Lymphovascular space invasion in endometrial carcinoma

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CHAPTER 7

DEFINING SUBSTANTIAL LYMPHOVASCULAR SPACE INVASION IN ENDOMETRIAL CANCER

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Abstract

Lymphovascular space invasion (LVSI) occurs in a minority of endometrial cancer (EC) cases, and the extent of LVSI is an important risk factor for recurrence and/or metastases. Our aim was to improve the reproducibility of measuring clinically meaningful LVSI by performing a quantitative analysis of the correlation between LVSI and the risk of pelvic lymph node recurrence (PLNR) in EC.

EC samples from the PORTEC-1 and PORTEC-2 trials were retrieved and used to collect quantitative data, including the number of LVSI-positive vessels per H&E-stained slide. Using a predefined threshold for clinical relevance, the risk of PLNR risk was calculated (Kaplan-Meier method, with Cox regression) using a stepwise adjustment for the number of LVSI-positive vessels. This analysis was then repeated in the DGCD (Danish Gynecological Cancer Database) cohort.

Among patients in the PORTEC-1 and PORTEC-2 trials who did not receive external beam radiotherapy, the 5-year PLNR risk was 3.3%, 6.7% (p=0.51), and 26.3% (p<0.001) when 0, 1-3, or ≥4 vessels had LVSI involvement; similar results were obtained for the DGCD cohort. Furthermore, both the average number of tumor cells in the largest embolus and the number of LVSI-positive H&E slides differed significantly between focal LVSI and substantial LVSI.

Based on these results, we propose a numeric threshold (≥4 LVSI-involved vessels in at least one H&E slide) for defining clinically relevant LVSI in EC, thereby adding supportive data to the semi-quantitative approach. This will help guide gynecologic pathologists to differentiate between focal- and substantial LVSI, especially in borderline cases.

Introduction

In endometrial cancer (EC), a combination of histopathological factors is typically used to assess clinical risk and forms the basis for recommendations regarding the use of adjuvant treatment. For example, lymphovascular space invasion (LVSI) is one of the most robust indicators of an increased risk of lymph node involvement, pelvic recurrence, and distant metastasis [1-5], and LVSI is commonly used in most risk-assessment algorithms, including the algorithm used in the ESGO-ESTRO-ESP guideline [6]. In addition, LVSI is often a critical factor in new risk models that incorporate molecular features [7], including the model used in the ongoing PORTEC-4a trial [8]. Moreover, recent studies have shown that the extent of LVSI—and not merely the presence or absence of LVSI—is the strongest predictor of recurrent regional and metastatic disease in patients with stage I disease [9, 10].

We previously reported that a three-tiered, semi-quantitative LVSI assessment based on no LVSI, focal LVSI, and substantial LVSI provided the strongest evidence with respect to recurrence among patients with intermediate-risk and high/intermediate-risk EC in the PORTEC-1 and PORTEC-2 trials [10]. Specifically, patients with substantial LVSI had a significantly higher risk of recurrent disease compared to patients with either no LVSI or focal LVSI. Moreover, substantial LVSI was the strongest independent prognostic factor for an increased risk of pelvic lymph node recurrence (PLNR), with a 5-year risk of 15.3% compared to 1.7% and 2.5% for no LVSI and focal LVSI, respectively. Importantly, among patients with substantial LVSI, external beam radiotherapy (EBRT) reduced the 5-year PLNR risk from 31% to 4.3% [10]. This finding suggests that the presence of substantial LVSI is clinically relevant with respect to recommending EBRT, as reflected by current guidelines.

For use in clinical practice, the definition and quantitative assessment of LVSI should be clear to practicing pathologists. In previous studies, however, “focal LVSI” was defined as a focus of LVSI, and “substantial LVSI” was defined as diffuse or multifocal LVSI present beyond the advancing tumor front [11]. Although the use of these semi-quantitative definitions provides a reasonable level of reproducibility among gynecologic pathologists—as we showed recently in an interobserver study [12]—using a numerical threshold to define clinically relevant LVSI may help distinguish between focal LVSI and substantial LVSI, particularly in borderline cases, thus providing improved standardization and reproducibility.

