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# The prognostic significance of microsatellite status and its relationship with tumor-infiltrating lymphocyte in endometrial cancer

Sahin Lacin[1,*], Gozde Elif Tasar[2], Alp Usubutun[2], Zafer Arık[3], Deniz Yüce[4], Mehmet Çoşkun Salman[5], Ayşe Kars[3]

[1] *Department of Medical Oncology, Yeditepe University, Faculty of Medicine, Koşuyolu Mah. Koşuyolu Cad. No:168 34718 Kadıköy/İstanbul, Turkey*

[2] *Department of Pathology, Hacettepe University, Faculty of Medicine, 06100 Altındağ, Ankara, Turkey*

[3] *Department of Medical Oncology, Hacettepe University, Cancer Institute, 06100 Altındağ, Ankara, Turkey*

[4] *Department of Preventive Oncology, Hacettepe University, Hacettepe Cancer Institute, 06100 Altındağ, Ankara, Turkey*

[5] *Department of Obstetrics and Gynecology, Hacettepe University, Faculty of Medicine, 06100 Altındağ, Ankara, Turkey*

*Correspondence: sahin.lacin@hotmail.com (Sahin Lacin)

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*Objectives*: The relationship between the microsatellite status (MS), tumor-infiltrating lymphocytes (TIL) and prognosis is unclear in endometrial cancer. We aim to examine the impact of MS and TILs on prognosis in patients with endometrioid type EC. *Methods*: The patients diagnosed with EC were retrospectively analyzed in the study. MS was evaluated by immunohistochemistry (IHC) based on expression of MLH1, MSH2, MSH6, and PMS2 proteins. The patients were stratified according to TIL patterns. TILs were classified as intratumoral (iTIL), stromal (sTIL), and peritumoral TILs (pTIL). *Results*: A total of 91 patients with different stages of endometrioid type EC. In terms of MS, 58 patients were microsatellite stable (MSS) and 33 patients were microsatellite instable (MSI). pTIL score was higher in patients with MSI than patients with MSS ($P < 0.0001$). We observed significant correlation between pTIL infiltration and MSI status. There was no statistically significant difference between the survival of patients with MSI and MSS irrespective of disease stage; median OS rates were 96 and 136 months, respectively ($P = 0.151$). Survival difference was not significant between patients with MSI and MSS early-stage disease: OS rates for patients with MSI and MSS were 95 and 139 months, respectively ($P = 0.087$). *Conclusion*: Our study identified a relationship between the extent of TIL infiltration and MSI status and reveals that EC with MSI attracts more immune cells to the tumor micro-environment. However, we could not find prognostic effect of microsatellite status in patients with EC.

*Keywords*

Endometrial cancer; Microsatellite instability; Survival; Tumor infiltrating lymphocyte

## 1. Introduction

Endometrial cancer (EC) is the first and the second most common cancer of the female genital tract in developed and developing countries, respectively [1]. The disease predominantly occurs in the post-menopausal period and the main risk factors for disease development are obesity, nulliparity, and somatic and inherited mutations [2]. Estrogen dependent endometrioid carcinoma (type I) is the most common histologic subtype and the less common subtype is non-estrogen dependent, non-endometrioid type II. Histologically EC is a heterogeneous disease [3]. As more evidence accumulates about the molecular genetic alterations that contribute to endometrial tumorigenesis, heterogeneity observed in this dualistic model broadens [4]. Endometrioid and serous carcinomas, represent the major phenotypes of types I and II endometrial carcinomas respectively, and have distinctive types of genetic instability and molecular alterations [5]. One of these molecular alterations associated with type I EC is microsatellite instability (MSI). The DNA mismatch repair (dMMR) deficiency leads to MSI by significantly increasing the rates of strand-slippage mutations.

