Prognostic Significance of CD3+ Tumor-infiltrating Lymphocytes in Patients with Endometrial Carcinoma

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Abstract. *Aim: The aim of the present study was to investigate tumor-infiltrating leukocytes in patients with endometrial carcinoma. Patients and Methods: Cluster of differentiation (CD)3$^+$, CD8$^+$ and C20$^+$ tumor-infiltrating lymphocytes (TILs) and CD68$^+$ tumor-associated macrophages (TAMs) were evaluated retrospectively by immunohistochemistry in tumor specimen from 124 patients with endometrial carcinoma. Results: A significant decrease of CD3$^+$ TILs and an increase of CD68$^+$ TAM count was associated with higher tumor stage. In patients with early-stage, high-risk tumors, low intraepithelial CD3$^+$ TIL counts were associated with significantly inferior survival. In multivariate analysis of patients with early-stage tumors, intraepithelial CD3$^+$ TIL counts were an independent predictor of survival. Conclusion: In patients with endometrial carcinoma a decrease of intraepithelial CD3$^+$ TIL counts is associated with advanced stage and high risk group. Intraepithelial CD3$^+$ TIL counts are an independent predictor of survival in patients with early tumors.*

Endometrial carcinoma is the most common gynecological cancer in developed countries and the sixth most common cancer in women worldwide. This neoplasia often produces symptoms such as vaginal bleeding at a relatively early stage. Consequently, the disease is diagnosed and treated in a timely manner, resulting in better prognosis compared to other gynecological malignancies. Multiple prognostic factors have been defined for endometrial carcinoma, including stage of the disease, histological type of the tumor, grade, myometrial invasion, angioinvasion, age at diagnosis and presence of lymph node metastases (1). Endometrial carcinoma comprises a histologically- and biologically-diverse group of neoplasms characterized by different pathogeneses and tumor biology. Estrogen-dependent type (type I) endometrial carcinoma characterized by endometroid histology encountered in younger peri-menopausal women is associated with a relatively good prognosis, while estrogen non-dependent type (type II) endometrial carcinoma, including serous and clear cell carcinoma, predominantly diagnosed in older women, is less related to sustained estrogen stimulation and has, in general, a poor prognosis.

In recent years, considerable progress has been made in understanding the role of the immune system in the progression and prognosis of malignant tumors. The presence of tumor-infiltrating mononuclear cells consisting of T-lymphocytes (helper and suppressor/cytotoxic), natural killer cells, B-lymphocytes and macrophages indicates the existence of active immune response of the host that may be directed against the tumor cells. It has been demonstrated across the spectrum of different primary tumors that the presence of tumor-infiltrating lymphocytes (TIL) correlates with better prognosis (2), and response to therapy (3). Among gynecological malignancies, the prognostic significance of TILs has been extensively studied in epithelial ovarian cancer (2, 4), and intraepithelial CD3$^+$ TIL counts were shown to be an independent prognostic biomarker in patients with epithelial ovarian cancer. On the other hand, the data on the prognostic significance of TILs in endometrial carcinoma are more limited (5-11). The aim of the present study was to analyze TILs and tumor-associated macrophages (TAMs) detected using immunohistochemistry in patients with endometrial carcinoma, including the impact of these parameters on overall survival.

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Patients and Methods

In the present study, tissue blocks of 124 consecutive patients aged (mean±standard deviation) 66±9 years (range=45-85 years), who underwent primary surgery between January 1999 and December 2004 at two departments performing gynecological surgery in Hradec Králové, Czech Republic, were retrospectively evaluated. The surgical specimens from these two hospitals were sent for histological examination exclusively to the Fingerland Institute of Pathology, Charles University Medical School Teaching Hospital in Hradec Králové, and the patients were referred for adjuvant radiotherapy to the Department of Oncology and Radiotherapy of Charles University Medical School Teaching Hospital in Hradec Králové. Abdominal hysterectomy and bilateral salpingo-oophorectomy, and, in selected patients, lymphadenectomy were performed. All patients were staged according to the Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) staging system (1).

Survival was measured from the date of diagnosis to death. Surviving patients were censored at the last follow-up. This study was part of a project approved by the Ethical Committee of the Charles University Medical School Teaching Hospital, Hradec Králové, Czech Republic.

