

The Ki-67 index and neutrophile-lymphocyte ratio are prognostic factors in patients with low-risk endometrial cancer

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Objective: To investigate the prognostic factors comparing clinical, histopathological, and laboratory parameters in low-risk endometrial cancer (EC). **Methods:** In the present single-center study, multivariate Cox regression analysis was performed on retrospective clinical and laboratory data and histopathological features obtained from the re-evaluation of 253 patients with low-risk EC. Receiver operating characteristic curves (ROC) were plotted for neutrophile-lymphocyte ratio (NLR), platelet-lymphocyte ratio, lymphocyte-monocyte ratio and Ki-67 index for recurrence. Kaplan-Meier analysis was employed for survival rates. **Results:** The median age was 58.5 years (32.0–75.4). Most of the patients were obese and post-menopausal. In nearly half of the patients, lymphadenectomy was performed in addition to hysterectomy and oophorectomy. The median tumor size was 30 mm (range 2–80), and the median Ki-67 index was 25 (1–90). According to the ROC curve analysis, the cut-off values for the Ki-67 index, NLR, PLR, and LMR were determined as ≥ 22 , ≥ 1.98 , ≥ 115.3 , and ≤ 4.71 , respectively. The log-rank test revealed that the patients with a Ki-67 index lower than 22% and NLR lower than 1.98 had statistically longer recurrence-free survival (RFS) ($p = 0.002$ for Ki-67 index and $p = 0.004$ for NLR). The multivariate analysis revealed that the Ki-67 index and NLR were statistically significant factors for RFS ($p = 0.012$ and $p = 0.029$, respectively). **Conclusion:** The present study highlights the prognostic implications of both the Ki-67 index and NLR in low-risk EC.

Keywords

Endometrial cancer; Low-risk; Ki-67; NLR; Survival

1. Introduction

Endometrial cancer (EC) is the leading gynecological cancer in developed countries and Turkey, accounting for almost one-third of malignancies originating from the female reproductive system [1]. The incidence of endometrial cancer increases over time [2], but most patients are diagnosed at an early stage [3]. The majority of EC affects post-menopausal women and has an endometrioid histological subtype [4].

The European Society for Medical Oncology (ESMO) consensus conference accepted new risk groups to guide clinicians for adjuvant therapy [5]. In June 2017, the ESMO Guidelines Committee updated the EC algorithms, recommending no adjuvant treatment after surgery in low-risk EC. 5-year recurrence-free survival (RFS) of patients with low-risk EC has been reported to be greater than 95% [6].

The Ki-67 protein is a non-histone nuclear DNA binding protein of which expression increases from the G1 phase to mitosis [7]. It is localized around the nucleolus and forms a perichromosomal layer with other nucleolar proteins such as fibrillarin, nucleolin, nucleophosmin [8]. This layer functions as a protective sheath and a platform during mitosis. Additionally, Ki-67 provides chromatin organization, compacting heterochromatin and prevents aggregation of mitotic chromosomes [9, 10]. After the M phase, the Ki-67 protein is degraded rapidly. These features of Ki-67 led to it being referred to as a cell proliferation marker.

Although Rudolf Virchow hypothesized that chronic inflammation enhanced cellular proliferation and might be the origin of cancer in the 19th century [11], the precise connection between inflammation and cancer remains unknown despite increased interest over the past fifty years. The systemic inflammatory response to cancer cells results in neutrophilia, lymphopenia, and thrombocytopenia via cytokines such as interleukin-6 and tumor necrosis factor α [12]. Indeed, the immune profile of these cells obtained from complete blood cell (CBC) counts have prognostic value in many cancer types [13].

The prognostic factors, including the patients' clinical characteristics, surgical procedures, pathological features, adjuvant treatments, genetic alterations, and hematological parameters, which are predictors for cancer-related inflammation, have been studied in many EC reports [14–21]. Nevertheless, most of the analyses included heterogeneous groups

of patients whose diseases had different stages or risk groups. Therefore this study aims to define the prognostic factors in low-risk EC, described in the consensus [5].

2. Patients and methods

2.1 Study population

The electronic medical records of patients admitted to the Department of Medical Oncology, or the Department of Gynecological Oncology of Bursa Uludag University between January 2010 and December 2019 due to EC were retrospectively reviewed. Fig. 1 demonstrates the profile of the study. Patients with non-endometrioid type EC, synchronous ovarian cancer, a history of malignancies at alternative sites, hematological disorders, corticosteroid use, and chronic inflammatory disease were excluded. After post-surgical staging, according to the International Federation of Gynecology and Obstetrics (FIGO) 2009, patients with low-risk disease, defined as FIGO stage I disease with histological endometrioid type and grade 1 or 2, invading less than one-half of the myometrium without lymphovascular space invasion (LVSI) [5], were included in the study.