Endometrial carcinomas develop both sporadically or in association with germ-line mutations in MLH1, MSH2, MSH6 and PMS2 genes so called MMR genes [6]. Lynch syndrome (LS), also known as hereditary nonpolyposis colorectal cancer, is an autosomal dominantly inherited disorder of cancer susceptibility associated with germline mutations in MMR genes. EC is known as the most common extracolonic malignancy in LS and 3 to 5% of all ECs are associated with LS. On the other hand, deficient MMR system is observed in 25–30% of sporadic ECs and is commonly associated with endometrioid histology [7]. LS is screened traditionally based on Amsterdam Criteria that use family history and this method was developed primarily for patients who had colorectal cancer [8]. Patients diagnosed with LS carry the germline variant that affects the proteins encoded by the DNA mismatch repair (MMR) genes MLH1, MSH2, MSH6, or PMS2. Immunohistochemical evaluation is one of the most commonly performed tests to detect this effect. As a preliminary test, the immunohistochemistry (IHC) method is a valuable screening test for MSI, with sensitivity ranging between 86–100% [9]. IHC screening detects the protein

expression of MMR genes and is found to have quite similar results to genetic analysis of the MSI test in detection of genetic abnormalities with high sensitivity [10]. Moreover, IHC screening can reduce overall LS detection cost dramatically through use of tissue microarrays without loss of accuracy and MSI genetic analysis requires tumor microdissection and evaluation of tissue in a molecular diagnostics laboratory [11]. On the other hand, TILs are well-recognized histologic markers associated with MS in the diverse tumor types, including EC [12]. Despite a positive relation between TILs and prognosis has been reported in these cancers, the presence of TILs has not been fully illuminated in endometrial cancer [13]. Similarly, MSI has been defined as a prognostic factor in colorectal cancer, the relation between MS status and the clinical outcome in EC remains debatable and controversial [14]. In this study, we aimed to evaluate the prognostic capacity of MSI and TILs detected by IHC and investigate other potential prognostic factors and their impact on the survival of patients with EC.

## 2. Materials and methods

This is a retrospective chart review of women with a diagnosis of EC treated at the university cancer institute between January 2004 and December 2017. Type I (endometrioid type endometrial cancer) EC patients with stage I–III and over 18 years of age except stage IA grade 1–2 were taken into the study. The data of one hundred patients with endometrioid type I EC was selected from the database, just 91 of them were eligible for the study. Stage 1A tumors having grade 1 or 2 morphology were excluded from the study as most of these tumors are associated with favorable prognosis and do not necessitates additional treatment. The hysterectomy specimens were re-evaluated, and the diagnosis and the grade of the tumor were confirmed by a gynecologic pathologist using surgical specimens. All patients had been discussed in an interdisciplinary tumor board, and treatment modalities such as surgery, systemic chemotherapy and radiotherapy had been recommended based on the consensus opinion. Seven patients with poor quality tissue samples were excluded and the study was carried out by performing additional analysis on specimens of 91 patients. Tumor diameter, pathological grade, disease stage, microsatellite status, and treatment modalities were the main parameters evaluated. The clinical and laboratory parameters of the patients were collected and patients were stratified according to the disease stage, recurrence and microsatellite status. Additionally, we stratified the patients according to the tumor-infiltrating lymphocyte patterns to evaluate the prognostic significance of lymphocyte infiltration patterns according to the guideline [15]. All the analyses were performed on H&E-stained sections. First, we selected the tumor area and defined the peritumoral, stromal, and intratumoral areas. Then we scanned these areas in low magnification and determined the type of inflammatory infiltrate. Finally, the infiltration severity or percentage was determined. Three distinct TIL infiltration patterns were identified: (I) lymphocytes within cancer cell nests (intratumoral lymphocytes (iTIL); (II) lymphocytes in the central cancer stroma (stromal lymphocytes (sTIL); and (III) lymphocytes present along the invasive margins (peritumoral lymphocytes (pTIL). TILs were enumerated on 10 high power fields. sTIL is the percentage of lymphocytic area to the central cancer stroma. pTIL is scored according to the intensity of lymphocytes on peritumoral area and is graded based on their intensity as 0, +1, +2 and 3+.