The surgical specimens were fixed in 10% formalin, processed, embedded in paraffin, and stained with hematoxylin-eosin. Hematoxylin-eosin-stained sections from each histological specimen were reviewed by a single pathologist who confirmed the histological diagnosis and the grade of the tumor according World Health Organization and selected one representative paraffin block for immunohistochemical analysis (2, 3).

For immunohistochemistry, 4-μm sections were prepared, and an indirect immunohistochemical method was used. After de-paraffinization, sections were immersed into pre-heated antigen-retrieval solution at pH 9.9 (S3308; DakoCytomation, Glostrup, Denmark), incubated at 95˚C for 40 min, and allowed to cool to room temperature (before the detection of CD3 and CD68). Before the detection of CD8 and CD20, sections were immersed in citrate buffer at pH 6 and treated in a microwave oven (320 W for 10 min). For the detection of $CD3^+$ TILs, primary rabbit polyclonal antibody to human CD3 (DakoCytomation) was used at a dilution of 1:100; for the detection of $CD8^+$ TILs, antibody to human CD8 (CD8/144B; DakoCytomation) was used at a dilution of 1:200; for the detection of $CD20^+$ TILs, antibody to human CD20 (L26; DakoCytomation) was used at a dilution of 1:300; and for the detection of $CD68^+$ TAMs, antibody to human CD68 (KP1; DakoCytomation) was used at a dilution of 1:400. Visualization was achieved using the EnVision+ peroxidase system (DakoCytomation). Control specimens underwent a similar staining procedure (2, 3).

Each tumor section was evaluated for TILs or TAMs by using a ×40 objective lens (0.196 mm^2), and six different areas with the most abundant TILs or TAMs were selected (three cancer epithelium and three stromal areas). The numbers of TILs and TAMs were then counted manually. The mean number of stromal or intraepithelial TIL counts in an area of 1 mm^2 of tumor for each patient was used for statistical analysis. The immunohistochemically stained sections were evaluated by a single pathologist without previous knowledge of the patient's clinical outcome.

Statistical analyses were performed using Number Cruncher Statistical System software (NCSS, Kaysville, UT, USA). The survival was analyzed by the Kaplan–Meier method. The prognostic significance of clinical, histological and immunohistochemical parameters on overall survival of patients was analyzed by log-rank test for univariate analysis and by Cox regression for multivariate analyses, with the results expressed as hazard ratio (HR) with 95% confidence intervals (CI). The values of non-categorical parameters were categorized according the median. The differences between subgroups of patients were analyzed with Mann–Whitney *U*-test. Correlations were investigated using the Spearman's rank correlation coefficient. The *p*-value of 0.05 or less was considered statistically significant.

Table I. *Tumor stage, histology and grade.*

Parameter	n	%
FIGO stage		
I	90	73
IA	21	17
IB	32	26
IC	37	30
II	19	15
IIA	9	7
IIB	10	8
III	13	10
IA	8	6
IB	0	0
IC	5	4
IV	2	2
IVA	1	1
IVB	1	1
Histological type		
Endometrioid	117	94
Mucinous	2	2
Clear cell	3	2
Serous	1	1
Adenosquamous	1	1
Grade		
G1	44	35
G2	63	51
G3	17	14
Low-risk, early-stage	50	40
High-risk, early-stage	49	40

FIGO= Fédération Internationale de Gynécologie et d'Obstétrique.

Results

The pathological characteristics of the patients included in the present study are summarized in Table I. The majority of patients had endometroid-type carcinoma, stage I and grade 2. Lymphadenectomy was performed in 36 patients. Eighty-seven patients underwent radiotherapy (external-beam radiation in 77 patients and vaginal brachytherapy in 75 patients), seven patients had additional adjuvant chemotherapy and four patients had chemotherapy in the palliative setting. Twenty-two patients received subsequent hormonal therapy with gestagens.

