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Zone Formation of Lymphocyte Infiltration at the Invasive Front Results in the Prolonged Survival of Individuals With Endometrial Serous Carcinoma and Endometrioid Carcinoma With Serous Component

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Abstract. *Background/Aim: This study aimed to evaluate the clinical significance of lymphocyte infiltration (LI) for patients with endometrial serous carcinoma and those with endometrioid carcinoma including serous component. Patients and Methods: Patients who underwent surgery at our hospital between 1990 and 2013 were identified. LI was classified into strong LI, defined as a continuous thick zone of LI, and weak LI, defined as the lack of zone or scattered small foci of LI at the invasive front. Results: Out of a total of 51 patients, 38 cases had weak LI and 13 had strong LI. The progression-free survival of patients with weak LI was worse (p=0.02). No significant difference of overall survival according to the status of LI was noted (p=0.054). Multivariate analysis revealed that LI was a prognostic factor of poorer progression-free survival (hazard ratio(HR)=5.05, p<0.01) and overall survival (HR=6.93, p=0.01). Conclusion: LI might be a new biomarker of such conditions.*

In developed countries, endometrial cancer is one of the most common gynecological malignancies (1). Its histological subtypes are in predicting the prognosis of patients with endometrial carcinoma in addition to the International Federation of Obstetrics and Gynecology (FIGO) stage (2). Histological subtypes are classified into two groups: estrogen-dependent moderately or highly differentiated type I carcinoma, and estrogen-independent poorly differentiated type II carcinoma (3). The prognosis of patients with type II tumors is worse than that of those with type II (4). Among the type-II tumors, uterine serous carcinoma (USC) is included, and its prognosis is naturally worse than type I (4). Patients in which USC comprised at least 5% of the total volume in endometrioid carcinoma cases had the same prognosis as those with pure-type USC (5, 6). In addition, less than 5% of USC in the total volume of endometrioid carcinoma was a risk factor of recurrence (7). Therefore, the presence of even small foci of serous carcinoma in endometrioid carcinoma results in a worse prognosis.

Recently, tumor-infiltrating lymphocytes (TIL) have been considered an important factor in predicting the prognosis of endometrial carcinoma (8-11). These reports evaluated TILs by counting the number of TILs in the stroma of tumor in tissue microarray. However, TILs in endometrial carcinomas were found to disproportionally emerge in some parts, such as the superficial tumor epithelium, invasive border, stroma of the tumor, and perivascular areas (9). Therefore, in our previous reports, TILs were evaluated using the formation of a lymphocyte zone at the invasive front regardless of the kinds of lymphocyte. Based on the results, TILs were an important factor predictive of the prognosis of endometrioid carcinoma, particularly grade 3 endometrioid carcinoma (12).

In our previous reports, the study participants were limited to those with endometrioid carcinoma. We assumed that TILs at the invasive front are associated with the prognosis of patients with serous carcinoma and endometrioid carcinoma, including even small foci of serous carcinoma, because grade 3 endometrioid carcinoma and serous carcinoma are classified as type II tumors.

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Key Words: Endometrial carcinoma, lymphocyte, serous carcinoma, prognosis.

Herein, we evaluated the clinical significance of the formation of a lymphocyte zone in endometrioid carcinoma, including even small foci of serous carcinoma and serous carcinoma.

Materials and Methods

Patients diagnosed with endometrial carcinoma who underwent primary surgery, including total hysterectomy and bilateral salpingo-oophorectomy, at our hospital between 1990 and 2013 were identified. Two observers (MM and HT) conducted a central pathological review of all cases according to the 2014 World Health Organization (WHO) criteria (13). Cases with serous carcinoma and mixed carcinoma consisting of endometrioid carcinoma and serous carcinoma of any grade using the 2014 WHO criteria were included in our study. In addition, cases of endometrioid carcinoma with serous carcinoma, which comprised less than 5% of the total incidence of serous carcinoma defined by a previous report (7), were included our study. The exclusion criteria were as follows: Other histology (clear-cell carcinoma and carcinosarcoma), cases complicated with other carcinomas (ovarian carcinoma), and lack of cancer in the surgical specimen.

