine, Denizli, Türkiye
[2]Department of Pathology, Pamukkale University Faculty of Medicine, Denizli, Türkiye
[3]Department of Social Pediatrics, Pamukkale University Faculty of Medicine, Denizli, Türkiye

Abstract

Objectives: The role of DNA mismatch repair (MMR) deficiency in the pathogenesis and prognosis of ovarian cancer has been a subject of considerable research. Deficiency in MMR genes result in accumulation of thousands of mutations in the genome, leading to a high mutation burden and subsequent activation of the immune system due to an increase in the number of "mutation-derived neoantigens". It has been increasingly reported that this process results in the number of tumors infiltrating lymphocytes with a favorable impact on prognosis. The aim here is to examine the association of mismatch repair (MMR) deficiency with tumor-infiltrating lymphocytes and other clinical and pathological characteristics in patients with ovarian cancer.

Methods: In a total of 81 patients with ovarian cancer, the microsatellite instability and presence of tumor infiltrating lymphocytes (CD3, CD8, CD4) were examined immunohistochemically. Negative test result in any of the markers MLH-1, MSH-2, MSH-6, or PMS-2 was considered to microsatellite instability (MSI). Also, with regard to tumor infiltrating lymphocytes, a proportion level of greater than 10% was considered positive.

Results: Fifty-one patient (53%) had locally advanced and metastatic disease, and 54 patients (66.7%) had high-grade tumors. Fifty-nine patients (72 %) had serous carcinoma. There was a loss of MMR protein expression in 28 patients (35%), and 53 (65%) were microsatellite stable. There were no significant associations between microsatellite status and age, grade, stage, lymphovascular invasion, CD3, and CD8. Among microsatellite stable patients, CD4 was statistically significantly higher (p=0.03). A reduction in CD3, CD8, and CD4 was found in 53 (64%), 57 (70%), and 54 (66 %) patients, respectively. A significant association between CD3 and lymphovascular invasion was found (p=0.011). CD3 levels are higher in patients with lymphovascular invasion. Survival analysis did not show any relationship between microsatellite instability, progression-free survival, and overall survival. Stage, grade, lymphovascular invasion, Ki-67, and CD8 were significant predictors of progression-free survival (p<0.001, p=0.011, p=0.022, and p=0.02, respectively). Also, there was a significant association between CD4 and overall survival (p=0.007).

Conclusion: We believe that assessment of tumor infiltrating lymphocytes holds the potential to provide valuable prognostic information as well as guidance for management strategies in the clinical practice.

Keywords: Ovarian cancer, microsatellite instability, immunotherapy, lymphocyte

Cite This Article: *Taşköylü BY, Bir F, Demiray AG, Değirmencioğlu S, Doğu GG, Yaren A, et al. The Association of Mismatch-Repair (MMR) Deficiency with Tumor Infiltrating Lymphocytes and Survival in Patients with Ovarian Cancer. EJMA 2024;4(1):22–27.*

Address for correspondence: Burcu Yapar Taşköylü, MD. Department of Medical Oncology, Pamukkale University Faculty of Medicine, Denizli, Türkiye
Phone: +90 505 580 75 59 **E-mail:** drburcuyapar@gmail.com

Submitted Date: October 11, 2022 **Revision Date:** October 11, 2022 **Accepted Date:** August 01, 2023 **Available Online Date:** August 29, 2024

Surgery, chemotherapy, and radiotherapy form the basis of cancer treatment. However, advanced stage and recurrent ovarian cancer are associated with poor prognosis, which is dependent on the grade, stage, histological type, and patient age.[1]

The interactions between cancer cells and the immune system are among the determinants of tumor progression. In recent years, significant number of studies have been published that examine the tumor-infiltrating lymphocytes.[2] In some epithelial ovarian tumors, better prognosis has been observed as a result of immune activation.[3]