Table II. *Tumor-infiltrating leukocyte counts [(mean±SD)n/mm^2] in patients with early- and advanced-stage endometrial carcinomas.*

Parameter	Whole cohort (n)	Early-stage tumors (n)	Advanced-stage tumors (n)	*p*-Value
CD3$^+$ lymphocytes				
Stromal	117±95 (121)	124±101 (96)	92±57 (25)	0.15
Intraepithelial	22±18 (120)	24±19 (95)	16±14 (25)	0.04
CD8$^+$ lymphocytes				
Stromal	47±42 (118)	48±46 (93)	44±27 (25)	0.65
Intraepithelial	15±17 (118)	16±17 (93)	10±12 (25)	0.11
CD20$^+$ lymphocytes				
Stromal	90±101 (118)	93±108 (93)	79±68 (25)	0.94
Intraepithelial	7±37 (118)	7±42 (93)	4±8 (25)	0.55
CD68$^+$ macrophages				
Stromal	49±38 (119)	47±39 (94)	58±31 (25)	0.03
Intraepithelial	20±19 (119)	21±20 (94)	15±11 (25)	0.37
Intraepithelial:stromal leukocyte ratio				
CD3$^+$	0.28±0.41 (120)	0.29±0.46 (95)	0.22±0.17 (25)	0.76
CD8$^+$	0.52±0.94 (118)	0.58±1.04 (93)	0.27±0.26 (25)	0.03
CD20$^+$	0.09±0.33 (111)	0.10±0.36 (86)	0.08±0.19 (25)	0.50
CD68$^+$	0.54±0.58 (119)	0.60±0.63 (94)	0.31±0.21 (25)	0.03
CD8$^+$:CD3$^+$ lymphocyte ratio				
Stromal	0.46±0.36 (117)	0.42±0.33 (92)	0.63±0.43 (25)	0.02
Intraepithelial	0.73±0.64 (119)	0.69±0.53 (91)	0.87±0.93 (25)	0.99

SD: Standard deviation.

The numbers of TILs were, in general, similar in patients with early-stage (IA-IIA) and advanced-stage (IIB and higher) tumors (Table II), and among patients with early-stage disease, patients with high-risk (defined as stage IC, IIA or grade 3 or clear cell or papillary serous histology) or low-risk tumors (Table III). In patients with advanced disease, intraepithelial CD3$^+$ TIL counts (Figure 1), and intraepithelial:stromal CD8$^+$ TIL and CD68$^+$ TAM ratios were significantly lower, and the numbers of stromal CD68$^+$ TAMs and stromal CD3$^+$:CD8$^+$ TIL ratio were significantly higher. In patients with low-risk early tumors, stromal CD3$^+$ TIL and stromal CD20$^+$ TIL counts were significantly lower compared to those with high-risk early tumors.

When stage was expressed on a numerical scale (stage IA=1, stage IB=2, stage IC=3, stage IIA=4, stage IIB=5, stage IIIA=6, stage IIIB=7, stage IIIC=8, stage IVA=9 and stage IVB=10), a significant correlation was observed between stage and grade (r_s=0.443; p<0.00001), stage and stromal CD68$^+$ TAM counts (r_s=0.245; p=0.007), grade and stromal CD20$^+$ TIL counts (r_s=0.208; p=0.024), grade and stromal CD8$^+$ TIL counts (r_s=0.192; p=0.037), grade and stromal CD68$^+$ TAM counts (r_s=0.322; p=0.0004), and grade and epithelial CD68$^+$ TAM counts (rs=0.301; p=0.0009).

Out of the 124 patients examined, 45 (36%) died during the observation period. One patient lost to follow-up was censored. The median survival in the whole cohort was not reached, and 61% of patients were alive after 92 months, with a 5-year survival rate of 72%. The median duration of follow-up of surviving patients was 102 months (range=64-145 months). None of the TIL populations was predictive of survival when the whole cohort was evaluated. In patients with early-stage, high-risk tumors, intraepithelial CD3$^+$ TIL counts below 17 per mm^2 were associated with significantly inferior prognosis (median survival=52.7 months *vs*. not reached, p=0.01; Figure 2). In multivariate analysis of patients with early tumors that included the TIL populations investigated and risk groups (high risk *vs*. low risk), intraepithelial CD3$^+$ TIL counts below 17 per mm^2 were associated with inferior prognosis (HR=4.56, 95% CI=1.77-11.75, p=0.002), while intraepithelial CD8$^+$ TIL counts below 9 per mm^2 were associated with improved prognosis (HR=0.28, 95% CI=0.10-0.76, p=0.01). High-risk classification itself was not a significant predictor of survival in this model. In patients with early-stage, high-risk tumors, CD3$^+$ TIL counts below 17 per mm^2 were associated with inferior prognosis in multivariate analysis (HR=3.79, 95% CI=1.34-10.71; p=0.01).