IHC was performed on paraffin-embedded formalin-fixed tissue, cut at 3 microns, by Leica Bond Autostainer machine, using antibodies against MLH1 (Leica, clone ES05, 1/50), MSH2 (Leica, clone 25D12, 1/100), MSH6 (Leica, clone PU29, 1/150) and PMS2 (Leica, clone MOR4G, 1/100). Pretreatment of MSH2 and MSH6 with epitope retrieval solution 1 (BOND Epitope Retrieval Solution 1 is a ready to use, citrate-based pH 6.0 epitope retrieval solution for the heat-induced epitope retrieval (HIER) of formalin-fixed, paraffin-embedded tissue on the BOND automated system.) were 20 minutes. This time for MLH1 and PMS2 were 10 minutes.

IHC for MMR enzymes such as MLH1, MSH2, MSH6, PMS2 were performed on all pathologic samples from hysterectomy specimens confirmed as endometrioid type EC. The patients with absent protein expression of any one of MLH1, MSH2, MSH6, PMS2 were classified as deficient MMR and patients with positive staining of all MMR enzymes were classified as intact or proficient MMR (Fig. 1).

Overall survival (OS) rate was the primary outcome as defined by the time from the date of diagnosis to death or censorship, in which individuals lost to follow-up were censored at the date they were last known to be alive. OS was calculated for all patients.

Differences in patient characteristics were compared between those with possible prognostic or predictive factors for microsatellite stable or microsatellite instable endometrioid type EC. For categorical variables, the number and percentage of patients in each category were provided, and Chi-square or Fisher' s exact test was used for statistical comparisons between the treatment groups. Survival rates were estimated by the Kaplan-Meier method and the log-rank test was used for comparisons between groups. Cox proportional hazards model was used for multivariate analyses for identifying independent prognostic factors of survival. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. All tests were 2-sided with a significance level of 0.05. Analyses were performed using SPSS version 22 statistical software (IBM Corporation, Somers, New York, USA).

## 3. Results

This study includes a total of 91 patients with EC type I whose treatment and follow-up were carried out at the university cancer institute. The median age of the patients was 60 years (26–81), and the median menopausal age was 50 years (38–54). The majority of the patients were postmenopausal (n = 79; 87.8%). The clinical characteristics of the

