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## Research Article

# Lymphovascular Space Invasion in Endometrial Cancer: Does it Matter Where and How Much to Sample? A Macroscopic Study of 208 Hysterectomies

Deniz Ates<sup>a</sup>, Sevilay Karahan<sup>b</sup>, Aleyna Oruç<sup>a</sup>, Alp Usubutun<sup>a,\*</sup>

<sup>a</sup> Department of Medical Pathology, Hacettepe University School of Medicine, Ankara, Turkey; <sup>b</sup> Department of Biostatistics, Hacettepe University School of Medicine, Ankara, Turkey

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## ABSTRACT

Lymphovascular space invasion (LVSI) is a key prognostic factor in endometrial cancer, guiding adjuvant treatment decisions. This retrospective study analyzed 208 hysterectomy specimens with confirmed LVSI to determine optimal sampling strategies for detecting substantial LVSI. Samples/blocks from tumor infiltration fronts were reviewed microscopically, and LVSI foci were counted per slide and summed across all slides. Cutoffs of  $\geq 5$ ,  $\geq 4$ , and  $\geq 3$  LVSI foci were evaluated. Only the  $\geq 5$  threshold significantly correlated with lymph node metastasis ( $P = .038$ ) compared with  $< 5$  LVSI. Both patients with  $\geq 5$  LVSI foci either on a single slide or those reaching this threshold by summing across slides were associated with nodal metastasis ( $P = .023$ ). However, significantly worse overall survival was observed only in patients with  $\geq 5$  foci on a single slide, not in those reaching the threshold by summing across multiple slides ( $P < .001$ ). Focal LVSI ( $< 5$  foci) showed no significant overall survival compared with substantial LVSI. Increased sampling from the tumor infiltration front improved LVSI detection rates ( $P < .001$ ), but gains in detecting substantial LVSI plateaued after 7 samples/blocks. Higher LVSI levels were associated with deep LVSI and cervical/endocervical LVSI ( $P < .001$ ), whereas cervical/endocervical LVSI showed no significant association with overall survival ( $P = .273$ ). Tumor grade, deep LVSI, and cervical involvement predicted nodal metastasis, whereas the microcystic, elongated, and fragmented pattern did not. These findings support using a threshold of  $\geq 5$  LVSI foci on at least 1 slide—as opposed to summing across slides—as a marker of worse overall survival. For optimal evaluation, at least 7 tumor infiltration front samples/blocks should be taken, including deep myometrium and cervical/endocervical canal, to ensure adequate assessment and identify patients at higher risk of nodal spread.

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## Introduction

Endometrial cancer is the most common gynecologic malignancy in developed countries.<sup>1</sup> Surgery plays a key role in the management of the disease, and surgical specimens are evaluated

for prognostic indicators. One of the most important prognostic factors is lymphovascular space invasion (LVSI). Cases with LVSI are associated with higher rates of lymph node metastasis, pelvic recurrence, and distant metastasis.<sup>2-4</sup> LVSI is defined as the presence of tumor cells within endothelium-lined spaces at the tumor infiltration front—the area where the tumor ends and the infiltrating myometrium begins, independent of the main tumor mass.

\* Corresponding author.

E-mail address: [alpusubutun@yahoo.com](mailto:alpusubutun@yahoo.com) (A. Usubutun).



Currently, it has been demonstrated that the presence of substantial LVSI, rather than just its presence, is crucial in determining prognosis. This information has been incorporated into the current guidelines for managing the disease and the latest Federation of Gynecology and Obstetrics (FIGO) staging.<sup>5</sup> There are a range of opinions regarding the number of LVSI that are considered substantial. The FIGO 2023 staging, 2020 World Health Organization Classification of Female Genital Tumors, and the European Society of Gynecological Oncology/European Society for Radiotherapy and Oncology/European Society of Pathology guidelines define substantial LVSI as  $\geq 5$ , whereas the National Comprehensive Cancer Network guideline defines it as  $\geq 4$ , and the International Collaboration on Cancer Reporting and IsGyP 2019 Endometrial Cancer Project recommendations define it as  $\geq 3$ .<sup>6-10</sup> Although substantial LVSI has been included in these guidelines, it remains one of the key challenges in the evaluation of LVSI in endometrial carcinoma, particularly with semi-quantitative use of 2-, 3-, or 4-tier scoring systems.<sup>11-13</sup> However, due to a lack of data, it is unclear whether these numbers should be obtained on a single slide or counted and summed across all slides. Additionally, it remains unknown whether the number of samples submitted from the tumor has any effect on the commonly defined substantial LVSI number, or whether the sampling site of the tumor (uterus or cervix) and the location of LVSI itself (uterus or cervix, surface or deep) are significant factors. This study aimed to assess the relationship between macroscopic/sampling parameters and LVSI count and to determine the optimal cutoff for distinguishing focal and substantial LVSI.