Discussion

The present study adds to the growing body of evidence indicating that the immune system plays an important role in the progression and prognosis of endometrial carcinoma. A significant trend of increasing CD68$^+$ TAM and decreasing CD3$^+$ TIL counts associated with higher stage or grade was recorded. Compared to patients with early tumors, stromal CD8$^+$ TIL counts were increased relative to intraepithelial CD8$^+$ TIL and stromal CD3$^+$ TIL counts in patients with

Table III. *Tumor-infiltrating leukocyte counts [(mean±SD) n/mm^2] in patients with early-stage tumors according to risk.*

Parameter	Low-risk tumors (mean±SD) (n)	High-risk tumors (n)	*p*-Value
CD3$^+$ lymphocytes			
Stromal	114±102 (48)	135±102 (48)	0.04
Intraepithelial	22±18 (47)	26±19 (48)	0.12
CD8$^+$ lymphocytes			
Stromal	44±44 (49)	52±47 (44)	0.23
Intraepithelial	14 ±15 (49)	19±20 (44)	0.06
CD20$^+$ lymphocytes			
Stromal	70±91 (49)	118±120 (44)	0.004
Intraepithelial	12±57 (49)	2±3 (44)	0.29
CD68$^+$ macrophages			
Stromal	42±35 (50)	53±43 (44)	0.09
Intraepithelial	18±16 (50)	24±24 (44)	0.43
Intraepithelial:stromal leukocyte ratio			
CD3$^+$	0.32±0.61 (47)	0.26±0.23 (48)	0.75
CD8$^+$	0.48±0.70 (49)	0.69±1.33 (44)	0.28
CD20$^+$	0.16±0.49 (44)	0.02±0.02 (42)	0.43
CD68$^+$	0.62±0.67 (50)	0.58±0.59 (44)	0.63
CD8$^+$:CD3$^+$ lymphocyte ratio			
Stromal	0.44±0.39 (48)	0.39±0.24 (44)	0.80
Intraepithelial	0.61±0.46 (47)	0.77±0.59 (44)	0.29

SD: Standard deviation.

advanced disease. Moreover, a significant difference was observed in patients with high-risk and low-risk early-stage tumors in stromal CD3$^+$ TIL and CD20$^+$ TIL counts. Most importantly, although none of the TIL populations studied was associated with prognosis when the whole cohort was examined, among patients with early-stage, high-risk tumors, intraepithelial CD3$^+$ TIL counts below median were predictive of poor prognosis. The Kaplan–Meier overall survival curves started to diverge at three years after diagnosis, and later, eight to nine years after diagnosis, both curves seemed to reach a plateau, with the difference in long-term survival rates of patients with high *versus* low intraepithelial CD3$^+$ TIL counts approaching almost 40%. In multivariate analysis of patients with early tumors, high intraepithelial CD3$^+$ TIL counts and low intraepithelial CD8$^+$ TIL counts were significant prognostic biomarkers that surpassed the prognostic significance of the risk classification itself, which was not a significant predictor of outcome in multivariate analysis. Interestingly, high intraepithelial CD3$^+$ and CD8$^+$ TIL counts had opposing effects on prognosis. In the sub-group of patients with high-risk early tumors, CD3$^+$ TIL counts were also an independent prognostic factor.