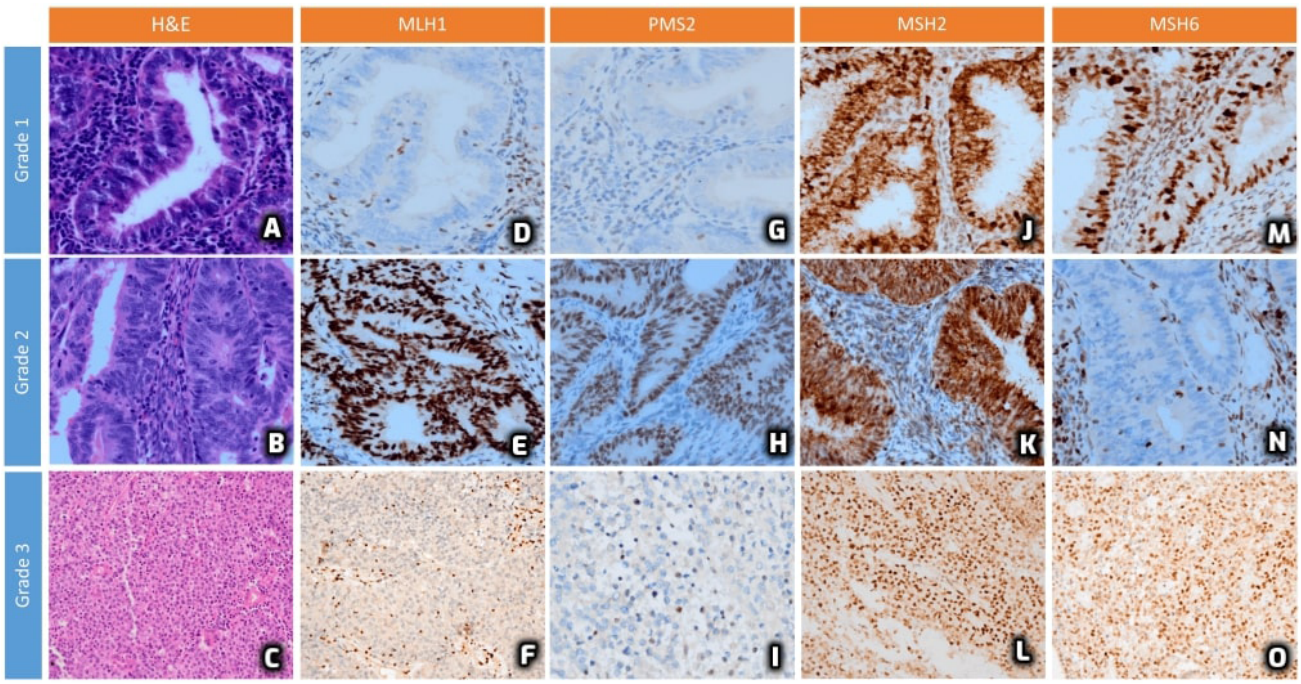


**Fig. 1. Endometrioid adenocarcinoma.** Grade I and III: Tumor cells show positive nuclear staining with MSH2 and MSH6 (J, L, M and O); expression loss with MLH1 and PMS2 (D and G). Grade II: Tumor cells show positive nuclear staining with MLH1, PMS2, MSH2 (E, H, and K) and nuclear expression loss with MSH6 (N) (Infiltrated lymphocytes showed nuclear staining).

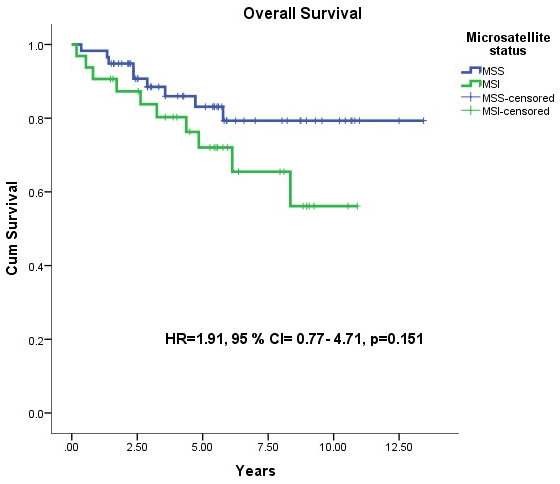


**Fig. 2. The survival of all patients according to microsatellite status irrespective of the disease stage (HR = 1.91, 95% CI = 0.77–4.71, *P* = 0.151).**

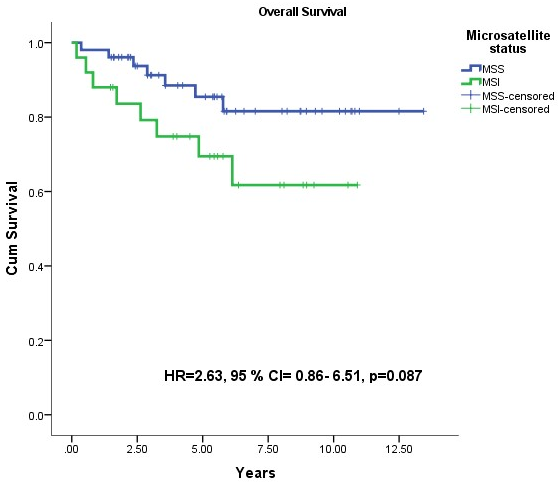


**Fig. 3. The survival of patients with the early-stage disease according to microsatellite status (HR = 2.63, 95% CI = 0.86–6.51, *P* = 0.087).**