As opposed to inflammation, tumor infiltrating lymphocytes are generally associated with better prognosis and survival in malignant diseases. CD8+ lymphocytes are cytotoxic cells that are able to kill the "target cells" via enzymes such as granzyme-B and perforin. On the other hand, CD4 + cells generally do not have cytotoxic properties. Macrophages are able to recruit and activate other cells such as B cells, dendritic cells, inflammatory cells, and other T cells.[4] In addition to functional classification systems, TILs may also be subcategorized based on their localization within the tumor. They are referred to as stromal TILs when they are present in the peritumoral space, while the term intraepithelial TIL is used when they invade the tumor islets. In a meta-analysis of 10 studies and 1815 patients with ovarian cancer, lower number of tumor infiltrating lymphocytes has been found to be associated with worse prognosis.[5] MMR system, on the other hand, plays a major role in the achievement of genomic stability, identifying and correcting biosynthetic errors occurring during DNA replication.[6] Loss of any of these MMRs (MLH1, MSH2, MSH6, and PMS2) genes leads to micro-satellite instability (MSI) and increased burden of tumor mutation.[7] The reported percentage of MSI in ovarian cancer is between 2% and 20%.[8-12] In solid tumors with MSI-H and MMR deficiency, immune checkpoint inhibitors have been reported to be effective. The first reported evidence regarding this was from metastatic colon cancer patients with high microsatellite instability and multiple lines of previous therapy who responded well to anti-PD-1 therapy. The objective response rate to pembrolizumab treatment in patients with microsatellite instability was 40%, while no benefit was observed in microsatellite stable patients.[13] The efficacy of immunotherapy in other cancer types with MMR deficiency was also explored and favorable results have been obtained.[14]

In MMR deficient tumors, elevated TIL levels and increased stimulation of the immune system has been observed, due to the high mutation burden and expression of neoantigens.[14] In our study we also investigated the association of MMR system, tumor infiltrating cells, and prognosis.

Methods

Patient Characteristics

A total of 81 patients diagnosed with ovarian cancer who were treated at our department between 2011 and 2019 were included in this study. Demographic data, tumor types, and chemotherapeutic regimens administered were retrieved from patients' medical records. Survival analyses were performed at the end of study.

Immunhistochemistry

Immunohistochemistry was performed on 3-micron sections cut from routinely processed formalin-fixed, paraffin-embedded tissue blocks. The tissue sections were deparaffinized and rehydrated, pretreated with 0.01 M citrate buffer (pH 6), and then stained for MLH-1 (MutL Protein Homolog 1) (Dako, clone ES05,Ready to use, Human Monoclonal Mouse Primary Antibody), MSH-2 (MutS Protein Homolog 2) (Dako, clone FE11, Ready to use, Human Monoclonal MousePrimary Antibody), MSH-6 (MutS Protein Homolog 6) (Dako, clone EP49, Ready to use, Human Monoclonal Rabbit Primary Antibody), PMS-2 (Postmeiotic Segregation İncreased 2) (Dako, clone EP51, Ready to use, Human Monoclonal Rabbit Primary Antibody),CD 3 (Dako, Ready to use, Human PoliclonalRabbit Primary Antibody),CD 4 (Dako, clone 4B12, Ready to use, Human Monoclonal Mouse Primary Antibody), CD 8 (Dako, clone C8/144B, Ready to use, Human Monoclonal Mouse Primary Antibody), antibodies by Ventana Benchmark ULTRA™ automatedimmunostainer. In addition, the ultraView Universal DAB detection kit was used for all staining. Positive and negative controls were used for each antibody, based on the manufacturer's prerequisites. The slides were evaluated by the pathologist (FB) with a Nikon eclipse e200 microscope. Tumor histotype was verified by light microscopic examination of H&E stained slides. Staining patterns were analyzed for each antibody; the percentage of positive staining and intensity (graded 0-3+) were determined at 40x magnification. MLH-1, MSH-2,MSH-6, PMS-2 were considered "positive"nuclear staining andCD3, CD4, CD8were considered "positive" for cytoplasmic staining. Negative test result in any of the markers MLH-1, MSH-2, MSH-6, or PMS-2 was considered to microsatellite instability (MSI). Also, with regard to tumor-infiltrating lymphocytes, a proportion level of greater than 10% was considered positive.

Statistical Analysis

SPSS v.23 software pack was used for statistical analyses. Descriptive statistics were expressed as mean, standard deviation, and percentages. The significance of the differences between groups was tested with Mann-Whitney U test. The associations between quantitative data were

determined with Spearman's correlation test. Progression-free and overall survival were estimated with Kaplan-Meier method. Statistical significance was set at a p level of <0.05.