## Materials and Methods

### Case Selection and Inclusion Criteria

This study was approved by the Institutional Review Board under research number GO-23-203. Of the 1278 patients diagnosed with endometrial cancer between 2014 and 2023, 760 cases reported as LVSI negative in pathology reports were excluded. A total of 518 patients were initially included: 269 with reported LVSI positivity and 249 with unspecified LVSI status. Hematoxylin and eosin slides were unavailable for 27 cases. Upon re-examination under light microscopy, LVSI was not identified in 235 cases—most of which (179 or 76.1%) were consultation cases lacking LVSI documentation in the original reports. An additional 46 cases were excluded due to contamination, autolysis, or other artifacts that precluded an objective assessment. In these cases, the extent of technical artifacts was such that grading and histotype determination of the tumor were not possible. Two further cases were excluded because the endomyometrial junction could not be identified. Ultimately, 208 hysterectomies that met the checklist criteria<sup>14</sup> (Supplementary Table) and demonstrated unequivocal LVSI positivity were included in the final analysis. The checklist criteria are detailed in the Supplementary Material. For these 208 cases, each hematoxylin and eosin slide containing a tumor infiltration front was thoroughly examined under light microscopy, and the number of LVSI foci per slide was recorded.

### Data Collection and Histopathology Review

The parameters obtained from pathology reports are as follows:

- Weight of the hysterectomy
- Largest tumor diameter
- Presence and localization of metastatic lymph nodes (isolated pelvic, pelvic + paraaortic, isolated paraaortic, others such as renal vein, parametrial, omental, etc.)
- Number of metastatic lymph nodes.

Microscopically assessed parameters included:

- Sampling site of the tumor (corpus uteri alone or both corpus uteri and cervix)
- Tumor grade (I, II, or III)
- Tumor histologic type
- Number of samples having a tumor infiltration front
- Number of LVSI per slide showing a tumor infiltration front and total number of LVSI
- Number of samples from the cervix or endocervix
- Number of samples that included the serosal surface
- Presence of microcystic, elongated, fragmented (MELF) pattern
- Depth of LVSI (superficial or combined superficial + deep)
- Localization of LVSI (only the corpus uteri or additionally cervix/endocervix)
- Lymph node metastasis: micrometastatic vs macrometastatic.