The aim of this study was to numerically define the threshold at which the extent of LVSI becomes clinically relevant with respect to the risk of PLNR, thereby establishing an objective threshold between focal LVSI and substantial LVSI. We therefore analyzed the number of LVSI-positive vessels, the number of tumor cells in the largest embolus, the distance between the deepest embolus and the serosal surface, and the number of H&E-stained slides containing LVSI-positive vessels in all previously annotated endometrioid EC (EEC) cases in the PORTEC-1 and PORTEC-2 trials [10]; in addition, we validated our findings using an independent Danish population-based EC cohort.

Materials and Methods

Study cohorts

For this study, we used the combined PORTEC-1 and PORTEC-2 [10] cohort and the Danish Gynecological Cancer Database (DGCD) cohort to quantitatively analyze the correlation between the extent of LVSI and the risk of PLNR. These two cohorts are described in detail below.

The PORTEC-1/PORTEC-2 cohort

The PORTEC-1 trial was a randomized controlled trial (RCT) involving 714 patients with intermediate-risk EC randomly assigned to receive either adjuvant EBRT or no adjuvant treatment following total abdominal hysterectomy and bilateral salpingo-oophorectomy without lymphadenectomy [13]. The PORTEC-2 trial was an RCT involving 427 patients with high/intermediate-risk EC randomly assigned to receive either adjuvant EBRT or adjuvant vaginal brachytherapy following total abdominal hysterectomy and bilateral salpingo-oophorectomy without lymphadenectomy [14]. All patients in PORTEC cohorts had endometrioid cancers. The risk classifications were based on ESMO-ESGO-ESTRO guidelines [15, 16].

The DGCD cohort

The second cohort in our study was derived from a Danish population-based study of patients with high-risk EC [6, 17] and is described in detail in our companion paper by Peters et al. [18]. Cases were collected from the DGCD, a Danish nationwide database that includes nearly all 4,707 patients diagnosed with EC from January 1, 2005 through December 31, 2012. All patients underwent a hysterectomy and 70.7% underwent systematic lymphadenectomy; 80% were FIGO stage I-II; 60% had non-endometrioid histology; and 70.3% of patients did not receive adjuvant therapy. Follow-up data were also registered in the DGCD and included the date and site of recurrence as well as the date and cause of death.

LVSI review

Four pathologists (authors EEMP, ALC, VTHBMS and TB) comprehensively reviewed the H&E–stained hysterectomy slides obtained from 926 cases in the PORTEC cohort and 401 cases in the DGCD cohort. The presence of LVSI was established when a tumor embolus was present in an endothelial-lined space within the myometrium beyond the invasive front of the tumor. The presence of LVSI was determined without the use of immunohistochemistry. For all cases, we noted the total number of H&E slides available, the number of tumor-positive H&E slides, the number of LVSI-positive H&E slides, and the number of LVSI-positive vessels per H&E slide. In addition, we noted the semi-quantitative assignment of LVSI (i.e., no LVSI, focal LVSI, or substantial LVSI). Focal LVSI was defined as a single focus of LVSI beyond the invasive border. Substantial LVSI was defined as diffuse or multifocal LVSI around the tumor [10]. For the PORTEC cohort only, we also measured the area of tumor-free myometrium per slide (in mm^2), the number of cells in the largest embolus, the distance between the deepest embolus and the

serosal surface (in mm), and the largest distance between the embolus and the invasive tumor front (in mm).

Statistics

Differences between two groups were analyzed using the Student's *t*-test. The time to event for PLNR was calculated from the date of randomization (for the PORTEC cohort) or the date of surgery (for the DGCD cohort) and analyzed using Cox regression and the Kaplan-Meier method with log-rank test, with stepwise adjustment of cut-off points by the highest number of tumor emboli counted in a H&E slide. If multiple slides were positive for LVSI, the highest number of emboli per slide was used for analyses. No calculations of the total number of emboli were made in case of multiple LVSI-positive slides. PLNR in PORTEC-1 and 2 was defined as any regional nodal recurrence and confirmed by a biopsy [13, 14]. For the DGCD cohort histology proven recurrences were located retrieving data from the Danish pathology database, non-histology verified recurrences were retrieved from medical records based on gynecological or radiological examination [17]. Because a small percentage of patients in the DGCD cohort received adjuvant treatment, the Cox regression analysis was adjusted for age, ASA classification score (in cases with comorbidity), lymph node resection, and adjuvant treatment. For the PORTEC cohort, the analyses were performed based on the patients in the PORTEC-1 and PORTEC-2 trials who were randomized to receive either vaginal brachytherapy or no additional treatment (n = 502, including 59 LVSI-positive patients). All patients (regardless of LVSI status) randomized to receive EBRT were excluded from this analysis, as EBRT has been shown to reduce the risk of pelvic failure [78]. The PORTEC data were analyzed using SPSS version 23.0 (IBM Corp., Armonk, NY), and the DGCD data were analyzed using Stata 11 (StataCorp LLC, College Station, TX). Differences with a p-value <0.05 were considered significant.

