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L1CAM as a Negative Prognostic Factor in Endometrioid Endometrial Adenocarcinoma FIGO Stage IA-IB

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Abstract. *Aims: In this study, we aimed to investigate how positivity for L1 cell adhesion molecule (L1CAM) was associated with outcome and relapse pattern in patients with Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) stage IA-IB endometrial cancer. Materials and Methods: This retrospective study included 358 patients who underwent surgical treatment for endometrial carcinoma. Tumor samples from 312 patients (87.2%) were available for L1CAM analysis by immunohistochemistry. Results: Of the 312 tumor samples analyzed, 93 (29.8%) were L1CAM-positive. L1CAM positivity was significantly more common in grade 3 compared to grade 1-2 carcinomas (p=0.02). Patients with L1CAM positivity more commonly experienced disease progression. Distant metastasis was significantly associated with L1CAM positivity (p=0.01). Progression-free interval and overall survival did not significantly differ between L1CAM-positive and L1CAM-negative cases. Conclusion: L1CAM is a promising independent prognostic marker associated with aggressive tumor behavior and recurrence risk, but not with overall survival.*

Endometrial cancer (EC) is the sixth most common malignancy in women worldwide (1). Based on clinical and histological features, ECs are divided into type I and type II tumors. Type I tumors constitute 85% of ECs, are most commonly found in younger women, and develop from a precursor lesion of atypical hyperplasia. These tumors tend to be endometrioid ECs (EECs), are usually well-differentiated, and typically exhibit minimal myometrial invasion; therefore, type I ECs often have a favorable outcome. Type II tumors account for a small percentage of ECs, are commonly found in older patients, and frequently develop in the setting of an atrophic endometrium. The type II category includes serous tumors, clear cell tumors and, possibly, grade 3 EECs. Notably, translational science data support the differentiation of these two groups at the molecular level. Molecular analysis further identifies four categories of EECs: polymerase epsilon ultramutated, microsatellite instability hypermutated, copy-number low, and copy-number high (2). Current guidelines stratify patients with EC based on tumor type, stage, depth of myometrial invasion, and presence of lymphovascular space invasion, delineating four risk groups: Low, intermediate, high-intermediate and high (3).

Although EEC type I tumors generally have a good prognosis, relapse occurs in some patients with low and intermediate risk. One current challenge is to understand the tumor biology as it relates to predicting recurrence and survival. Several recent studies show that positivity for L1 cell adhesion molecule (L1CAM) is strongly associated with more aggressive EEC behavior (4), risk of recurrence, and poor survival (5-10). L1CAM belongs to the immunoglobulin superfamily of cell adhesion molecules. Initially identified in the nervous system, L1CAM plays key roles in nervous system development, neuronal migration, neurite outgrowth on Schwann cells, and neurite fasciculation and myelination (11). In cancer cells, L1CAM promotes cell proliferation, migration, invasion, and metastasis. Its expression is associated with tumor progression in many types of cancers, including colorectal, gastric, renal, breast cancer and melanoma (12-18).

In the present study, we aimed to identify the clinicopathological features of L1CAM-positive Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) stage IA-IB EC in patients, and to confirm the prognostic value of L1CAM.

Materials and Methods

Study cohort. This study enrolled patients who underwent primary surgical treatment for endometrial carcinoma at the University Hospital Ostrava between 2007-2015. Cases with histologically confirmed endometrioid adenocarcinoma, and with available complete surgical and follow-up data, were eligible for study

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inclusion. Patients with other types of adenocarcinoma (clear cell, serous, *etc*.) or with incomplete data were excluded from the study. A totaI of 358 eligible patients were identified but it was not possible to analyze histopathological samples from 46 of these cases; thus, the final number of patients included was 312. Clinical data were collected from the secure hospital database and from the patients' charts. Data were recorded during the initial hospital stay and follow-up visits. The follow-up period was at least 2 years. Written informed consent was obtained from all patients, and this study was approved by the Local Ethic Committee (approval number: 353/2015).