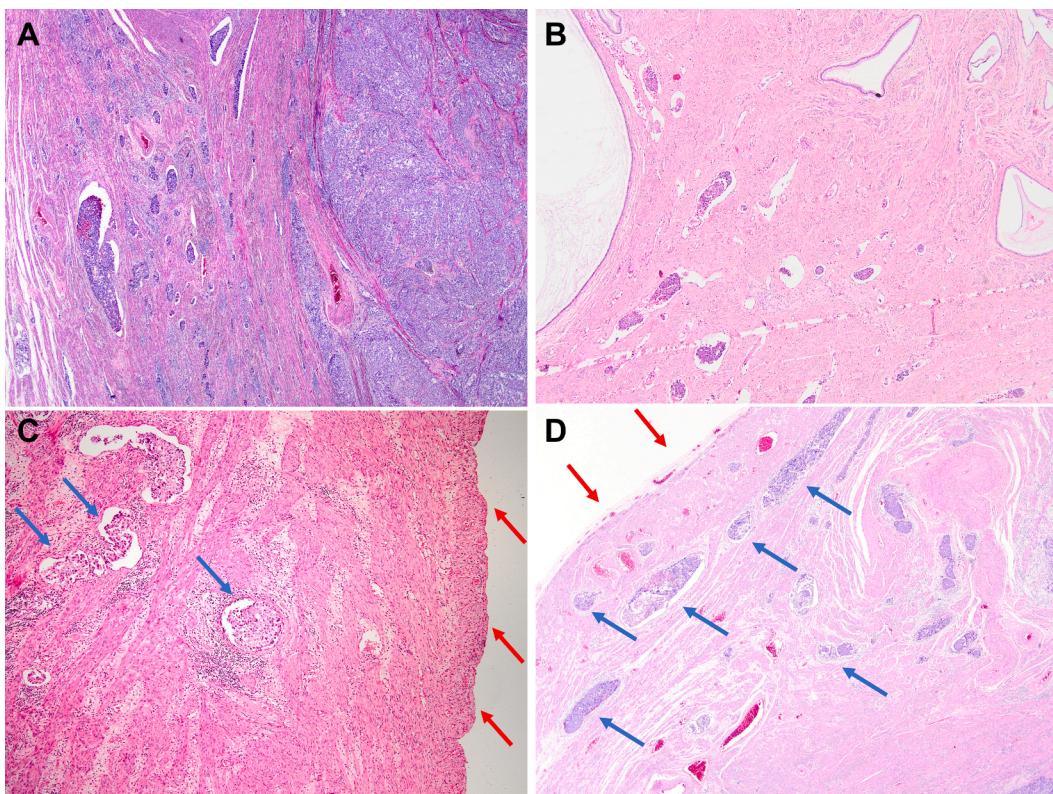
Slides without a tumor infiltration front (ie, slides containing only tumor tissue or no tumor) were excluded from the study conducted to determine the optimal number of sections to be sampled. The LVSI, which was located within the tumor stroma (observed in a few cases), was not included in the LVSI count according to checklist recommendation<sup>14</sup> (Supplementary Table).

Our institution's established standard protocol was utilized for tissue submission, which allows for the submission of 2 cervical and 2 endocervical canal samples, as well as at least 4 samples containing full-thickness myometrium and tumor from a hysterectomy specimen with endometrial carcinoma. However, this number may increase as needed. For instance, if endocervical stromal invasion is suspected, additional samples can be submitted.

The tumor infiltration front was defined as an area at least 1 mm deep marking the interface between tumor and stroma. If a single slide showed  $>20$  LVSI foci poses a spray-like pattern (Fig. 1A), the number was recorded as " $>20$ " and assigned the value of 30 during statistical analysis. LVSI numbers from all relevant slides were summed and grouped as follows:  $\geq 5$  and  $<5$ ,  $\geq 4$  and  $<4$ , or  $\geq 3$  and  $<3$ . The number of LVSI foci was recorded separately for each slide with a tumor infiltration front.

LVSI in the cervix/endocervix is defined as the presence of lymphovascular invasion within stromal vessels located beneath the endocervical or ectocervical epithelium (Fig. 1B). Deep myometrial LVSI was defined as LVSI located at least 3 mm beyond the invasive tumor front in the outer half of the myometrium. This included either (1) cases where no tumor was seen in the endometrium but LVSI was detected in the outer myometrium, especially in the tumor-free endomyometrial sample adjacent to the tumor, or (2) cases with deep myometrial invasion, where LVSI was observed  $>3$  mm beyond the deepest point of tumor infiltration into the outer myometrium (Fig. 1C, D). Tumors with deep invasion and a tumor infiltration front near the serosa were not classified as having deep LVSI. However, LVSI located near the serosa but distant from the tumor front was considered deep LVSI.

The authors noted that in some hysterectomies, most slides showed no LVSI, whereas 1 to 2 slides showed a high number of

**Figure 1.**

(A) More than 20 foci of LVSI in a spray-like pattern, representing substantial LVSI (H&E,  $\times 40$ ). (B) LVSI in the endocervical canal\* (H&E,  $\times 40$ ). (C, D) LVSI in the deep myometrium\*, located at a distance from the main tumor mass (H&E,  $\times 20$ ). Blue arrows indicate LVSI; red arrows indicate the serosal surface. \*Cases with LVSI in the deep myometrium and cervix/endocervical canal typically show a higher total LVSI count, are more likely to be classified as substantial LVSI, and are frequently associated with nodal metastases and a greater number of metastatic lymph nodes. H&E, hematoxylin and eosin; LVSI, lymphovascular space invasion.

foci, usually at the deepest invasive area, indicating substantial lymphovascular invasion. To reflect the impact of macroscopic sampling and slide count on distribution, a *variation score* was calculated to express deviation between slide-specific LVSI counts. Patient follow-up data, including overall survival status, were retrieved from hospital and national health databases and confirmed via patient phone calls when necessary.

