Detection of lymph vessel invasion and blood vessel invasion using double D2-40 and CD31 immunohistochemistry reveals independent prognostic significance of blood vessel invasion in endometrial cancer

Ani Mihaljević Ferari[1], Marko Klarić[2], Maja Krašević[3], Senija Eminović[3], Jasna Marušić[1], Iva Plišić[2], Gabrijela Sopta[2], Emina Babarović[3]

[1] Department of Radiotherapy and Oncology, Clinical Hospital Centre, Rijeka, Croatia
[2] Department of Obstetric and Gynaecology, Clinical Hospital Centre, Rijeka, Croatia
[3] Department of Pathology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

OBJECTIVE: Lymph-vascular space invasion (LVSI) is adverse prognostic factor in endometrial cancer (EC). Due to lack in histologic criteria and interobserver variability its role in administering adjuvant treatment and predicting recurrence isn't well defined. The aim of our study was to utilize sensitive immunohistochemical method for separate detection of lymph vessel invasion (LVI) and blood vessel invasion (BVI) in patients with EC and to investigate their prognostic impact.

METHODOLOGY: We retrospectively identified from the institutional database 217 patients with EC and with complete follow-up, between 2002 and 2006. Tumor specimens were retrieved and two-colored double staining immunohistochemistry (D2-40/CD31) was performed to highlight lymphatic and blood vessels in the same slide. Tumors were divided in three groups: LVSI negative, LVI positive and BVI positive.

RESULTS: In the original pathology reports, LVSI was misclassified in 36 (16.6%) tumors. In this study by using immunohistochemistry LVI was detected in 64 (29.5%) and BVI in 32 (14.7%) cases. All tumors with positive BVI also had accompanying LVI. BVI had the strongest impact on the risk for recurrence (OR 9.27 CI 4.47–19.22; p<0.0001) and was, also significantly associated with decreased survival outcomes (overall survival, 59% compared with 96% in LVI group and 98% in LVSI negative group, p<0.0001 and disease-free survival, 5-year rate 42% compared with 96% in both other groups, p<0.0001) in univariate analysis. In multivariate analysis (including: age, tumor type, grade, stage, treatment) BVI remained independent prognostic factor for recurrence (HR 7.1 CI 2.9–16.8), overall survival (HR 8.2 CI 4.1–16.4) and disease-free survival (HR 6.2 CI 2.9–12.8).

CONCLUSIONS: Our results suggest that BVI could have greater clinical value in contrast to isolated LVI, and that therapeutic decisions should be based on its presence. We hope that these preliminary results will encourage further application of this inexpensive, readily available technique.

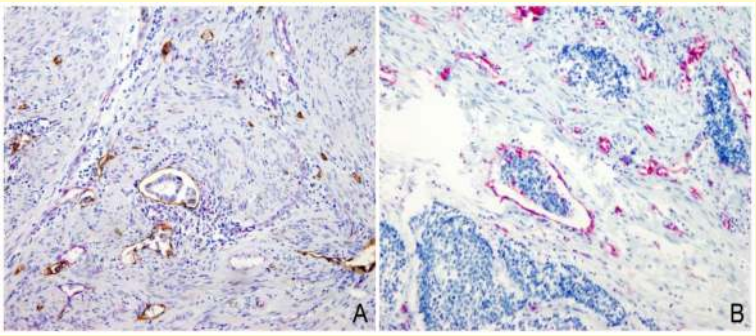

Figure 1 Examples of double immunohistochemical staining with pan-endothelial marker CD 31 and lymph endothelial marker podoplanin (D2-40). Lymphatic vessel invasion was determined as the presence of tumor cells within brown, D2-40-stained vessel (A, original magnification x100). Blood vessel invasion was determined as the presence of tumor cells in the red, CD31 stained vessel (B, original magnification x200).