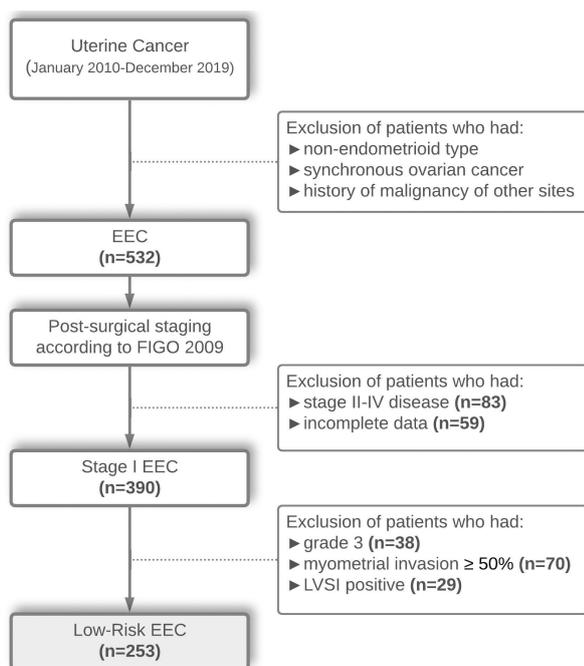


Fig. 1. Patients selection flow chart.

2.2 Data collection

The following demographic, clinical, and laboratory features were extracted from electronic records for all the participants: age, menopausal status, parity, body mass index, history of diabetes mellitus, hemogram parameters, surgical procedure, and adjuvant treatment. Patients with a body mass index of 30 and above were considered obese. The absolute neutrophil, lymphocyte, and platelet counts were obtained from CBC within 21 days before surgery. CBC re-

sults before three weeks or after surgery were not used and accepted as a missing value. Neutrophil lymphocyte ratio (NLR), lymphocyte monocyte ratio (LMR), and platelet lymphocyte ratio (PLR) were determined by dividing absolute neutrophil by lymphocyte count, lymphocyte by monocyte count, and platelet by lymphocyte count, respectively. Due to the lack of optimal NLR, LMR, and PLR, the ratio's cut-off was determined using the receiver operating characteristic (ROC) curve.

2.3 Surgical procedure and adjuvant treatment

In the author's center, total hysterectomy and bilateral salpingo-oophorectomy (TAH and BSO) is the surgical treatment of EC. Intraoperative frozen section analysis was routinely performed in all cases. Pelvic and paraaortic lymphadenectomy is also performed for patients whose frozen section analysis reveals a tumor type other than endometrioid, grade 3 histology, cervical stromal invasion, myometrial invasion greater than 50% depth, tumor size greater than 2 cm, and in case of suspicion of lymph node involvement during intraoperative evaluation. Brachytherapy was applied as adjuvant therapy to the patients with stage 1A disease, most of which consist of low-risk disease, in the presence of high-risk factors (LVSI and age 60) and grade 3 EC. The dose was delivered to the vaginal 1/3 apex area, 5 mm deep from the vaginal surface, with a high dose rate brachytherapy device using the Ir-192 source. The doses applied to the vaginal mucosa, rectum, and bladder were calculated according to International Commission on Radiation Units and Measurements. A total dose of 18–24 gray (Gy) was planned with a fraction dose of 6–7 Gy.

2.4 Pathological assessment

Histopathological features, including tumor size, grade, myometrial invasion, lower uterine segment involvement, LVSI, squamous and mucinous differentiation, were obtained from the patients' pathology reports. Hematoxylin-eosin stained tissue slides were evaluated in terms of grade, myometrial invasion, squamous and mucinous differentiation, and immunostained slides were examined for Ki-67 by two expert pathologists (FOA and MO). The slides of all cases were evaluated using a light microscope (model BX51TF, Olympus, Tokyo, Japan). Histological grading was performed using the FIGO grading system. Myometrial invasion depth was categorized into three; without invasion, less than 50%, or more than 50% in the slide with the deepest tumor penetration. The patients were classified into two groups as either absent or present according to squamous and mucinous differentiation in re-evaluations of each sample [22, 23]. Squamous differentiation was expected to account for at least 10% of the tumor.