#### Statistical Analysis

All analyses were conducted using IBM SPSS, version 23.0. Descriptive statistics for histologic diagnosis, hysterectomy weight, tumor size, number of samples submitted from the tumor infiltration front, tumor localization, number of LVSI foci, presence of MELF pattern, and metastatic lymph node data (number and site) were summarized using mean  $\pm$  SD, median (25th-75th percentile), or frequency/percentage as appropriate.

Associations with lymph node metastasis were assessed using the Mann-Whitney *U*-test, Spearman correlation, or  $\chi^2$  test based on variable type and parametric assumptions. Receiver operating characteristic (ROC) curve analysis was used to determine a cutoff value for total LVSI. As data for total LVSI were nonparametric and the substitution would not affect results, the value of 30 was used for any case where LVSI count exceeded 20.

The SD of LVSI counts across slides from each patient was used to represent variation. The significance threshold was set at  $P < .05$ .

The survival probabilities were estimated using the Kaplan-Meier method. The log-rank test was used to compare the survival curves of different groups. Cox regression analysis was used to determine factors affecting survival probabilities.

#### Results

##### Descriptive Results

Two hundred eight hysterectomies with LVSI (45 consults, 163 in-house) were included in the study. The mean age of patients was 61.37 years (range, 36–85; SD, 9.93). One hundred fifty cases (72.1%) had  $\ge 5$  LVSI foci. For alternative cutoffs, 167 cases (80.3%) had  $\ge 4$ , and 180 cases (86.5%) had  $\ge 3$ . Of the 208 hysterectomies, 13 patients did not undergo lymphadenectomy, metastatic status was unknown in 38 patients, 84 patients had no metastatic lymph nodes, and 73 patients had lymph node metastases.

The mean number of slides demonstrating the tumor infiltration front was 6 (range, 0–15; SD, 2.45). Slides showing the serosal surface had a mean count of 4.7 (range, 0–15; SD, 2.2). The mean number of sections obtained from the cervix and endocervix was 3.6 (range, 0–8; SD, 1.4). The details of the evaluated parameters and study cohort are provided in Table 1.

##### Results in Correlation With Total Lymphovascular Invasion

Three literature-based cutoffs for total LVSI ( $\ge 5$  vs  $<5$ ,  $\ge 4$  vs  $<4$ , and  $\ge 3$  vs  $<3$ ) were evaluated in 208 hysterectomies.

**Table 1**

Study cohort and descriptive results

Parameter (n = 208)	Study cohort
Age of the patients (y)	Mean: 61.37 (min: 36; max: 85; SD: 9.93)
Weight of the hysterectomy (g)	Mean: 169 (min, 36; max, 1486; SD, 150.86)
Total no. of LVSI (n = 208)	<5: 58 (27.9%) ≥5: 150 (72.1%) <4: 41 (19.7%) ≥4: 167 (80.3%) <3: 28 (13.5%) ≥3: 180 (86.5%)
Lymph node status	Reactive: 84 Metastatic: 73 (26 isolated pelvic, 34 pelvic + paraaortic, 8 isolated paraaortic, 5 other) (64 macromet, 8 micromet, 1 isolated tumor cells) No lymph node dissection: 13 Lymph node status unknown: 38
No. of metastatic lymph nodes	Mean: 5.76 (min: 1; max: 24 SD: 5.72)
Tumor localization	Localized to corpus uteri: 139 Cervix/endocervical involvement: 69
Tumor type	Endometrioid: 168 Serous: 32 Dedifferentiated: 5 Clear cell: 2 Clear + endometrioid: 1
Tumor grade	I: 60 II: 49 III: 99 (59 endometrioid, 32 serous, 5 dedifferentiated, 2 clear cell, 1 endometrioid+clear cell)
No. of samples showing the tumor invasion front	Mean: 6 (min: 1; max: 15; SD: 2.45)
No. of samples showing the serosal surface	Mean: 4.7 (min: 0; max: 15; SD: 2.2)
No. of samples from the cervix or endocervix.	Mean: 3.6 (min: 0; max: 8; SD: 1.4).
Microcystic elongated fragmented pattern	Yes: 51 (24%) No: 157 (66%)
Depth of LVSI	Superficial: 181 Superficial+ deep: 27
LVSI in cervix or endocervix	Yes: 39 No: 151 Unknown: 18