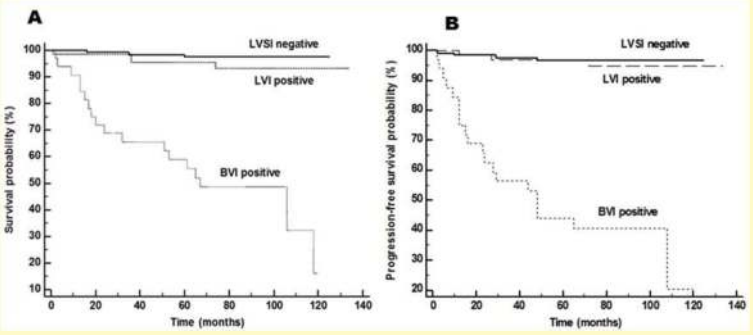

Figure 2 Kaplan-Meier survival plots according to detection of LVI and BVI in patients with EC: (A) significantly shorter overall survival in patients with positive BVI (p<0.0001). (B) disease-free survival, the log-rank test showed significantly shorter disease-free survival in patients with positive BVI (p<0.0001).

Table 1. Clinicopathologic characteristics of study cohort

Features	All	LVSI negative	LVI positive	BVI positive	P value
Patients	217	121	64	32	
Age (years) Median (Range)	65 (33-89)	62 (46-87)	69 (44-89)	66.5 (33-89)	**0.001¶**
Type of tumor	N (%)	N (%)	N (%)	N (%)	
Type 1	197 (90.8)	114 (94.2)	57 (89.1)	26 (81.2)	0.067*
Type 2	20 (9.2)	7 (5.8)	7 (10.9)	6 (18.8)	
Grade					
1	111 (51.2)	81 (66.9)	22 (34.4)	8 (25.0)	**< 0.0001***
2	68 (31.3)	31 (25.6)	28 (43.7)	9 (28.1)	
3	38 (17.5)	9 (7.4)	14 (21.9)	15 (46.9)	
Lymph nodes					**< 0.0001***
Negative	133 (95.0)	81 (100)	38 (100)	14 (66.7)	
Positive	7 (5.0)	0 (0)	0 (0)	7 (33.3)	
FIGO stage					
1A	128 (59.0)	97 (80.2)	24 (37.5)	7 (21.9)	**< 0.0001***
1B	54 (24.9)	13 (10.7)	28 (43.7)	13 (40.6)	
2	24 (11.1)	11 (9.1)	11 (17.2)	2 (6.2)	
3 and 4	11 (5.1)	0 (0)	1 (1.6)	10 (31.2)	
T-stage					**< 0.0001***
1a	128 (59.0)	97 (80.2)	24 (37.5)	7 (21.9)	
1b	54 (24.9)	13 (10.7)	28 (43.7)	13 (40.6)	
2	27 (12.4)	11 (9.1)	11 (17.2)	5 (15.6)	
3 and 4	8 (3.7)	0 (0)	1 (1.6)	7 (21.9)	
Postoperative therapy					**< 0.0001***
None	126 (58.1)	93 (76.9)	30 (46.9)	3 (9.4)	
Radiotherapy	76 (35.0)	24 (19.8)	30 (46.9)	22 (68.7)	
Chemotherapy	6 (2.8)	2 (1.7)	4 (6.2)	0 (0)	
Concomitant chemoradiotherapy	9 (4.1)	2 (1.7)	0 (0)	7 (21.9)	
Recurrence					
No	190 (87.6)	117 (96.7)	61 (95.3)	12 (37.5)	**< 0.0001***
Vaginal	3 (1.4)	1 (0.8)	2 (3.1)	0 (0)	
Pelvis	9 (4.1)	2 (1.7)	0 (0)	7 (21.9)	
Distant metastatic sites	15 (6.9)	1(0.8)	1 (1.6)	13 (40.6)	
Died of disease [N (%)]	25 (11.5)	3 (2.5)	4 (6.2)	18 (56.2)	**< 0.0001***

¶Mann-Whitney U test; *Chi-squared test