ER assay clone used was SP1, and the Ki-67 assay clone used was 30-9. Only nuclear staining was considered positive immunostaining for ER and Ki-67 staining, and the staining was scored according to the percentage of nuclear staining (independent of immunostaining intensity). Staining of >1%

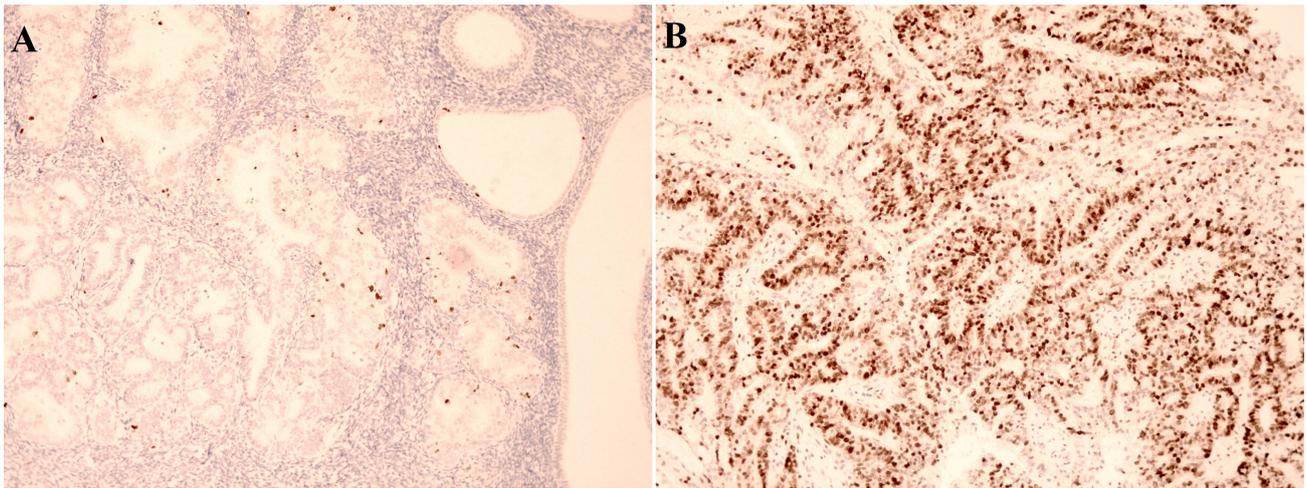


Fig. 2. Ki-67 immunohistochemical staining of low-risk endometrial cancer. (A) Low Ki-67 proliferation index (5%). (B) High Ki-67 proliferation index (70%) (magnification, 20 \times).

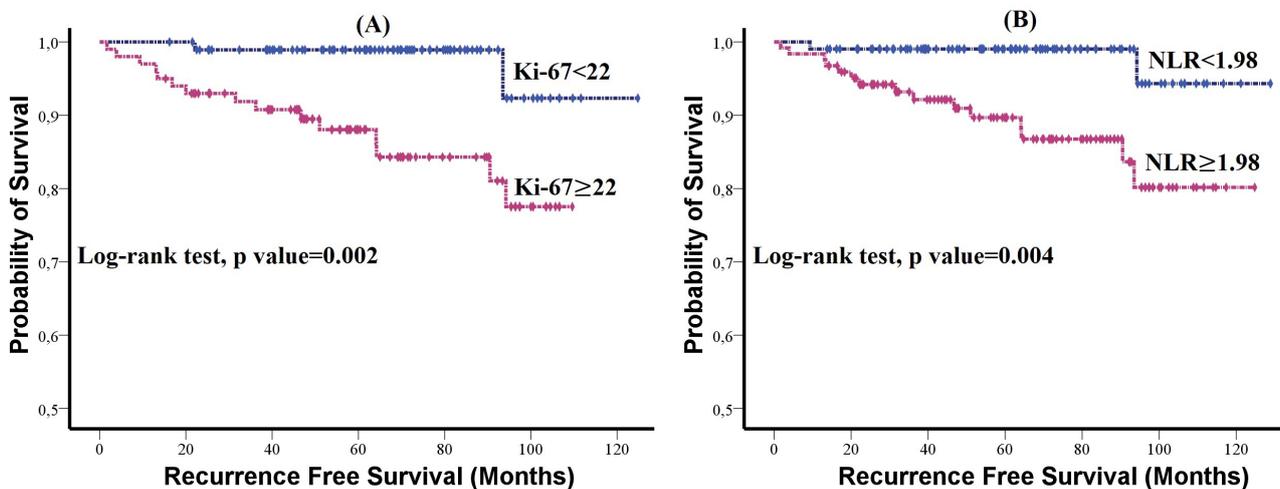


Fig. 3. Kaplan-Meier curves for recurrence-free survival (RFS). (A) RFS according to the Ki-67 index. (B) RFS according to the neutrophil-to-lymphocyte ratio (NLR).