Criteria used to establish a numerical threshold for clinically relevant LVSI

Ideally, a clinically relevant threshold should indicate an increase in the risk of recurrence sufficient to justify providing adjuvant treatment despite the associated risk of side effects. In low-risk EC, the 5-year recurrence-free survival rate is 95% [19]; thus, the remaining 5% risk is not sufficient to justify adjuvant treatment. For our analysis, a PLNR risk of 10% was consider the upper range of acceptable risk for not recommending EBRT. Additionally, we took the Hazard Ratios (HRs) into consideration, seeking for a trade-off between the highest possible PLNR HR for substantial LVSI, versus the lowest possible PLNR HR for focal LVSI.

Results

We first analyzed a variety of quantitative factors measured in the H&E slides from the PORTEC cohort for any possible association with the number of LVSI-positive vessels. For this analysis, H&E-stained slides were available from 108 of the 129 (83.7%) LVSI-positive EC cases. For each LVSI-positive EC case, an average of 1.8 (range: 1-5) tumor-containing H&E slides were available, including 70 cases with focal LVSI and 38 cases with substantial LVSI. If LVSI was present, this

was not present in all samples taken from the tumor and myometrium. As shown in Table 1, the focal LVSI cases and the substantial LVSI cases differed significantly with respect to the number of H&E slides containing LVSI-positive vessels, the number of LVSI-positive vessels per H&E slide, and the number of cells contained in the largest embolus; in contrast, we found no significant difference with respect to the number of tumor-positive H&E slides, the myometrial area per H&E slide, the distance between the deepest tumor-positive vessel and the serosa, or the distance between the deepest tumor-positive vessel and the invasive border. The largest embolus in focal LVSI contains on average 31 cells (SD 32 cells), whereas for substantial LVSI the average size is 55 cells (SD 53) ($p = 0.002$). Focal LVSI was associated with less LVSI positive H&E's than substantial LVSI (1.19 (SD 0.46) H&E's and 1.5 (SD 0.73) H&E respectively; $p = 0.019$).

Table 1. Summary of pathological findings in LVSI positive cases in the combined PORTEC-1 and PORTEC-2 cohort, based on the extent of LVSI.

	Focal LVSI (n = 70)		Substantial LVSI (n = 38)		*p*
	Mean	(SD)	Mean	(SD)	
Number of H&E's with tumour	1.71	(0.94)	1.97	(1.1)	0.199
Number of H&E's with LVSI	1.19	(0.46)	1.5	(0.73)	0.019
Myometrium surface per H&E (mm^2)	157	(100)	159	(92.6)	0.851
Distance of deepest embolus to serosa (mm)	4.6	(2.9)	3.8	(2.4)	0.107
Distance of deepest embolus to advancing front (mm)	2.2	(2.2)	2.5	(1.9)	0.395
Number of LVSI-positive vessels	1.9	(2.5)	5.6	(6.4)	< 0.001
Number of tumour cells in the largest embolus	31	(32)	55	(53)	0.002