patients at baseline are summarized in Table 1. Patients were stratified according to the revised FIGO staging system: 5 (5.5%) patients had stage IA grade 3, 64 (70.3%) patients'stage IB grade 3, 8 (8.8%) patients stage II and 14 (15.4%) patients stage III. Fifty-eight patients (63.7%) were classified as MSS and 33 (36.3%) as MSI according to the immunohistochemistry analyses. The OS of MSS and MSI groups was 136 and 96 months, respectively ($P = 0.151$) (Fig. 2). There were 77 patients with type I early-stage cancer (stage IA, IB, and II) in our cohort, and 51 of these patients (66.2%) were classified as MSS and 26 (33.8%) as MSI. In patients with early-stage disease, there was a clinically but not statistically significant difference between the OS of the MSS and MSI groups (139 months vs. 95 months, respectively, $P = 0.087$) (Fig. 3). We observed a statistically significant relationship between the microsatellite status and the peritumoral lymphocyte infiltration scores. Indeed, MSI patients tended to have higher lymphocyte infiltration scores compared to MSS patients ($P < 0.001$) (Table 2). Moreover, there was a significant difference between the sTIL scores in MSS vs MSI tumors Lymphocyte

**Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.**

| | | | % |
|---|---|---|---|
| Median age at diagnosis (min-max) | | 60 (26–81) | 100 |
| Median age at menopause (min-max) | | 50 (38–54) | 87.1 |
| Menopausal status | Pre-menopause | 4 | 4.4 |
| | Peri-menopause | 8 | 8.8 |
| | Post-menopause | 79 | 86.8 |
| Microsatellite status | MSI | 33 | 36.3 |
| | MSS | 58 | 63.7 |
| MLH 1 | Positive | 60 | 65.9 |
| | Negative | 31 | 34.1 |
| MSH 2 | Positive | 91 | 100 |
| | Negative | - | - |
| MSH 6 | Positive | 89 | 97.8 |
| | Negative | 2 | 2.2 |
| PMS 2 | Positive | 60 | 65.9 |
| | Negative | 31 | 34.1 |
| Tumor stage | Stage IA | 5 | 5.5 |
| | Stage IB | 64 | 70.3 |
| | Stage II | 8 | 8.8 |
| | Stage III | 14 | 15.4 |
| Tumor grade | Grade 1 | 43 | 46.3 |
| | Grade 2 | 23 | 24.7 |
| | Grade 3 | 25 | 29 |
| Peritumoral Lymphocyte infiltration score | 1+ | 44 | 48.4 |
| | 2+ | 41 | 45.1 |
| | 3+ | 6 | 6.5 |
| Co-morbidities | Diabetes Mellitus | 33 | 35.4 |
| | Hypertension | 52 | 55.9 |
| | Thyroid dysfunction | 18 | 19.4 |
| | Coronary arterial disease | 16 | 17.2 |
| | Asthma | 12 | 12.9 |