Lymphocyte infiltration (LI) along the tumor–myometriaI junction at the invasive front was categorized into two patterns, as in our previous report (12). In brief, strong LI was defined as a continuous thick zone of LI without tear-shaped LI into the myometrium or only parts of scattered tear-shaped LI into the myometrium. Weak LI was defined as the lack of zone of LI or scattered small foci of LI at the invasive front. During the review of pathological specimens, any discrepancies between the two observers were resolved *via* a discussion.

Serous carcinoma according to the 2014 WHO criteria was defined as pure type. Mixed endometrial carcinomas that included endometrioid carcinoma and serous carcinoma of any grade according to the 2014 WHO criteria and endometrioid carcinomas that included less than 5% of serous carcinoma were defined as non-pure type.

JMP Pro 14 software (SAS Institute Inc., Cary, NS, USA) was used for statistical analysis. Moreover, chi-square test, Fisher exact test, and Mann–Whitney *U*-test for unpaired data were used for statistical analysis. Progression-free survival (PFS) and overall survival (OS) curves were obtained using the Kaplan–Meier method. Comparisons of the survival distribution were conducted with log-rank test. Multivariate analysis of PFS and OS was performed with Cox proportional hazards model. A *p*-value of less than 0.05 was considered statistically significant.

This study was approved by the Ethics Committee of the National Defense Medical College, Tokorozawa, Japan (No. 2614).

Results

During the observational period, 51 patients were included in our study. Among them, 38 had weak LI and 13 had strong LI. The evaluation results of three (5.8%) cases were different between observers, and these discrepancies were resolved *via* a discussion. Representative images of strong and weak LI are presented in Figure 1A and B, respectively. The characteristics of all patients is presented in Table I. A higher number of patients with weak LI received adjuvant therapy compared with those with strong LI (p=0.02). No statistically significant differences were observed in terms of the other factors between the two groups.

Table I. *The characteristics of all patients according to status of lymphocyte infiltration.*

Variable	Weak lymphocyte infiltration, n (%) n=38	Strong lymphocyte infiltration, n (%) n=13	*p*-Value
Age			
≥65 Years	21 (55%)	5 (39%)	0.35
<65 Years	17 (45%)	8 (61%)	
Serous type			
Pure	23 (61%)	5 (39%)	0.21
Non-pure	15 (39%)	8 (61%)	
FIGO stage			
I	18 (47%)	9 (69%)	0.50
II	2 (6%)	0	
III	13 (34%)	4 (31%)	
IV	5 (13%)	0	
Adjuvant therapy			
Yes	26 (68%)	4 (31%)	0.02
No	12 (32%)	9 (69%)	

FIGO: International Federation of Gynecology and Obstetrics.

The PFS of patients with strong LI was better than that of patients with weak LI (p=0.02), as depicted in Figure 2A. However, no statistically significant difference was observed in terms of OS between groups (Figure 2B, p=0.054). Multivariate analysis revealed that weak LI was a prognostic factor of poorer PFS (hazard ratio=5.05, p<0.01) and OS (hazard ratio=6.93, p=0.01) (Table II).

Discussion

Our present study showed that a strong LI at the invasive front was observed in 25% of cases, and it was considered a biomarker of better prognosis of PFS and OS of patients with pure-type and non-pure type. The rate of discrepancy between observers for TIL evaluation was 5.8%.

Endometrial carcinoma with mutations in the exonuclease domain of polymerase ε (*POLE*) or with microsatellite instability (MSI) induced high neoantigen loads and was positively associated with the number of TILs (14). According to the Cancer Genome Atlas, *POLE* mutation and MSI were frequently identified in endometrioid carcinoma and rarely in serous carcinoma (15). Meng *et al*. reported that *POLE* mutations were discovered in eight out of 53 (15%) individuals with grade 3 endometrioid carcinoma and none of 25 (0%) individuals with serous carcinoma (16). In addition, Lax *et al*. showed MSI in 16 out of 57 (28%) cases of endometrioid carcinoma but in none out of 34 cases of serous carcinoma (17). In endometrioid carcinoma of any grade, Zighelboim *et*