LVSI, lymphovascular space invasion.

Although lymph node metastasis was significantly associated with the ≥5 cutoff ( $P = .038$ ), there was no significant association for the ≥4 and ≥3 thresholds ( $P = .082$  and  $P = .425$ , respectively) (Table 2). Based on these results, the threshold for substantial LVSI was set at ≥5 in this study. Detailed analyses according to LVSI counts are presented in Table 3. Total LVSI increased with larger tumor diameter, histologic type (serous vs endometrioid), tumor grade (1 + 2 vs 3), and the number of tumor infiltration front samples ( $P = .04$ ,  $P < .001$ ,  $P < .001$ ,  $P < .001$ , respectively). In contrast, LVSI was not increased in tumors with the MELF pattern ( $P = .757$ ). Moreover, the rate of substantial (≥5) LVSI detection rose with a higher number of samples with tumor infiltration front ( $P < .001$ ). LVSI was also more common in tumors involving the cervix or endocervical canal ( $P < .001$ ). Lymphovascular invasion in the cervix/endocervix and deep myometrium distant from the tumor was significantly associated with increased total LVSI (both  $P < .001$ ). Substantial (≥5) LVSI was also significantly more frequent in these subgroups ( $P = .007$  and  $P = .020$ , respectively).

There was a positive correlation between the number of samples with tumor invasive front and total LVSI ( $r = 0.212$ ,  $P < .001$ ). This significance held when cases were grouped as ≥5 versus <5 LVSI ( $P = .045$ ). An ROC analysis determined the number of infiltration front samples needed to detect substantial LVSI. Detection of ≥5 LVSI increased with up to 7 tumor-infiltrated samples; beyond 7, detection rates plateaued (sensitivity, 50.7%; specificity, 64%; area under the curve, 59.1). To examine if this threshold was influenced by tumor size, the number of infiltration front samples per centimeter of tumor was

calculated. However, total LVSI did not increase with a higher sample/cm ratio ( $P = .816$ ).

#### Correlation of Metastatic Lymph Nodes and the Presence of Metastatic Lymph Nodes

Of the 208 patients, 157 had known lymph node status (84 benign; 73 with metastasis, including 64 macrometastatic, 8 micrometastatic, and 1 with isolated tumor cells) (Table 1).

Table 4 details parameters related to the presence and number of metastatic lymph nodes. Both were significantly associated with increasing tumor diameter and tumor grade ( $P = .032$  and  $P = .025$ ). Serous tumors were associated with a higher number of metastatic lymph nodes ( $P = .008$ ). The MELF pattern did not predict lymph node metastasis ( $P = .971$  and  $P = .686$  for presence and number, respectively). Cervical or endocervical involvement correlated with higher presence ( $P = .023$ ) and number ( $P < .001$ ) of lymph node metastases. Moreover, patients with LVSI in the cervix/endocervix or LVSI in deep myometrium had more lymph node metastases (presence:  $P = .017$  and 0.026; number:  $P = .003$  and <.001, respectively).