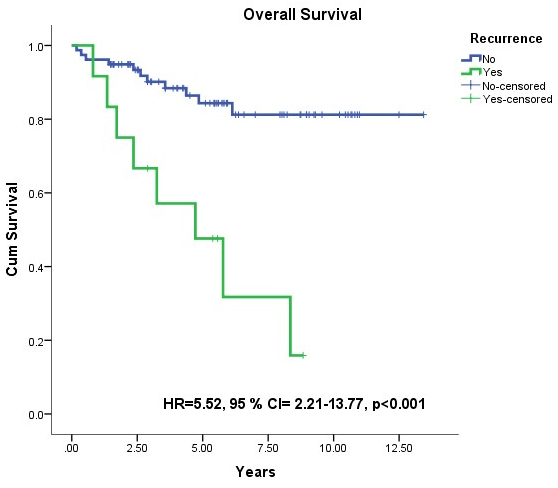


**Fig. 4. The survival of patients according to recurrence status (HR = 5.52, 95% CI = 2.21–13.77, $P < 0.001$).**

infiltration in MSI tumors was more prominent compared to MSS tumors ($P < 0.001$). Although MSI patients had higher intratumoral lymphocyte infiltration than MSS patients, this difference did not reach statistical significance ($P = 0.088$). In terms of tumor grade, 43 patients (47.3%) had grade 1, 23 patients (25.3%) had grade 2, and 25 patients (27.4%) had grade 3 tumors. We observed a significant association between tumor grade and tumor stage ($P = 0.022$). Obese patients comprised 52.5% of the study population and there were no underweight patients in the group. We didn't observe an association between microsatellite status and body mass index ($P = 0.8$). In terms of treatment modality, fifty-nine (72.8%) patients were treated with brachytherapy, 4 (4.9%) had both brachytherapy and external radiotherapy, 3 (3.7%) had chemotherapy and 15 (18.5%) had both chemotherapy and radiotherapy. Twelve patients had recurrent disease, 6 (10.3%) in the MSS and 6 (18.2%) in the MSI group, without a statistically significant difference ($P = 0.30$). 58% of these recurrences were local. The OS in patients with recurring disease was significantly lower than the OS of patients with non-recurring disease and the median OS were 138.1 months and 59.0 months, respectively ($P < 0.001$) (Fig. 4). The correlation analyses between patients' characteristics and microsatellite status were listed in Table 2. Diabetes (35.2%), hypertension (56%), coronary arterial disease (16.5%), thyroid dysfunction (19.8%) and asthma (13.2%) were the most common comorbidities in our patient population (Table 1). Cox regression analysis and prognostic factors for survival are summarized in Table 3. The median follow-up is 73.6 months.

**Table 2. The correlation between patients' characteristics and Microsatellite status.**

| Patient's characteristic | | Microsatellite Instable (MSI) N (%) | Microsatellite stable (MSS) N (%) | *P*-Value |
|---|---|---|---|---|
| Lymphocyte infiltration | Positive | 29 (87.9) | 18 (69) | <0.001* |
| | Negative | 4 (12.1) | 40 (31) | |
| Tumor Grade | Grade 1 | 13 (39.4) | 30 (51.7) | 0.18 |
| | Grade 2 | 1 (36.4) | 11 (19) | |
| | Grade 3 | 8 (24.2) | 17 (29.3) | |
| Recurrence status | No | 27 (81.8) | 52 (89.7) | 0.3 |
| | Yes | 6 (18.2) | 6 (10.3) | |
| Disease stage | Stage 1A | 1 (3) | 4 (6.9) | 0.6 |
| | Stage 1B | 22 (66.7) | 42 (72.4) | |
| | Stage 2 | 3 (9.1) | 5 (8.6) | |
| | Stage 3 | 7 (21.2) | 7 (12.1) | |
| Treatment Modalities | Brachytherapy | 22 (73.3) | 37 (72.5) | 0.2 |
| | Brachytherapy and External Radiotherapy | 0 (0) | 4 (7.8) | |
| | Chemotherapy | 1 (3.3) | 2 (3.9) | |
| | Chemotherapy and Radiotherapy | 7 (23.4) | 8 (15.7) | |

*: statistically significant.