of tumor cell nuclei is considered positive for ER staining. For Ki-67, at least 1000 cells were counted at $\times 400$ magnification from the hot-spot areas in each sample (Fig. 2). Because the literature has not defined an optimal Ki-67 value in EC, the ROC curve was used to determine the cut-off value.

2.5 Outcomes

Recurrence-free survival was calculated from the time of surgery until locoregional or distant recurrence, which was confirmed by histological examination or imaging modalities, and disease-specific survival (DSS) was determined from the time of diagnosis until death. Non-cancer-related death (death without confirmation of recurrence) was censored during RFS and DSS estimation.

2.6 Statistical analysis

Statistical analyses were performed using IBM SPSS version 22 software (IBM, New York, NY, USA). Contin-

uous and categorical variables were expressed as median (minimum-maximum) values and frequency values, respectively. ROC curves were plotted for NLR, PLR, LMR, and Ki-67 index for recurrence, considering the sensitivity and specificity. Kaplan-Meier analysis was employed for survival rates with comparisons made with the log-rank test. The possible factors affecting RFS were examined using Cox regression analysis. Backward stepwise model was used with parameters having a p -value below 0.25. A 5% alpha error level was used to infer statistical significance.

3. Results

The study included 253 patients. The demographic, pre-operative laboratory, and histopathological characteristics of them is presented in Table 1. The median age was 58.5 years (32.0–75.4). More than one-fourth of the patients were obese, and 82% were post-menopausal. In nearly half

Table 1. Clinical, preoperative laboratory, and pathological characteristics of the patients.

Characteristic		N (253)	(%)
Age	Median (range), years	58.5	(32.0–75.4)
Body mass index	<30 kg/m ²	57	(22.5)
	≥30 kg/m ²	196	(77.5)
Diabetes mellitus	Present	116	(45.8)
	Absent	137	(54.2)
Parity	0	34	(13.4)
	≥1	219	(86.6)
Menopausal status	Pre-menopausal	44	(17.4)
	Post-menopausal	209	(82.6)
NLR	Median (range)	2.03	(0.60–17.53)
LMR	Median (range)	4.65	(1.01–31.96)
PLR	Median (range)	112.6	(40.8–563.2)
Surgery	TH with BSO	130	(51.4)
	TH with BSO and lymphadenectomy	123	(48.6)
Tumor size	Median (range), mm	30	(2–80)
Grade	1	151	(59.7)
	2	102	(40.3)
Myometrial invasion	Present (<1/2)	189	(74.7)
	Absent	64	(25.3)
Lower uterine segment involvement	Present	39	(15.4)
	Absent	214	(84.6)
Squamous differentiation	Present	50	(19.8)
	Absent	203	(80.2)
Mucinous differentiation	Present	69	(27.3)
	Absent	184	(72.7)
Ki-67 index	Median (range), %	25	(1–90)
	ER-positive	215	(85.0)
Estrogen receptor status	ER-negative	16	(6.3)
	Missing	22	(8.7)
	Brachytherapy	94	(37.2)
Adjuvant treatment	Observation	159	(62.8)

Abbreviations: TH with BSO, total hysterectomy with bilateral salpingo-oophorectomy; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio.

of the patients, lymphadenectomy was performed in addition to TAH and BSO. The median tumor size was 30 mm (2–80), and the median Ki-67 index was 25% (1–90). The majority of the patients (63%) were observed without adjuvant brachytherapy. The values of the area under the curve (AUC), sensitivity, and specificity in ROC analysis are shown in Table 2. The cut-off values for the Ki-67 index, NLR, PLR, and LMR were determined as ≥22, ≥1.98, ≥115.3, and ≤4.71, respectively.