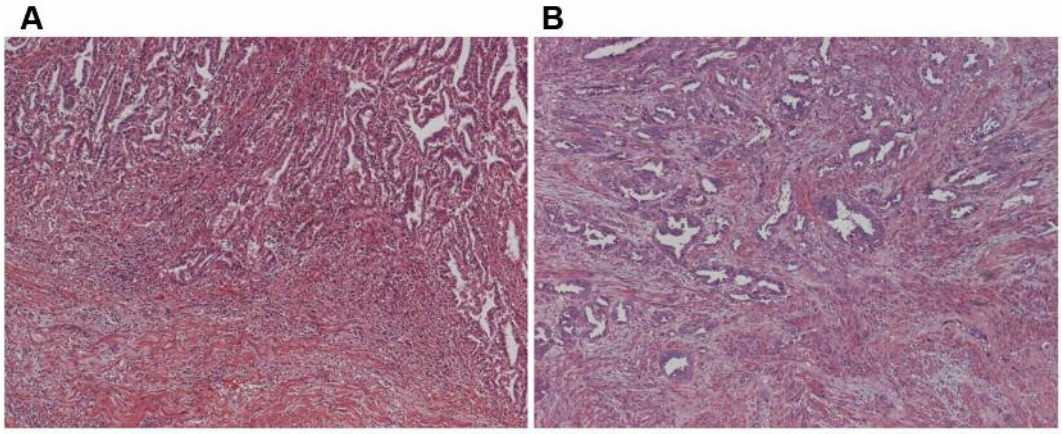

Figure 1. *Representative images of zone formation of lymphocyte infiltration at the invasive front (LI) showing strong (A) and weak (B) LI, respectively. Original magnication, ×40.*

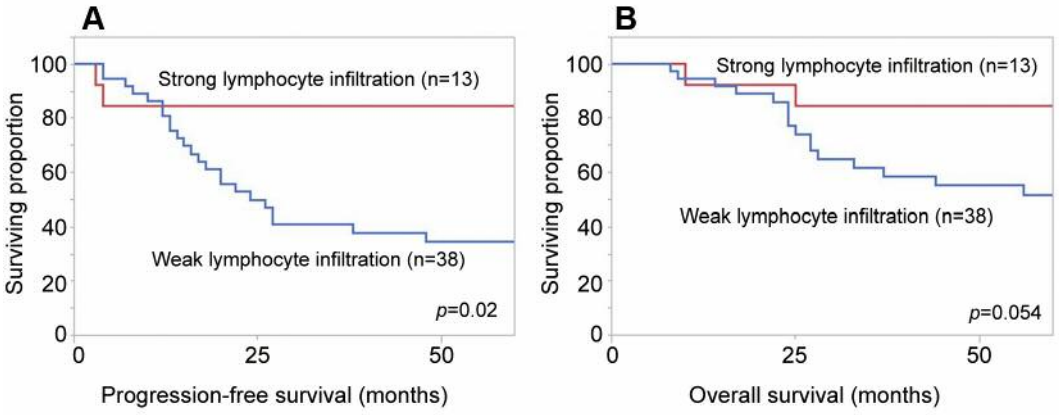

Figure 2. *Progression-free (A) and overall (B) survival of 51 patients with serous carcinoma and endometrioid carcinoma with serous component according to tumor lymphocyte infiltration.*

Table II. *Multivariate analysis for progression-free and overall survival considering the whole cohort of patients.*

		Progression-free survival		Overall survival	
Variable	Comparator *vs*. referent	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age	≥65 *vs*. <65 Years	0.51 (0.22-1.20)	0.12	0.49 (0.17-1.33)	0.16
FIGO	II/III/IV *vs*. I	7.86 (2.77-25.1)	<0.01	22.6 (4.80-139.9)	<0.01
Serous type	Pure *vs*. non-pure	1.43 (0.57-3.81)	0.45	1.55 (0.54-4.94)	0.42
Adjuvant therapy	Yes *vs*. no	0.23 (0.08-0.66)	<0.01	0.12 (0.03-0.52)	<0.01
Lymphocyte infiltration	Weak *vs*. strong	5.05 (1.49-23.4)	<0.01	6.93 (1.53-51.2)	0.01