**Table 3. Cox regression model for the prognostic factors, N = 91.**

| Factor | HR | 95% CI of HR | *P* |
|---|---|---|---|
| **Grade** | | | **0.09** |
| Grade 1 | 1.000 | | |
| Grade 2 | 2.347 | 0.716–7.696 | |
| Grade 3 | 3.233 | 1.052–9.871 | |
| **Recurrence** | | | **< 0.001*** |
| Non-recurrent | 1.000 | | |
| Recurrent | 5.520 | 2.213–13.771 | |
| **Microsatellite status** | | | **0.16** |
| MSS | 1.000 | | |
| MSI | 1.913 | 0.777–4.710 | |
| **pTIL** | | | **0.6** |
| Lymphocyte 0+ | 1.000 | | |
| Lymphocyte 1+ | 1.492 | 0.597–3.925 | |
| Lymphocyte 2+ | 2.010 | 0.417–9.696 | |
| **iTIL** | | | **0.96** |
| Continuous | 1.000 | 0.983–1.018 | |
| **sTIL** | | | **0.16** |
| Continuous | 1.014 | 0.994–1.033 | |

HR, Hazard ratio; CI, Confidence Interval; MSS, microsatellite stable; MSI, microsatellite instability; pTIL, peritumoral lymphocytes; iTIL, intratumoral lymphocytes; sTIL, stromal lymphocytes.

*: statistically significant.

## 4. Discussion

The aim of this study was to examine the relationship between TIL, microsatellite status (MS), and prognosis in patients with type I EC and their roles in terms of patient survival. Despite the degree of TIL infiltration correlates with improved prognosis in patients with numerous types of cancer, the relationship between MS status and the clinical outcome in EC is controversial [16]. In our study, there was no statically significance difference between the survival rate of our patients with microsatellite unstable tumors and patients with microsatellite stable tumors irrespective of the disease stage (Figs. 2,3). Consistent with earlier reports, patients with MSI tumors had more prominent peritumoral lymphocyte infiltration compared to those who had MSS tumors.

The MMRd can be detected in both type I endometrial cancer and sporadic endometrial cancers. This situation is rarely related to Lynch syndrome [17]. Lynch syndrome-associated endometrial cancer and sporadic endometrial cancers represent different types of deficient MMR system. The Lynch syndrome is associated with germline mutation, in contrast, sporadic endometrial cancers are associated with hypermethylation of MLH1 [6]. Consequently, microsatellite status can be affected by two different types of genetic alterations in responsible genes. The proportion of our patients with a deficient MMR system was consistent with the literature. However, as we didn't perform molecular assays, we cannot dissect the exact mechanisms leading to dMMR in our study.

Although deficient MMR system has been associated with improved prognosis in early-stage colorectal cancer the relationship between MMR status, the clinical outcome in EC is controversial [18]. There are reports suggesting a favorable prognosis conferred by MSI [19, 20], however, these studies remain debatable for various reasons such as the lack of genetic confirmation of Lynch syndrome or the characteristics of the study population that included [21]. However, there are in contrast claims regarding the favorable prognostic effect of MMRd on the survival of patients [22]. On the other hand, in a meta-analysis that included 23 studies, 74% of which were retrospective case series, conclusive evidence of an association between dMMR and poor survival in endometrial cancer could not be found [23]. Even though the outcomes in our study may have been affected by some confounding factors such as the disease stage and grade, patient age, treatment type, patient distribution and comorbidities, the findings were consistent with the medical literature. The OS of the patients with MSI tumors was worse compared to the OS of patients with MSS tumors. However, we didn't observe a statistically significance, which could be small number of patients in our cohort (Fig. 2).

The tumor-infiltrating immune cells encompassing T and B lymphocytes, natural killer cells, and macrophages are regulators of the immune response against tumors, and each T-lymphocyte subset has a unique role in antitumor response. While cytotoxic T-lymphocytes infiltrating ovarian and colorectal cancer are associated with improved survival, regulatory T lymphocytes have suppressive roles on antitumor response and hence are associated with poor prognosis [24]. Although TILs have been reported to have prognostic and predictive roles in certain tumors, the prognostic role of TILs in endometrial carcinoma is still not settled [24, 25]. Low intraepithelial TIL counts are detected in the advanced stage, high-risk groups of endometrial carcinomas and this emerges as an independent predictor of poor survival [26]. In our study, we couldn't find a significant relationship between increased TILs (intratumoral lymphocytes, peritumoral lymphocytes, and stromal lymphocytes) and patients'survival. However, we haven't performed lymphocyte subtype analysis and therefore we cannot make any further comments on this issue.