Endometrial cancer usually manifests early by vaginal bleeding, resulting in timely diagnosis and curative surgical therapy. In contrast to other gynecological malignancies, *e.g.* epithelial ovarian carcinoma, most patients with endometrial carcinoma are cured by surgery-alone or in combination with external-beam radiation with or without vaginal brachytherapy. Consequently, compared to epithelial ovarian carcinoma, patients with advanced/metastatic disease are rare such that metastatic endometrial carcinoma represents an almost 'orphan' disease, and the information on the systemic treatments and utilization of biomarkers in endometrial cancer is limited (12). There is an unmet medical need for new treatments and biomarkers that would allow the prediction of prognosis and response to therapy. Currently, tumor stage, depth of myometrial invasion, and tumor grade are commonly used to predict clinical outcome and to plan treatment modalities in patients with endometrial carcinoma (13). Precise histological assessment of myometrial invasion and cervical involvement is crucial in the staging of early disease. Patients with more than 50% myometrial invasion are at increased risk for extrauterine metastasis and may require more aggressive surgical staging. Vascular and lymphatic vessel invasion are encountered in approximately one-fifth of cases of endometrial carcinoma and correlate significantly with the extension of the primary tumor, depth of myometrial invasion and histological grade. The presence of vascular invasion has been shown to be a powerful independent indicator for increased risk of recurrence and diminished survival in patients with clinical stage I endometrial carcinoma. The grading system relies firstly on the architectural pattern of the glands and secondarily on the cytological and nuclear appearances, but grading itself, as well as the subtyping of high-grade endometrial carcinoma, was found to be frequently subjective and poorly-reproducible. Among parameters that can be assessed using immunohistochemistry, estrogen receptors and progesterone receptors expression play a major role. Approximately 60-70% of endometrial carcinomas express both receptors, which seem to characterize clinically less aggressive tumors, with a better chance of response to endocrine therapy. Data have also been reported on the significance of other molecular alterations, such as *p53* mutation which is more frequently detected in serous endometrial tumors, microsatellite instability, mutation of phosphatase and tensin homologue on chromosome ten, β-catenin, or Kirsten retrovirus-associated DNA sequences genes that characterize the pathogenesis of endometrial tumors (13). Potential prognostic significance has also been attributed to markers of proliferation of tumor cells, such as Ki-67.

Considerable progress has been made in the past few years in understanding the role of the immune system and host–tumor interactions in tumor progression (14). The presence of TILs within the tumor microenvironment is considered to represent a biomarker of the host immune response against the tumor. T-Cells and macrophages

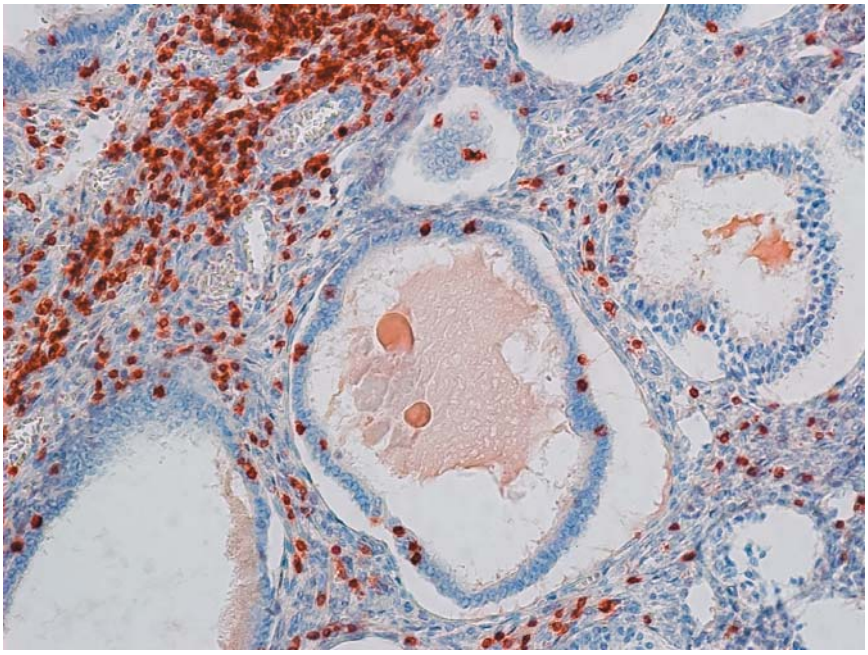

Figure 1. *Intraepithelial and stromal tumor-infiltrating lymphocytes. Immunohistochemically-stained cluster of differentiation (CD3)$^+$ lymphocytes in endometrial carcinoma are shown. Both stromal and intraepithelial CD3$^+$ lymphocytes are evident (original magnification, ×200).*