The median follow-up time was 65.0 months (13.8–128.9). Seventeen (6.7%) patients had recurrences. The median time to recurrence was 31.5 months (1.7–94.2); nearly two-thirds of them had recurrent disease before the 36th month. Thirteen of those had locoregional recurrence, of which vaginal recurrence was most common, and four patients presented with distant metastasis. Twelve-month, 36-month and 60-month RFS rates were 98.8%, 96.3% and

Table 2. Receiver operating characteristic curve analyses for recurrence-free survival.

Curve	AUC	95% CI	p-value	Cut-off value	Sensitivity	Specificity
Ki-67 index	0.746	0.622–0.870	0.001	22%	88.2%	52.5%
NLR	0.655	0.532–0.777	0.034	1.980	88.2%	48.6%
LMR	0.649	0.508–0.790	0.041	4.715	76.5%	50.0%
PLR	0.660	0.520–0.799	0.029	115.3	76.5%	56.7%

Abbreviations: AUC, the area under the curve; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio.

95.8%, respectively. Twelve-month, 36-month, and 60-month DSS rates were 100%, 99.1%, and 98.6%, respectively.

The univariate and multivariate Cox regression analyses for RFS were performed (Table 3). The multivariate analysis revealed that the Ki-67 index and NLR were statistically significant factors for RFS ($p = 0.012$ and $p = 0.029$, respectively). Fig. 3 shows the Kaplan-Meier curves of RFS, according to the Ki-67 index (A) and NLR (B). The log-rank test revealed that the patients with a Ki-67 index lower than 22% and NLR lower than 1.98 had statistically longer RFS ($p = 0.002$ for Ki-67 index and $p = 0.004$ for NLR).

4. Discussion

The present study consisted of a relatively high number of patients EC whose median follow-up time was longer than five years and evaluated potential clinical, laboratory, and histopathological factors affecting RFS in low-risk EC. The Ki-67 index and NLR were found to be independent risk factors of which high values resulted in shorter RFS.

Although the diagnostic, prognostic, and predictive roles of Ki-67 have been studied in many cancers, the Ki-67 index is not recommended to be evaluated routinely by international guides except for breast cancer and neuroendocrine tumors [24–26]. In breast cancer and neuroendocrine tumors, the Ki-67 index is used to classify the disease and decide the optimal treatment and follow-up strategy. Although an increasing number of reports have supported the prognostic effect of the Ki-67 index on EC, its value is not precise due to studies claiming the opposite were published [27, 28]. In 2004, Stefansson *et al.* [29] reported that tumor cell proliferation estimated by Ki-67 rather than mitotic count was an independent prognostic factor for DSS in a multivariate analysis of EC patients. Similarly, Suthipintawong *et al.* [30] detected longer overall survival (OS) in patients with a Ki-67 index of 35% or less. In another study evaluating 473 patients with stage I–III EC by Jia *et al.* [31], a combined ratio obtained from percentages of estrogen, progesterone, Ki-67, and TP53 was reported to be a statistically significant factor for recurrence in multivariate analysis. Recently Jiang *et al.* [32] reported that the Ki-67 index was an independent prognostic factor in stage I and II EC. Because the Ki-67 index was also a significant factor in an univariate analysis in low-risk disease, they claimed that it could be helpful in the de-

Table 3. Univariate and multivariate cox regression analysis of the predictors for recurrence.

Factor		Univariate analysis			Multivariate analysis		
		HR	95% CI	p	HR	95% CI	p
Age	years	1.033	0.973–1.096	0.291			
Body mass index	<30 (R) vs. ≥30 (kg/m ²)	0.881	0.287–2.702	0.824			
Diabetes mellitus	absent (R) vs. present	0.790	0.301–2.075	0.632			
Parity	nulliparous (R) vs. ≥1 parity	0.842	0.241–2.939	0.787			
Menopausal status	pre-men (R) vs. post-men	1.628	0.372–7.120	0.671			
NLR	<1.98 (R) vs. ≥1.98	6.625	1.515–28.976	0.012	5.251	1.189–23.188	0.029
LMR	>4.71 (R) vs. ≤4.71	2.779	0.900–8.578	0.076			
PLR	<115.3 (R) vs. ≥15.3	3.677	1.198–11.281	0.023			
Lymphadenectomy	no (R) vs. yes	1.518	0.581–3.964	0.394			
Tumor size	mm	1.028	0.998–1.059	0.067			
Grade	1 (R) vs. 2	3.216	1.131–9.142	0.028			
Myometrial invasion	absent (R) vs. present	5.416	0.718–40.886	0.101			
Lower uterine segment involvement	absent (R) vs. present	1.259	0.405–3.919	0.691			
Squamous differentiation	absent (R) vs. present	1.387	0.485–3.969	0.542			
Mucinous differentiation	absent (R) vs. present	1.005	0.353–2.865	0.992			
Ki-67 index	<22 (R) vs. ≥22	7.292	1.663–31.971	0.008	6.606	1.503–29.032	0.012
Estrogen receptor status	positive (R) vs. negative	2.080	0.475–9.119	0.331			
Adjuvant treatment	no (R) vs. yes	1.484	0.572–3.854	0.417			