Results

Microsatellite instability and tumor infiltrating lymphocytes (CD3, CD8, and CD4) were immunohistochemically examined among 81 ovarian cancer patients. Thirty patients (47%) had Stage 1-2, 51 (53%) had Stage 3-4 disease. High-grade tumors were observed in 54 patients (66.7%), and 59 patients (72.6%) had serous carcinoma histopathologically. CD3 levels were low in 53 patients (64.2%), and high in 29 (35.8%). CD8 was low in 57 patients (70.4%) and high in 24 (29.6%). CD4 was low in 54 cases (66.4%), and high in 27 (33.3%) (Table 1). Micro-satellite status had no statistically significant association with age, stage, grade, lymphovascular invasion, CD3, and CD8. However, microsatellite instable patients had significantly reduced CD4 (p=0.03) (Table 2). Also, there was a significant association between CD3 level and lymphovascular invasion (p=0.011) (Table 3). Survival analysis did not show any relationship between microsatellite instability and progression-free and overall survival. Stage, grade, lymphovascular invasion, Ki-67, and CD8 had significant impact on progression-free survival (p=0.007) (Fig. 1). CD 3 and CD4 levels have no impact on overall survival.Univariate (Table 4) and multivariate analyses (Table 5) showed statistically significant associations between CD8 level and progression-free survival (Fig. 2).

Table 1. Patient and tumor characteristics

Characteristics	n	%
Histology		
Serous	59	72
Borderline	7	8
Granulosa	6	7
Müsinous	2	2
Endometrioid	3	3
Clearcell	4	4
FIGO stage		
1-2	30	47
3-4	51	53
Histologicalgrade		
Low grade (G1-G2)	27	33
High grade (G3)	54	66
MSI		
Stabile	53	65
Instabile	28	35
CD3		
Low	53	64
High	29	35
CD8		
Low	57	70
High	24	29
CD4		
Low	54	66
High	27	33

Discussion

Advanced ovarian cancer is associated with most unfavorable prognosis among all gynecological cancers and the search for treatments to improve the survival continues. [15] Immune system plays a major role in the pathogenesis and progression of ovarian cancer.[16] In our study, we found an association between CD4 levels and overall survival (p=0.007). Also, in both univariate and multivariate analyses, CD8 levels were also significantly associated with progression-free survival (p=0.02). Previous studies also showed that intraepithelial TIL is associated with survival. For instance, in a study by Pinto et al., the CD4 and CD3 within the tumor tissue were associated with progression free survival (PFS) and overall survival (OS), while CD8 was associated with PFS.[17] James et al. found

Table 2. Associations between histopathological characteristics of patients and MSI

Characteristic	MSS n	MSI n	p
Age			
≤65	41	22	0.90
>65	12	6	
Stage			
1-2	18	12	0.43
3-4	35	16	
Grade			
Low	16	11	0.40
High	37	17	
Lymphovascular invasion			
Absent	17	13	0.20
Present	36	15	
CD3			
Low	34	18	0.99
High	19	10	
CD8			
Low	39	18	0.38
High	14	10	
CD4			
Low	31	23	0.03
High	22	5	

Table 3. Associations between histopathological characteristics of patients and CD3, CD8, CD4

		CD3		CD8		CD4	
	n	Mean±SD	p	Mean±SD	p	Mean±SD	p
Stage							
1-2	30	13±16	0.052	10±8	0.227	26±27	0.661
3-4	51	16±13		12±11		24±23	
Grade							
Low	27	11±12	0.104	8±6	0.050	17±15	0.176
High	54	17±15		13±12		28±27	
Lymphovascular invasion							
Absent	30	10±12	0.011	9±8	0.149	18±20	0.166
Present	51	18±15		13±12		28±26	
Ki-67							
Negative	28	11±12	0.068	8±6	0.094	17±5	0.149
Positive	53	17±15		13±12		29±27	

*Univariate analysis is obtained and analysed by using Mann Whitney U and Kruskal-Walles tests.