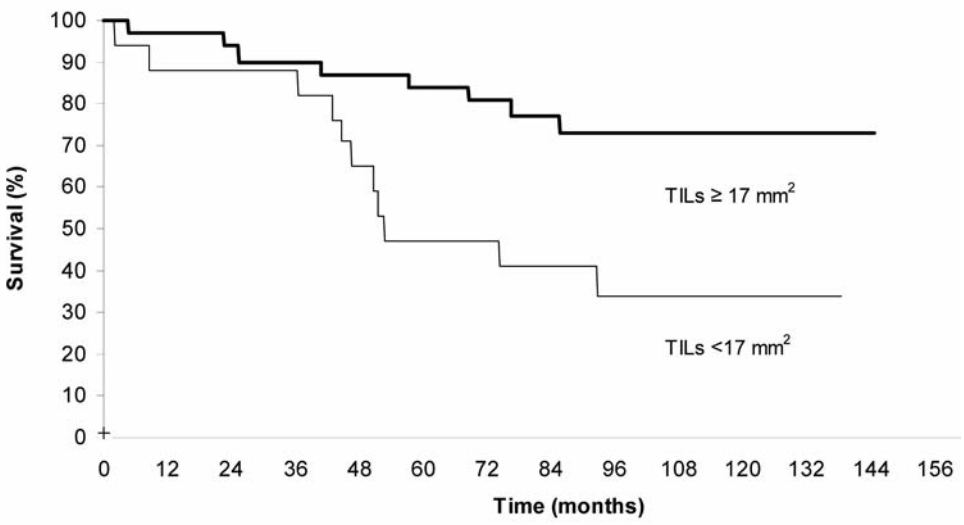

Figure 2. *Survival of patients with early-stage, high-risk endometrial carcinoma according the intraepithelial cluster of differentiation (CD3)$^+$ tumor-infiltrating lymphocyte (TIL) counts. The Kaplan–Meier survival curves of patients with intraepithelial CD3$^+$ TIL counts <17/mm^2 and ≥17/mm^2 in patients with early-stage, high-risk tumors are shown (median survival of 52.7 months versus not reached, log-rank test, p=0.01).*

constitute major components of tumor-infiltrating leukocytes in epithelial malignancies. Depending on the function and phenotype, T-cells may either act as effectors in tumor cell destruction, or induce immune tolerance. Macrophages may also play a more complex role in tumor progression and metastasis. Depending on the context of the tumor microenvironment, macrophages may represent potent effectors of the host antitumor response and a crucial component of the stroma contributing to tumor cell proliferation and angiogenesis (15-17). In different reports

examining patients with endometrial carcinoma, the presence of TILs was associated with microsatellite instability (11), expression of B7-H7, a membrane protein that negatively regulates T-cell response (10), and the expression of indoleamine-2,3-dioxygenase, the enzyme responsible for tryptophan depletion (6). The reported results on the prognostic significance of the presence of TILs in patients with endometrial carcinoma are inconsistent, or even conflicting because of differences in size of the cohorts examined, number of events or differences in methodology, including differences in lymphocyte populations analyzed or methods for the detection of lymphocytic infiltration. In an early report, the presence of higher numbers of intraepithelial $CD8^+$ TILs at the invasive border was an independent predictor of improved overall survival (7). The association of higher $CD8^+$ TIL counts with prolonged time to recurrence and improved overall survival was also reported in a large cohort of 368 patients with endometrial carcinoma (8). A positive association of overall survival with TILs that exhibit $CD45RO^+$ (memory) T-cell phenotype has also been noted (8). In some studies, the prognostic significance of forkhead box P3 $(FOXP3)^+CD4^+$ TILs was examined (5, 8, 9), and an association of high $FOXP3^+CD4^+$ TIL counts with disease-free survival (5) and overall survival (8) has been reported in patients with endometrial carcinoma. The lack of prognostic significance of $FOXP3^+CD4^+$ TILs reported in another study may be attributed to an insufficient number of events (9). The presence of $FOXP3^+CD4^+$ TILs was also demonstrated to correlate with clinical and pathological characteristics associated with high risk, including vascular density, stage, differentiation, and lymphovascular space invasion (5, 9). In a study that demonstrated the association between indoleamine-2,3-dioxygenase expression and tumor lymphocyte infiltration, high stromal $CD3^+$ TIL counts were reported to independently predict better relapse-free survival (6). Less is known about the significance of TAMs in patients with endometrial carcinoma. In one study, $CD68^+$ TAM counts at the invasive margin were associated with tumor stage, grade, myometrial invasion, lymph node metastasis and vascular space invasion (18). Intratumoral $CD68^+$ TAM counts were positively associated with Ki-67 expression and microvascular density. High $CD68^+$ TAM counts at the invasive margin were associated with poor prognosis in univariate, but not multivariate analyses (18).