To assess whether LVSI should be measured per slide or summed, 150 substantial LVSI cases (≥5) were divided as follows:

- 124 had ≥5 LVSI on at least 1 slide
- 26 reached ≥5 by summing across slides

**Table 2**

Results for total lymphovascular space invasion with cutoffs ( $\geq 5$  and  $< 5$ ,  $\geq 4$  and  $< 4$ , or  $\geq 3$  and  $< 3$ ) and correlation with presence of lymph node metastasis in terms of patient number

Parameter	Total LVSI cutoff	<i>P</i> values for diffuse vs focal LVSI	
Is there lymph node metastasis?		$< 5$	$\geq 5$
Yes (n = 73)	15 (20.5%)	58 (79.5%)	.038
No (n = 84)	53 (63.1%)	31 (36.9%)	
Unknown (n = 51)			
Yes (n = 73)	10 (13.7%)	63 (86.3%)	.082
No (n = 84)	22 (26.2%)	62 (73.8%)	
Unknown (n = 51)			
	$< 4$	$\geq 4$	
Yes (n = 73)	8 (11%)	65 (89%)	.425
No (n = 84)	14 (16.7%)	70 (83.3%)	
Unknown (n = 51)			

LVSI, lymphovascular space invasion.

No significant difference was found in metastatic lymph node count ( $P = .463$ ) or presence ( $P = .944$ ) between these groups.

#### Lymphovascular Space Invasion Distribution Per Slide

LVSI counts were recorded per slide. In some hysterectomies, only 1 to 2 slides showed clustered LVSI, suggesting extensive invasion despite many negative slides. To evaluate variability, a variation score was calculated for LVSI distribution across slides. As variation increased, total LVSI increased ( $P < .001$ ), but variation was not associated with the number of samples ( $P = .952$ ). Cases with substantial ( $\geq 5$ ) LVSI had significantly higher variation than focal cases ( $P < .001$ ).

#### Results of the Overall Survival Analysis

Of the 208 patients, 134 were alive, 60 had died, and the survival status of the remaining 14 was unknown. The mean follow-up duration was 44.3 months (range, 1-131; SD, 30).

No significant difference in overall survival was found between focal and substantial LVSI cases. Cutoff-based survival analysis using thresholds of  $\geq 5$ ,  $\geq 4$ , and  $\geq 3$  yielded  $P$  values of .248, .244, and .817, respectively. However, deeper LVSI was significantly associated with worse overall survival than superficial LVSI ( $P = .001$ ; Fig. 2A). No overall survival difference was observed based on cervical/endocervical LVSI ( $P = .273$ ). Among cases with  $\geq 5$  total LVSI, those with  $\geq 5$  on at least 1 slide had significantly worse overall survival than those who reached  $\geq 5$  by summing ( $P = .023$ ; Fig. 2B). A multivariate Cox regression analysis, including tumor grade, histologic type, presence of metastatic lymph nodes, tumor location (uterus vs uterus and cervix), and having deep LVSI and  $\geq 5$  total LVSI (those with  $\geq 5$  on at least 1 slide vs summing), revealed that serous histology ( $P < .000$ ; hazard ratio, 3.770; CI, 1.865-7.621) and having a positive metastatic lymph node were associated with poorer overall survival ( $P = .022$ ; hazard ratio, 2.343; CI, 1.131-4.854).

#### Discussion

LVSI is currently an important parameter in guiding treatment and determining prognosis.<sup>5-8</sup> Not only its presence but also its extent is clinically significant. Numerous studies have

investigated the amount of LVSI.<sup>12,13,15-17</sup> The prognostic value of substantial LVSI is now widely accepted and has been incorporated into the World Health Organization 2020 classification and guidelines.<sup>6</sup> The FIGO 2023 staging system defines substantial LVSI as  $\geq 5$  foci,<sup>5</sup> although interobserver agreement among pathologists remains suboptimal,<sup>18</sup> and there is still no clear consensus on the exact cutoff number. Some studies have proposed using thresholds of  $\geq 3$ ,<sup>9,10</sup> or  $> 4$  LVSI foci.<sup>8,12,17</sup>

Regarding the commonly used LVSI cutoffs in the literature, we observed that a threshold of  $\geq 5$  vs  $< 5$  was significant in predicting lymph node metastasis in our study, both in terms of the number of metastatic lymph nodes and lymph node positivity. In contrast, cutoffs of 4 vs  $< 4$  and  $\geq 3$  vs  $< 3$  were not statistically significant. However, no difference in overall survival was observed between focal LVSI and any of the aforementioned cutoffs. Although overall survival is not the sole clinical parameter indicative of prognosis, this does not necessarily imply a lack of prognostic value. These findings suggest that, when determining a cutoff for LVSI to predict lymph node metastasis, a threshold of  $\geq 5$  foci appears to be the most appropriate.