Abbreviations: HR, hazard ratio; CI, confidential interval; R, reference category; pre-men, pre-menopausal; post-men, post-menopausal; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio. Bold numbers imply statistical significance.

cision of performing an adjuvant treatment. Although most patients in the present study had local recurrences, likely due to the inclusion of patients with low-risk disease in which local recurrence is more commonly observed, Di Danota *et al.* [33] published that a high Ki-67 value was correlated with distant recurrence in high-risk EC. In addition to its prognostic value, there are also studies indicating that the high Ki-67 index is associated with the histopathological findings of more aggressive disease (such as non-endometrioid histotype, grade, lymphovascular space invasion, myometrial invasion and lymph node metastasis) [21, 34–38]. Although the studies mentioned above included heterogeneous diseases consisting of patients with different risk groups and were designed with a varying Ki-67 index cut-off, they in general support the findings of our study.

Chronic inflammation is known to be both an etiological factor for cancer and also an indispensable component of it [39, 40]. Neutrophils are among the most critical cells in the tumor microenvironment and play a significant role in cancer-favored inflammation by various mechanisms such as releasing reactive oxygen species and secreting cytokines like transforming growth factor-beta and vascular endothelial growth factor [41]. Unlike neutrophils, lymphocytes exhibit anti-cancer activity via direct cytolytic effects and by producing an immune response by introducing cancer cells to the immune system [42]. Therefore, increased NLR was published to be associated with poor prognosis in EC, as in many cancers [13, 43]. Although some investigators have reported that NLR was not a prognostic factor for survival [44, 45], a meta-analysis of nine studies revealed the prog-

nostic value of NLR on both DFS and OS. All the studies included in the meta-analysis consisted of patients with stage I–IV disease. Holub *et al.* [46] reported the only research in the literature investigating the effect of NLR on survival in patients with non-metastatic disease (stage I–III). They concluded that NLR was an independent factor for OS but not for other cancer-specific survival and progression-free survival.

Although patients with low-risk disease have an excellent prognosis, 3% to 7% of these patients have recurrences [14, 18], usually within three years [19]. However, the clinicopathological and laboratory parameters associated with disease recurrence are uncertain. To the author's knowledge, this present study is the first study to investigate the parameters in patients with low-risk disease and revealed both Ki-67 and NLR as independent factors in recurrence. Supporting the prognostic value of these factors by large-scale prospective studies with a higher number of patients with recurrence may change the adjuvant treatment and follow-up strategies in patients with low-risk EC.

The current study has some limitations. First, it is a retrospective design and consists of patients of Turkish descent only from a single center. Secondly, a genomic analysis could not be performed in the present study. Although the integrated genomic-pathologic classification of EC was recommended and genomic classification was reported to have a prognostic value [47, 48], the mutational analysis may not be available in all centers like the authors' center. In addition, this analysis may not be cost-effective in particularly low-risk EC.

5. Conclusions

The present study highlights the prognostic implications of both the Ki-67 index and NLR in low-risk EC. By studies designed for scores combining both histopathological and hemogram parameters predictors for cancer inflammation, practical and cost-effective prognostic models and new follow-up strategies can be developed. Furthermore, such models can help clinicians select which patients may benefit from adjuvant treatment in low-risk EC.

Author contributions

ABS, EC, and TE conceived and designed the study. ABS, BO, UH, AD, MO, KO, HO, CDA, and MM performed the research. FOA and MO reevaluated the stained slides. ABS, UH, and MM analyzed the data. ABS and EC wrote the paper. TE, BO, FOA, AD, HO, KO, and CDA edited the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The clinical research ethics committee of Bursa Uludag University Faculty of Medicine approved the study (Approval number: 2020-19/19) and waived the need for informed consent because of the retrospective nature. The study was in accordance with the institutional research committee's ethical standards and the 1964 Declaration of Helsinki.

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Conflict of interest

The authors declare no conflict of interest.

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