Establishing a clinically relevant threshold based on the number of LVSI-positive vessels
Repeated Cox regression and Kaplan-Meier analyses were used to calculate the risk of PLNR in order to establish a numerical threshold for defining clinically relevant LVSI. Table 2 shows the risk for PLNR of the study cohort with application of the semi-quantitative and several quantitative definitions We found that PLNR risk increased as the number of tumor-positive vessels per slide increased. In the study cohort, when LVSI was present in ≥3 vessels, the 5-year PLNR risk was 20.8%; in patients with <3 LVSI-positive vessels, this risk was 5.3%, which was slightly higher than the risk in LVSI-negative patients (3.3%). At a threshold of <6/≥6 LVSI-positive vessels, the 5-year PLNR risk in patients with <6 LVSI-positive vessels was 10.2%, which exceeds the predefined upper border of clinical acceptability. The lowest HR for PLNR was reached when focal LVSI was defined as <3 positive vessels per slide. The highest HR for PLNR was reached if substantial LVSI was defined as ≥6 positive vessels. The optimal trade-off was found when at least ≥4 LVSI-positive vessels were present in at least one H&E slide and is shown in a Kaplan-Meier curve in Figure 1.

Review of LVSI in 401 EC cases in the DGCD validation cohort resulted in 321 cases (80.0%) without LVSI, 37 cases (9.2%) with focal LVSI, and 43 cases (10.7%) with substantial LVSI. Over a five-year follow-up period, a total of 25 cases had PLNR, of which 12 cases were LVSI-positive. The results with respect to PLNR were analysed using a similar numerical approach as

Table 2. Repeated Kaplan-Meier analyses of the risk of pelvic lymph node recurrence (PLNR) based on stepwise increases in the total number of LVSI-positive vessels.

N emboli	patients	events	*p*	HR	95% CI			5-year PLNR risk
No LVSI	443	18	*NA*	*NA*		*NA*		3.3
focal	38	3	0.256	2.0	0.6	-	6.9	8.3
substantial	21	5	< 0.001	8.2	3.0	-	22.2	30.2
< 2	12	0	0.978	0.0	0.0			0.0
≥ 2	47	8	< 0.001	5.1	2.2	-	11.7	19.8
< 3	20	1	0.822	1.3	0.2	-	9.4	5.3
≥ 3	39	7	< 0.001	5.4	2.2	-	12.9	20.8
< 4	32	2	0.513	1.6	0.4	-	7.0	6.7
≥ 4	27	6	< 0.001	6.9	2.7	-	17.5	26.3
< 5	36	3	0.193	2.3	0.7	-	7.7	9.0
≥ 5	23	5	< 0.001	6.5	2.4	-	17.6	25.8
< 6	42	4	0.091	2.6	0.9	-	7.5	10.2
≥ 6	17	4	< 0.001	7.6	2.4	-	22.7	30.4
< 10	46	4	0.111	2.4	0.8	-	7.1	9.6
≥ 10	13	4	< 0.001	9.1	3.1	-	27.1	34.4
< 11	48	5	0.034	2.9	1.1	-	7.9	11.4
≥ 11	11	3	< 0.001	7.7	2.3	-	26.3	31.4
< 12	52	5	0,052	2,67	0,99	-	7,20	10,50
≥ 12	7	3	< 0,001	13,35	3,91	-	45,64	50,00
< 13	54	7	0,004	3,62	1,51	-	8,68	14,40
≥ 13	5	1	0,082	5,98	0,80	-	44,96	25,00
< 15	55	7	0,005	3,54	1,48	-	8,49	14,10
≥ 15	4	1	0,042	8,15	1,08	-	61,34	33,30
< 18	57	8	0,001	3,91	1,70	-	9,01	15,70
≥ 18	2	0	0,983	0,00	0,00			0,00

HR: hazard ratio, 95% CI: 95% confidence interval, PLNR: pelvic lymph node recurrence. The hazard ratio (HR) with 95% CI is based on the "no LVSI" reference group. This analysis included 502 patients in the combined PORTEC-1 and PORTEC-2 cohort who received either adjuvant vaginal brachytherapy or no adjuvant treatment but did not receive external beam radiotherapy.

described above and are summarized in Table 3. When we set the threshold to ≥4 LVSI-positive vessels, the 5-year PLNR risk was 26.1%, and 8.9% in cases with <4 LVSI-positive vessels. When we set the threshold to ≥5 LVSI-positive vessels, the 5-year PLNR risk for cases with <5 LVSI-positive vessels was 12.1%, which exceeded the predefined upper border of clinical acceptability. The lowest HR for PLNR in case of focal LVSI was 2,5 at the threshold of <4/≥4

vessels. With a HR of 7,4, the highest HR for PLNR was reached for substantial LVSI at the same threshold; i.e. at least 4 LVSI positive vessels in a H&E slide.