Disease stage was determined using the FIGO 2009 classification all cases recorded before 2009 were reclassified according to this norm (19). We did not perform a specialized gynecopathological case review to exclude potential mixed-type carcinomas containing components other than endometrioid type. Risk for recurrence was classified following published guidelines (3). Surgery and adjuvant treatment were performed according to the preoperative and perioperative (frozen section) staging procedure. In general, surgery comprised extrafascial hysterectomy and bilateral salpingo-oophorectomy alone or with pelvic/para-aortic lymphadenectomy. Surgery was adapted to risk and performance status.

Immunohistochemistry. The immunohistochemical procedure used was essentially that originally described by Bosse *et al*. (5). Formalin-fixed paraffin-embedded tissue blocks were cut into 4-μm slices and placed on superfrost microscope slides. Immunohistochemistry was then performed using a Ventana Discovery automated immunostaining system (Ventana Medical Systems, Tucson, AZ, USA). Briefly, after deparaffinization with an inorganic buffer and rehydration, antigen retrieval was achieved by incubating the slides in Cell Conditioning Solution 1 (CC1; Ventana Medical Systems). The primary L1 antibody (CD171) (BioLegend, Dedham, MA, USA) was then applied at a 1:40 dilution, followed by a 32-min incubation. Next, diaminobenzidne- and horseradish peroxidase-containing detection kit was used (iView Universal DAB detection Kit; Ventana Medical Systems), and performed counterstaining with hematoxylin and bluing reagents.

Immunohistochemical evaluation of L1CAM and scoring were performed by two experienced pathologists dedicated for this study. Only unequivocal membranous staining was considered specific. A case was classified as L1CAM-positive if over 10% of tumor cells were scored positive (10).

Statistical analyses. Statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria; https:// www.R-project.org/). Between-group differences were analyzed using the chi-squared and Fisher's exact probability tests. A *p*-value of less than 0.05 was considered to indicate a statistically significant difference.

Results

Of the 312 analyzed FIGO stage I endometrioid endometrial carcinomas, 93 (29.8%) were L1CAM-positive. Patients with L1CAM positivity were more likely to experience disease progression. However, progression-free interval (PFI) and overall survival did not significantly differ between L1CAM-positive and L1CAM-negative cases. With regards to type of disease progression, L1CAM positivity was significantly associated with distant metastasis (p=0.01). L1CAM positivity was significantly less common in grade 1-2 carcinomas compared to grade 3 carcinomas (p=0.02). Only a small number of patients (n=13) had grade 3 tumors, and these cases did not show disease progression.

The median age at diagnosis for the entire study population was 63.4 years (range=27-89 years). L1CAM status was not related to patient age at diagnosis. Diabetes mellitus and obesity, which are classical epidemiological risk factors for type I EC, were not associated with L1CAM status or disease progression. The mean body mass index was 32.4 kg/m^2 in the L1CAM-negative group, and 31.6 kg/m^2 in the L1CAM-positive group (p=0.17). A total of 28 patients died during follow-up, including 16 deaths due to disease progression (Table I).

Discussion

The present study was designed to evaluate the association of L1CAM positivity with outcome in patients with FIGO IA-B stage EC, and to compare relapse patterns between L1CAM-positive and L1CAM-negative carcinomas.

Recent studies show that L1CAM positivity in EC is strongly associated with more aggressive tumor behavior, greater recurrence risk, and worse overall survival (5-10). L1CAM expression rates are reportedly higher in non-endometrioid cancer than in endometrioid cancer, and prior reports describe L1CAM expression rates ranging from 7-26.6% in endometrioid cancer (5-10, 20). While previous studies have excluded non-endometrioid histotypes, we did not perform a retrospective specialized gynecopathological case review to exclude potential mixed-type carcinomas containing components. Thus, our sample better reflects cases in daily clinical practice, and we found a L1CAM expression rate of 29.8%, which is the highest rate in the published literature as far as we are aware.