The presence of an association between microsatellite status and tumor-infiltrating lymphocytes is well known. Especially a positive correlation between microsatellite instability and the proportion of lymphocytic infiltration has been demonstrated in colorectal carcinomas [27]. The presence of high TIL counts in colorectal cancer has been associated with MSI, with a rate of 80% sensitivity and 60% specificity to predict MSI. Similarly, significantly higher TIL counts have been reported in endometrial cancer patients with MSI compared to MSS tumors [28]. We found a statistically significant relationship between microsatellite status and peritumoral TILs, and patients with MSI tumors had higher peritumoral lymphocyte scores than patients with MSS tumors. Furthermore, sTIL counts were significantly higher in MSI compared to MSS tumors.

A potential limitation of our study is that we chose IHC rather than MSI testing for screening our patients to detect MMR system deficiency. However, we know that IHC is almost equally sensitive compared to MSI for screening Lynch syndrome and its sensitivity was found as high as 94% in a study [10]. Additionally, the concordance between IHC and MSI testing has been reported to be 93% in colon tumors when four MMR proteins were evaluated, and positive staining for MLH1 or MSH2 predicted an intact mismatch repair system in 95% of patients [29]. based on high concordance between microsatellite instability evaluated through genotyping and mismatch repair defects evaluated through immunohistochemistry, we believe that mismatch repair protein expression is both feasible and reliable in evaluation of mismatch repair status in endometrial cancer. The second limitation of our study may be the lack of POLE and P53 mutation analyzes in our patients. Because 5–8% of the POLE mutation and much less frequently P53 mutation can be seen in endometrioid type endometrial cancer and have potential effects on the course of the disease [30].

Stage IA grade I–II patients were not included in our study because of their low risk of recurrence, and follow-up for patients younger than 60-years old, not having deep invasion and lymphovascular involvement (LVSI) is recommended in all guidelines. In general, there is no controversy in the treatment of patients with non-endometrioid type endometrial cancer, and chemotherapy is recommended for these patients. Various treatment modalities have been proposed for patients with high intermediate-risk and high-risk endometrioid type endometrial cancer, such as surveillance, brachytherapy, pelvic radiation treatment or chemotherapy [31]. In our study, we investigated the possible effects of MSI or MSS status on treatment decisions in early-stage endometrioid endometrial cancers which included high intermediate and high-risk stage I and II patients. And we tried to figure out if there is any prognostic importance of MSI or MSS status in EC that not clearly demonstrated in the literature.

## 5. Conclusions

In conclusion microsatellite status in endometrial carcinoma is important, but not well recognized. In our study, we did not find any difference between the survival of patients with MSI and MSS endometrial cancer. We found significantly increased TIL in microsatellite unstable patients like in previous studies, however, despite high peritumoral lymphocyte infiltration, we couldn't find a significant difference in terms of patients'survival. Our findings may contribute to the information on the prognosis of patients with MSI in patients with endometrial cancer which at present is conflicting. Multi-institutional studies with a large number of patients and longer follow-up are needed to identify the role of microsatellite instability in this disease.

## Author contributions

SL: Conception and design, analysis and interpretation of data, drafting of the manuscript. EGT: Conception and design, analysis and interpretation of data. AU: Conception and design, analysis and interpretation, and drafting of the manuscript. ZA: Analysis and interpretation of data. DY: Analysis, Acquisition of data. MCS: Conception and design of study. AK: Conception, drafting of the manuscript, advices and final approval. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The study was designed and conducted following the Helsinki declaration. Approval of the study was obtained from the Hacettepe University Ethics Committee (approval date and number: 26/08/2017 and 17/80). Written informed consent was obtained.

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## Conflict of interest

The authors declare no competing interests.

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