Adjuvant radiation prevents only locoregional relapse, and the benefit of adjuvant chemotherapy in endometrial carcinoma is controversial. In fact in most prospective trials, the benefit of adjuvant chemotherapy was not evident. The lack of demonstrable benefit of adjuvant systemic treatment could be due to the fact that subsequent distant metastases occur infrequently in patients with early-stage disease. A prognostic biomarker would first allow identification of a high-risk patient population to be enrolled into prospective studies of adjuvant systemic therapy and, subsequently, serve as a biomarker for treatment decisions. Future systemic therapies may include not only cytotoxic chemotherapy regimens, but also targeted-therapy. Conceptually, targeted treatments aim at one or more hallmarks of cancer. Hallmarks of cancer are defined as pathological features that enable tumor growth and escape from control, and one of the eight hallmarks currently recognized is the escape of tumor from destruction by the immune system (14). $CD3^+$ TIL counts could serve as a biomarker for targeted therapies that aim at manipulating the immune system, *e.g.* ipilimumab or nivolumab.

In the present study, no prognostic significance of intraepithelial $CD3^+$ TIL counts was detected when the whole cohort of patients was examined; an association with prognosis was found only in patients with early-stage tumors. However, patients with advanced endometrial cancer represent a subgroup of patients that is different from a point of view of prognosis and biology. The outcome of patients with advanced endometrial carcinoma is generally poor, and other factors, including therapy, age or comorbidity, may modify the effect of the immune response on patient prognosis. Moreover, patients with advanced disease constituted only 20% of patients in the present study. Despite the fact that the prognostic significance of $CD3^+$ TIL counts was not evident in the whole cohort, $CD3^+$ TIL counts were independent predictors of prognosis in patients with early-stage tumors, *i.e.* patients for whom the decision on the choice of therapy is the most urgent and controversial. Of note, patients with early-stage tumors in the present cohort were almost equally split between the low- and high-risk groups. In fact, the identification of patients with high risk is most crucial in patients with early disease, some of whom would have excellent prognosis without any adjuvant treatment, while others will eventually experience relapse.

As in other reports (2, 3), the presence of $CD3^+$ TILs in the present study was more relevant in the tumor epithelium compared to the tumor stroma. In general, lymphocytic infiltration is more abundant in the stroma compared to the tumor epithelium, but intraepithelial lymphocytes are more likely to be associated with the immune response to the tumor than lymphocytes encountered in the tumor stroma. The finding of prognostic significance for the intraepithelial $CD3^+$ TIL count is in contrast with a report demonstrating prognostic significance of stromal $CD3^+$ TIL counts that, however, analyzed progression-free survival based on a smaller number of events (6). Interestingly, high $CD8^+$ TIL counts were associated with adverse prognosis in the present cohort, in contrast with studies reporting positive prognostic significance of high $CD8^+$ TIL counts in patients with endometrial carcinoma (7, 8).

The current study has obvious limitations that are inherent to its retrospective nature. Because of the retrospective nature of the study, overall survival rather than relapse-free survival

or progression-free survival was analyzed in the present cohort. For obvious reasons, overall survival represents the most reliable end-point in a study that attempts to identify prognostic biomarkers. From the current perspective, surgical management of the patients in the present cohort may appear suboptimal. On the other hand, the strong association of intraepithelial $CD3^+$ TIL counts with overall survival observed in the present study indicates the activity of the immune system determines the natural history of this cancer.

In conclusion, the present data demonstrate that in patients with endometrial carcinoma, a decrease of intraepithelial $CD3^+$ TIL count is associated with advancing stage and risk group. The intraepithelial $CD3^+$ TIL count is also an independent predictor of survival. Future studies should address the role of the intraepithelial $CD3^+$ TIL count as a prognostic and predictive biomarker in adjuvant therapy of endometrial carcinoma.

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