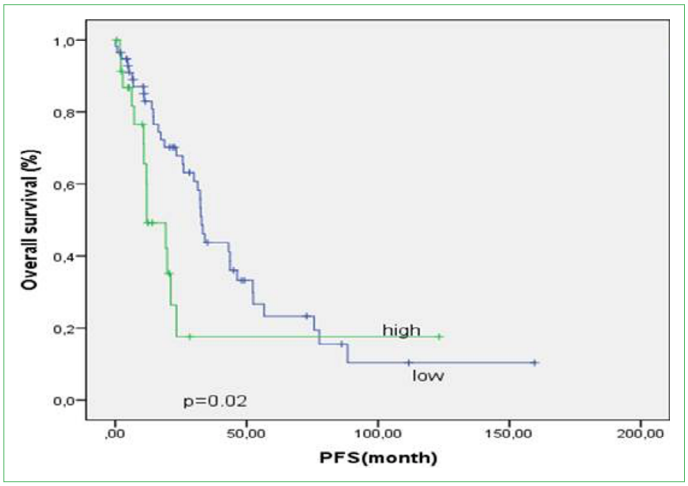

Figure 1. Kaplan Meier curves for progression free survival of ovarian cancer patients according to CD8.

that more marked lymphocyte infiltration in the ovarian cancer tissue was associated with better prognosis.[18] In Goode et al.'s study, higher CD8 levels were associated with better survival in high-grade ovarian cancer.[19] In a 2017 meta-analysis by Li et al., involving 21 studies and 2903 patients, a link between tumor infiltrating lymphocytes and survival was reported.[20] Again, in another study, the 5-year survival rate in patients with high TIL count in the tumor was 73.9% vs. 11.9% among those with less marked TIL.[21] In the current study, a statistically significant association between CD3 levels and lymphovascular invasion was found (p=0.01). Presence of elevated CD3 was associated with higher lymphovascular invasion. Also, the association between CD3 and disease stage was

Table 4. Prognostic factors related to progression free survival and overall survival

Characteristics	n	p	
		pfs	os
Stage			
Early	30	<0.001	0.148
Late	51		
Grade			
Low	27	0.011	0.372
High	54		
Lymphovascularinvasion			
Negative	30	0.022	0.964
Positive	51		
Ki 67			
Low<%50	28	0.022	0.362
High≥%50	53		
CD3			
Low	52	0.247	0.055
High	29		
CD8			
Low	57	0.020	0.427
High	24		
CD4			
Low	54	0.646	0.007
High	27		
MSI			
Stabile	53	0.204	0.545
Instabile	28		

*p values are obtained by Kaplan Meier analysis. p<0.05 is accepted to be statistically significant.

Table 5. Prognostic factors for progression free survival and overall survival of ovarian cancer in multivariate analysis

	Hazard ratio	95%CI	p
Progression free survival			
Grade	0.645	0.184-2.261	0.493
Lymphovascularinvasion	0.745	0.216-2.571	0.641
MSI	1.569	0.856-2.877	0.145
CD3	1.360	0.631-2.931	0.433
CD8	0.388	0.173-0.869	0.021
CD4	1.270	0.650-2.483	0.485
Overall survival			
Grade	0.180	0.032-1.001	0.050
Lymphovascularinvasion	3.074	0.573-16.482	0.190
MSI	1.785	0.616-5.170	0.286
CD3	3.465	0.616-19.492	0.158
CD8	0.852	0.153-4.742	0.855
CD4	7.059	0.903-55.209	0.063

*p values are obtained by Cox multivariate analysis.p<0.05 is accepted to be statistically significant.

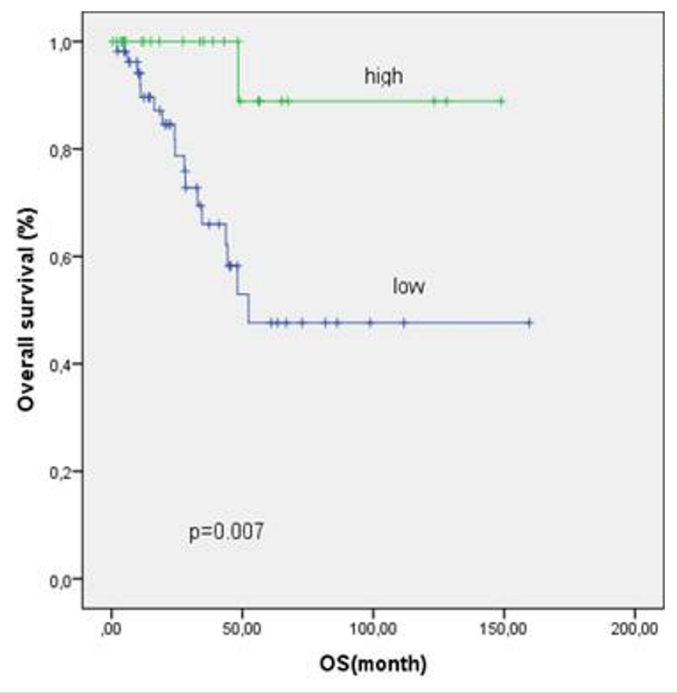

Figure 2. Kaplan Meier curves for overall survival of ovarian cancer patients according to CD4.

close to statistical significance (p=0.052). Again, the association between CD8 and histological grade reached near statistical significance (p=0.05). Our literature search did not reveal any studies that examined the link between tumor infiltrating lymphocytes and histopathological characteristics of ovarian cancer.