Zeimet *et al*. performed the largest multicenter cohort study, and reported L1CAM to be the strongest prognostic factor in FIGO stage I type I ECs (10). Their results showed L1CAM-positive cancer to be associated with a shorter disease-free interval and worse overall survival. Pasanen *et al*. demonstrated that L1CAM positivity predicted poor disease-specific survival in endometrioid carcinoma, but not in non-endometrioid cancer (8). Based on these findings, Kommoss *et al*. proposed that the definition of the low-risk category should be limited to L1CAM-negative EC (21). In contrast, our present results showed that disease-free interval and overall survival did not significantly differ between the L1CAM-negative and L1CAM-positive groups, which is in agreement with the findings of Dellinger *et al*. (6) and Smogeli *et al*. (4). We also found that L1CAM status was not related to diabetes mellitus or obesity, which are classical epidemiological risk factors for type I EC (8, 10).

Table I. *Patient characteristics*.

Characteristic	L1CAM, N (%)		Total, N (%)	*p*-Value
	Negative	Positive)		
Patients, n (%)	219 (70.2)	93 (29.8)	312 (100)	-
Age, years				
Mean (range)	63.0 (27-89)	64.4 (44-83)	63.4 (27-89)	0.30
Adjuvant therapy, n (%)	49 (22.4)	22 (23.7)	71 (22.8)	0.80
Pelvic lymphadenectomy, n (%)*	91 (41.6)	28 (30.1)	120 (38.3)	0.056
Disease characteristics				
FIGO stage, n (%)				
IA	162 (74.0)	72 (77.4)	234 (75.0)	0.52
IB	57 (26.0)	21 (22.6)	78 (25.0)	
Grade 1, n (%)				
1	150 (68.5)	54 (58.1)	204 (65.4)	0.02
2	64 (29.2)	31 (33.3)	95 (30.4)	
3	5 (2.3)	8 (8.6)	13 (4.2)	
Disease progression, n (%)	10 (4.6)	10 (10.8)	20 (6.4)	0.04
Progression-free interval, months				
Mean (range)	24.1 (4-73)	24.8 (1-50)	24.5 (1-73)	0.93
Death, n (%)	9 (4.1)	7 (7.5)	16 (5.1)	0.25
Overall survival, months				
Mean (range)	44.6 (24-80)	53.1 (28-80)	48.3 (24-80)	0.42

L1CAM: L1 cell adhesion molecule; FIGO: Fédération Internationale de Gynécologie et d'Obstétrique. *With/without para-aortic lymphadenectomy.

In Zeimet *et al*.'s study, 117 patients (11.5%) experienced recurrence over a median follow-up of 54.3 months (10). Recurrence was detected in 51.4% of L1CAM-positive tumors, and 2.9% of L1CAM-negative tumors. In our study, recurrence was detected in only 6.4% of patients with stage I EC, with recurrence rates of 4.6% among L1CAM-negative tumors and 10.8% among L1CAM-positive tumors (p=0.04).

Zeimet *et al*. also found that extra-abdominal relapses were more frequent in L1CAM-positive (13.2%) than L1CAM-negative (1.9%) FIGO stage I endometrioid carcinomas (p<0.0001). In contrast, other site-specific relapses showed no association with L1CAM status (10). Bosse *et al*.'s study revealed that L1CAM positivity is significantly correlated with risk of distant recurrence (hazard ratio=5.1) but not with vaginal relapses (5). Similarly, other authors reported a greater frequency of distant relapses in L1CAM-positive cases (4, 8). In agreement with these findings, our present data regarding type of disease progression indicated that only distant metastasis was significantly associated with L1CAM positivity. One strength of our present study was that it included an unselected cohort of patients who underwent treatment at a tertiary care center, following a standardized surgical technique and regular follow-up. A weakness of our study was its retrospective design and the small number of recurrences. As in all studies focusing on cancer with limited malignant potential, we faced the statistical dilemma of having to calculate survival rates based on a limited number of disease-specific events during follow-up.

In conclusion, we herein report that L1CAM is a promising independent prognostic marker that was strongly associated with more aggressive cancer behavior and recurrence risk, but not with overall survival. Prospective trials are needed to evaluate the clinical significance of L1CAM for risk assessment among low-risk patients, and to guide potential alterations of surgical and adjuvant treatment strategies.

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