FIGO: International Federation of Gynecology and Obstetrics; HR: hazard ratio; CI: confidence interval.

al. reported that MSI was identified in 15 out of 68 (22%) cases of grade 3 endometrioid carcinoma (18). Therefore, due to these genetic backgrounds, TILs were more frequently found in grade 3 endometrioid carcinoma than in serous carcinoma because a higher number of cases with grade 3 endometrioid harbored *POLE* mutations or MSI. In our previous report, strong LI was observed in 33/51 (65%) of individuals with grade 3 endometrioid carcinoma (12). In the present study,

strong LI was observed in 5/28 (18%) of cases of pure type serous carcinoma. Clearly, the frequency of strong LI in pure-type serous carcinoma was statistically lower ($p<0.01$). Our results might be associated with these genetic backgrounds. In addition, regardless of the frequency of POLE mutations and MSI in serous carcinoma, only few cases with strong LI in pure-type serous carcinoma were observed. This result might suggest other mechanisms are associated with TILs.

A recent study showed that some cases with mixed carcinoma consisting of endometrioid and serous component shared several gene mutations in two distinct components, although this was not observed in other cases (19, 20). Coenegrachts *et al*. reported that MSI in both components was discovered in 3/23 (13%) cases (19). Moreover, Köbel *et al*. showed that *POLE* mutation in both components was observed in 1/11 (9%) of cases. Therefore, these genetic changes might be associated with our result showing that strong LI was discovered in 8/23 (35%) of individuals with non-pure type. However, because *POLE* mutation and MSI were observed in few cases of mixed carcinoma consisting of endometrioid and serous component, other functions associated with TILs that are similar to pure-type serous carcinoma may be possible.

Among several types of TIL, cluster of differentiation $(CD)8^+$ cytotoxic T-lymphocytes, forkhead box P3 $(FOXP3)^+$ regulatory T-lymphocytes, $CD45R0^+$ memory T-lymphocytes, and CD3 lymphocytes in tumor were associated with the prognosis of endometrial carcinoma (8-11). Among the T-lymphocytes, $FOXP3^+$ regulatory T-lymphocytes suppress immune reaction, and the distribution of TILs is an important factor of tumor-specific immune reaction (10). In addition, the expression of programmed death-1 (PD1) and its ligand PD-L1 was associated with TILs (13). Furthermore, coexpression of cytotoxic T-lymphocyte antigen-4, lymphocyte activation gene-3, and T-cell immunoglobulin mucin protein-3 by TILs was associated with immune reaction (21). Therefore, further studies must be conducted to explore the association between the distribution of TILs, immune checkpoints, and genetic background.

In our study, 13/51 (25%) cases presented strong LI, but there were only few of these cases. However, LI was an important predictive factor of PFS and OS. LI was an important prognostic factor in cases of pure- and non-pure endometrioid carcinoma. In addition, using our evaluation method, low discrepancies were observed. Therefore, LI was easy to utilize and useful in predicting the prognosis of endometrioid carcinoma.

A limitation of our study included the use of a small sample size. Moreover, it was conducted at a single institution. Our study did not examine for important genetic changes, distribution of lymphocytes, not expression of immune checkpoint proteins. However, this evaluation method was easy to utilize and useful in predicting prognosis with a low discrepancy between observers.

In conclusion, LI at the invasive front was associated with the prognosis of patients with pure- and non-pure endometrioid carcinoma.

Conflicts of Interest

The Authors declare that they have no conflict of interest in regard to this study.

Authors' Contributions

Protocol/project development: Morikazu Miyamoto, Masashi Takano, Hitoshi Tsuda; Data collection or management: Hiroki Ishibashi, Hiroaki Soyama, Hiroko Matsuura, Takahiro Sakamoto, Tadashi Aoyama, Hideki Iwahashi; Data analysis: Morikazu Miyamoto, Hitoshi Tsuda; Manuscript writing/editing: Morikazu Miyamoto, Masashi Takano, Kenichi Furuya.

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