In 28 of our patients (34.6%), there was a loss of MMR protein expression. MSI was detected in 19 of the 59 patients with serous carcinoma, 3 of the 4 patients with clear cell carcinoma (75%), 2 of the 3 patients with endometroid carcinoma (66%), 2 of the 6 patients with granulosa carcinoma (33%), 1 of the 2 patients (50%) with mucinous carcinoma, and 1 of the 7 patients with borderline tumor (14%). In the meta-analysis by X. Xiao et al., micro-satellite instability was found in 7%, 17%, 13%, and 21% of the patients with serous, clear cell, endometrioid, and mucinous carcinoma, respectively. In that study, high MSI among those with mucinous carcinoma is remarkable.[22] In a study by Yamashita et al., 6 of the 136 patients (4.4%) had MSI, which was present in 2.6%, 57.7%, 8.7%, and 4.2% of their patients with serous, mucinous, endometrioid, and clear cell carcinoma, respectively.[23] Also, in other previous studies, MSI was mostly reported in non-serous carcinomas, although the samples were generally small.[24,25] In our study, CD4 was statistically lower in patients with microsatellite instability (p=0.03). To the best of our knowledge, no previous studies investigated the association between microsatellite instability and tumor infiltrating CD4 lymphocytes. In Yamashita et al.'s study, no statistically significant associations between MSI and tumor infiltrating CD8 lymphocytes were reported.[23] Xiao et al., found MSI in 18 of their 419 patients (4.3%), with elevated numbers of tumor infiltrating CD3 and CD3 lymphocytes. These patients with MSI also had better PFS.[22] However, in our survival analyses, no associations between microsatellite instability, progression-free survival, and overall survival were observed.

Conclusion

When one considers the complexity of the immune system, it is very likely that a single biomarker will not be fully predictive of the response to immunotherapy. Therefore, search for biomarkers that can accurately guide the choice of immunotherapy and examination of the associations between these markers have prognostic and therapeutic implications in patients with ovarian cancer.

Disclosures

Ethics Committee Approval: The study was approved by Pamukkale University Faculty of Medicine Ethics Committee in compliance with Helsinki Declaration (Approval number: 60116787-020/28709, approval date: 22.04.2019.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – B.Y.T., T.S.; Design – B.Y.T, A.G.D.; Supervision – G.G.D., A.Y.; Materials – T.S., A.E.; Data collection &/or processing – F.B.,C.K., B.C.D., T.D., M,Ö.; Analysis and/or interpretation – S.,D., A.E.; Literature search – C.K., B.C.D., T.D., M.Ö.,T.G.K.; Writing – B.Y.T.; Critical review – G.G.D., A.Y.