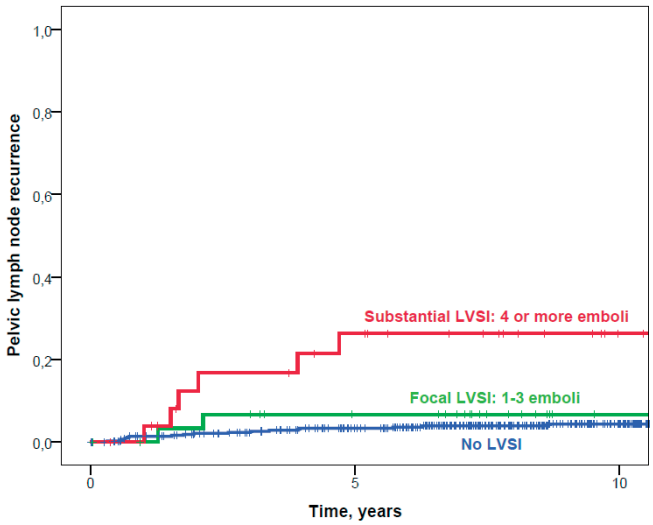

Figure 1. Kaplan-Meier curves for the 5-year risk of pelvic lymph node recurrence in the combined PORTEC-1 and PORTEC-2 cohort, for no LVSI, 1 to 3 LVSI positive vessels or 4 or more LVSI positive vessels.

Table 3. Pelvic lymph node recurrence (PLNR) based on stepwise increases in the total number of LVSI-positive vessels.

N emboli	patients	events	*p*	HR	95% CI		5-year PLNR risk
No LVSI	321	13	*NA*	*NA*	*NA*		4.7
focal	37	4	p = 0.015	4.1	1.3	12.8	14.5
substantial	43	8	p < 0.001	6.7	2.6	16.8	24.3
< 3	21	2	p = 0.173	2.8	0.6	12.5	11.6
≥ 3	59	10	p < 0.001	5.7	2.5	13.0	22.8
< 4	27	2	p = 0.242	2.5	0.6	11.1	8.9
≥ 4	53	10	p < 0.001	7.4	3.1	17.8	26.1
< 5	29	3	p = 0.050	3.6	1.0	12.9	12.1
≥ 5	51	9	p < 0.001	6.7	2.7	16.4	24.7
< 6	38	4	p = 0.017	4.0	1.3	12.4	14.0
≥ 6	42	8	p < 0.001	6.9	2.7	17.4	25.1

HR: hazard ratio, 95% CI: 95% confidence interval, PLNR: pelvic lymph node recurrence. Repeated analyses of the risk of PLNR based on stepwise increases in the total number of LVSI-positive vessels. The hazard ratio (HR) with 95% CI is based on the "no LVSI" reference group.

Discussion

Several studies have shown that the extent of LVSI is clinically relevant and can be used to improve the stratification of patients with intermediate/high-risk and high-risk EC [9, 10, 20]. Moreover, introducing the concept of measuring the extent of LVSI resulted in pathologists

reporting the extent of LVSI using a semi-quantitative approach (i.e., focal LVSI vs. extensive/substantial LVSI), with acceptable interobserver variation [12]. Despite the advantages associated with this semi-quantitative approach, the addition of a strictly quantitative (i.e., numerical) threshold is likely to increase reproducibility, particularly in cases in which no clear distinction can be made between focal LVSI and substantial LVSI. In this respect, our results show that the presence of LVSI is a biological variable, with the risk of PLNR increasing as the number of LVSI-positive vessels increases. In the PORTEC cohort, the threshold of ≥5 LVSI-vessels was sufficient to achieve the predefined upper border of a 10%increase in 5-year PLNR risk without the need for EBRT. In an independent cohort (the DGCD cohort), the upper border was reached when ≥4 vessels were involved, yielding a similar 5-year PLNR risk. This slight difference in threshold between the two cohorts (i.e., 5 vs. 4 vessels in the PORTEC and DGCD cohorts, respectively) may be explained—at least in part—by the inclusion of predominantly grade 3 and non-endometrioid tumors in the DGCD cohort and/or fewer H&E slides available in the PORTEC cohort. Nevertheless, in both cohorts the difference between choosing 4 or 5 vessels as the threshold would have affected the classification of only 4 patients in the PORTEC cohort and 2 patients in the DGCD cohort. Although the semi-quantitative (i.e., no LVSI, focal LVSI, or substantial LVSI) approach did not perform poorly in this study, we recommend to replace it with a numerical threshold of ≥4 LVSI-positive vessels in at least one H&E slide for the diagnosis of substantial LVSI, which will also improve acceptance and reproducibility.