References

1. Agarwal R, Kaye SB. Prognostic factors in ovarian cancer: How close are we to a complete picture? Ann Oncol 2005;16(1):4–6.
2. Gasparri ML, Attar R, Palaia I, Perniola G, Marchetti C, Di Donato V, et al. Tumor infiltrating lymphocytes in ovarian cancer. Asian Pac J Cancer Prev 2015;16:3635–8.
3. Powell DJ Jr, Singh N, Coukos G. Immunotherapy for ovarian cancer: What's next? Clin Oncol 2011;29(7):925–33.
4. Turner TB, Buchsbaum DJ, Straughn JM Jr, Randall TD, Arend RC. Ovarian cancer and the immune system - the role of targeted therapies. Gynecol Oncol 2016;142:349–56.
5. Hwang W, Adams S, Tahirovic E, Hagemann S, Coukos G. Prognostic significance of tumor-infiltrating T-cells in ovarian cancer: A meta-analysis. Gynecol Oncol 2012;124(2):192–8.
6. Li GM. Mechanisms and functions of DNA mismatch repair. Cell Res 2008;18(1):85–98.
7. Abdel-Rahman WM, Mecklin JP, Peltomäki P. The genetics of HNPCC: Application to diagnosis and screening. Crit Rev Oncol Hematol 2006;58(3):208–20.
8. Aysal A, Karnezis A, Medhi I, Grenert JP, Zaloudek CJ, Rabban JT. Ovarian endometrioid adenocarcinoma: Incidence and clinical significance of the morphologic and immunohistochemical markers of mismatch repair protein defects and tumor microsatellite instability. Am J Surg Pathol 2012;36:163–72.
9. Xiao X, Dong D, He W, Song L, Wang Q, Yue J, et al. Mismatch repair deficiency is associated with MSI phenotype, increased tumor-infiltrating lymphocytes and PD-L1 expression in immune cells in ovarian cancer. Gynecol Oncol 2018;149:146–54.
10. Lu Y, Liu XS, Wang YX, Song HY, Zhong N. Study of microsatellite instability in epithelial ovarian tumors. Beijing Da Xue Xue Bao Yi Xue Ban 2006;38:62–5.
11. Huan Z, Nakayama K, Nakayama N, Ishibashi M, Yeasmin S, Katagiri A, et al. Genetic classification of ovarian carcinoma based on microsatellite analysis: Relationship to clinicopathological features and patient survival. Oncol Rep 2008;19:775–81.
12. Rambau PF, Duggan MA, Ghatage P, Warfa K, Steed H, Perrier R, et al. Significant frequency of MSH2/MSH6 abnormality in ovarian endometrioid carcinoma supports histotype-specific Lynch syndrome screening in ovarian carcinomas. Histopathology 2016;69:288–97.
13. Le DT, Uram JN, Wang H, Bjarne R, Bartlett BS, Holly Kemberling RN, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509–20.
14. Nelson BH. The impact of T-cell immunity on ovarian cancer outcomes. Immunol Rev 2008;222:101–16.
15. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Niksic M, et al. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): Analysis of individual records for 37,513,025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 2018;391:1023–75.
16. Charbonneau B, Goode EL, Kalli KR, Knutson KL, Derycke MS. The immune system in the pathogenesis of ovarian cancer. Crit Rev Immunol 2013;33:137–64.
17. Pinto M, Balmaceda C, Bravo L, Kato S, Villarroel A, Gareth I, et al. Patient inflammatory status and CD4+/CD8+ intraepithelial tumor lymphocyte infiltration are predictors of outcomes in high-grade serous ovarian cancer. Gynecol Oncol 2018;151:10–7.
18. James FR, Linan MJ, Alsop J, Mack M, Song H, Brenton JD, et al. Association between tumour infiltrating lymphocytes, histotype and clinical outcome in epithelial ovarian cancer. BMC Cancer 2017;17:657.
19. Goode EL, Block MS, Kalli KR, Vierkant RA, Chen W, Fogarty ZV, et al. Dose-response relationship of CD8+ tumor infiltrating lymphocytes and survival time in high-grade serous ovarian cancer. JAMA Oncol 2017;3(12):e173290
20. Li J, Wang J, Chen R, Bai Y, Xin LX. The prognostic value of tumor-infiltrating T lymphocytes in ovarian cancer. Oncotarget 2017;8(9):15621–31.
21. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003;348:203–13.
22. Xiao X, Melton DW, Gourley C. Mismatch repair deficiency in ovarian cancer: Molecular characteristics and clinical implications. Gynecol Oncol 2014;132:506–12.
23. Yamashita H, Nakayama K, Ishikawa M, Ishibashi T, Nakamura K, Sawada K, et al. Relationship between microsatellite instability, immune cells infiltration, and expression of immune checkpoint molecules in ovarian carcinoma: Immunotherapeutic strategies for the future. Int J Mol Sci 2019;20(20):5129.
24. Malander S, Rambech E, Kristoffersson U, Halvarsson B, Ridderheim M, Borg A, et al. The contribution of the hereditary nonpolyposis colorectal cancer syndrome to the development of ovarian cancer. Gynecol Oncol 2006;101:238–43.
25. Rosen DG, Cai KQ, Luthra R, Liu J. Immunohistochemical staining of hMLH1 and hMSH2 reflects microsatellite instability status in ovarian carcinoma. Mod Pathol 2006;19:1414–20.