For our analysis, we used a PLNR risk of 10% as the upper border of the acceptable risk associated with not recommending EBRT in these patients. This threshold was based on the following factors: 1) in low-risk EC the 5-year recurrence-free survival rate is 95% with general agreement that adjuvant treatment is not indicated, despite the remaining 5% risk; 2) the use of EBRT in patients with substantial LVSI reduces the risk of PLNR to <5%; and 3) there is general agreement among patients and clinicians that intensive adjuvant therapy should be recommended if it can reduce risk by 10% [21].

In this study, we extensively characterized LVSI, defined as a tumor embolus in an endothelial-lined space within the myometrium and beyond the invasive front of the tumor. In addition to counting the number of LVSI-positive vessels per H&E-stained slide, we also measured the area of tumor-free myometrium per slide, the number of cells in the largest embolus, the distance between the deepest embolus and the serosal surface, the largest distance between the embolus and the invasive tumor front, and the number of LVSI-positive H&E slides. Among these factors, we found that both the number of tumor cells in the largest embolus and the number of LVSI-positive H&E slides were associated with an increased risk of PLNR. This finding suggests that histological factors should be taken into account when assessing the extent of LVSI. The distribution of cell counts is skewed and there is great overlap in size between emboli found in focal and substantial LVSI. Therefore, cell counts on its own are not reliable to

distinguish focal from substantial LVSI. Additionally, cell counting is time consuming and prone to errors and therefore not applicable for every-day practice. Acknowledging LVSI is often present in multiple H&E slides as opposed to focal LVSI can aid to decide about the extent of LVSI, but is not an argument on its own.

Determining an evidence-based threshold in histopathology can be challenging, and such thresholds should ideally be established based on clinically annotated series, as the biological behavior of any given tumor is multifactorial. Therefore, we used well-documented cohorts of EC cases with high-quality pathology review data and robust data based on long-term clinical outcome [22]. Although these cohorts are relatively large, substantial LVSI is relatively uncommon and therefore challenging to study. Another aspect requiring vigilance is the risk of statistical noise that is easily introduced when studying phenomena with low incidence such as LVSI. A minor change in numbers of cases or events can have major effect on risk calculation. We excluded patients who received EBRT from our analyses, as EBRT can effectively prevent pelvic recurrence and would potentially have concealed the effect of LVSI on PLNR. Furthermore, fewer slides were available for review from the PORTEC cohort compared to what we would expect in routine practice, potentially causing an underestimation of the prevalence and/or extent of LVSI. The chance of underestimating the prevalence of LVSI was minimal because for the previous study a successful effort was made to collect as many H&E slides from cases that were LVSI positive [10]. For the current study less H&E slides from PORTEC were available for measurements and counting of emboli. Nevertheless, our review of LVSI in the DGCD cohort—in which the number of H&E slides was similar to routine practice—yielded similar outcome results. Moreover, approximately 70% of patients in the DGCD cohort did not receive adjuvant treatment and were therefore well suited to demonstrate the risk of PLNR associated with LVSI, adjusted for adjuvant treatment.

In conclusion, our results provide the first numeric threshold for clinically relevant LVSI in EC. This threshold—namely, ≥4 LVSI-positive vessels in at least one H&E slide—provides valuable supportive information that can help gynecologic pathologists classify the extent of LVSI, particularly in cases in which the distinction between focal LVSI and substantial LVSI is unclear. We therefore recommend pathologists to report the number of emboli in equivocal cases. The semi-quantitative approach has shown to be meaningful and reproducible in a population-based validation cohort and will generally be sufficient in most EC cases. By correlation of quantitative data, we were able to provide additional supportive factors such as the number of tumor cells in the largest LVSI embolus. We encourage other research groups to validate our proposed threshold in independent cohorts.

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