One of the ongoing controversies in LVSI evaluation is whether the number of foci should be assessed on at least a single slide or summed across all slides. This study also aimed to address this question. Among patients with substantial LVSI ( $\geq 5$ , n = 150), there was no significant difference in lymph node metastasis or the number of metastatic lymph nodes between those with  $\geq 5$  foci on at least 1 slide (n = 124) and those with  $\geq 5$  foci only when summed across slides (n = 26). The literature on this issue remains inconclusive. For example, in the study by Peter et al.,<sup>17</sup> substantial LVSI was defined as the presence of  $\geq 4$  foci on a single slide, and pelvic lymph node recurrence was used as a clinical endpoint. In contrast, our study divided patients with substantial LVSI into 2 groups: 1 with  $\geq 5$  foci on at least 1 slide (n = 124), as in the study by Peter et al.,<sup>14</sup> and another with  $\geq 5$  foci only when summing across multiple slides (n = 26). Although no difference in lymph node positivity was observed between these 2 groups at diagnosis, overall survival analysis revealed that patients with  $\geq 5$  foci on at least a single slide had a worse clinical prognosis. These findings highlight the importance of clearly defining how substantial LVSI is determined in pathology reports. Further investigation in larger cohorts—including patients with negative LVSI, substantial LVSI across multiple slides, and substantial LVSI on a single slide—is warranted.

The International Collaboration on Cancer Reporting guideline identifies several artifacts that may mimic LVSI, the most notable

**Table 3**

Results for total lymphovascular space invasion—comparison of total LVSI with related parameters

Parameter	Comparison with total LVSI		
No. of samples having the tumor invasion front	Total LVSI increases as the no. of samples increases with correlation coefficient ( $r$ ): 0.212 $P \leq .001$		
No. of metastatic lymph node	No. of metastatic lymph nodes increases as total LVSI increases with correlation coefficient ( $r$ ): 0.316 $P \leq .001$		
Is there a lymph node metastasis? 50p (25p-75p)	Yes 16 (6-60.5)	No 7 (3-17.75)	
Is there a tumor in the cervix or endocervical canal? 50p (25p-75p)	Yes 18 (6.5-74)	No 9 (3.75-20.25)	
Tumor grade 50p (25p-75p)	Yes 7 (3-14)	No 9 (4-17)	Grade III tumors contain more total LVSI than grade I and II tumors $P \leq .001$ (1 + 2 vs 3) 3 21 (6-61)
Is there a MELF pattern? 50p (25p-75p)	Yes 11 (5-25)	No 11 (4-31)	No significant association between having a MELF pattern and total LVSI $P = .757$
LVSI localization: Is there LVSI in the cervix/endocervix? 50p (25p-75p)	Yes 40 (9-120)	No 9 (4-23)	Those with LVSI in the cervix and/or endocervical canal have more total LVSI $P \leq .001$
LVSI depth: Is there LVSI deep into the myometrium? 50p (25p-75p)	Superficial 9 (4-23)	Superficial + deep 64 (31-114)	Those containing deeper LVSI contain more total LVSI $P \leq .001$
Tumor size (cm)	As tumor size increases, the total no. of LVSI increases with correlation coefficient ( $r$ ): 0.209 $P = .04$		
No. of samples per centimeter (no. of samples having the tumor infiltration front/tumor diameter)	As no. of samples per cm increases, the total no. of LVSI increases with correlation coefficient ( $r$ ): 0.017 $P = .816$		
Tumor type	Endometrioid 9 (4-23)	Serous 23 (4.5-63.25)	Serous type has more LVSI than endometrioid type $P \leq .001$

LVSI, lymphovascular space invasion; MELF, microcystic elongated fragmented.

being autolysis-induced interpretive difficulty and intravascular seeding. In our archive, 46 cases in which evaluation was compromised due to such artifacts were excluded from the analysis. Unfortunately, endometrial autolysis remains a persistent issue, particularly in hysterectomy specimens from developing countries. It is imperative that medical centers address this issue with the utmost seriousness. When necessary, preventive measures should be implemented through close collaboration between pathologists and clinicians, supported by comprehensive training programs for technical and ancillary staff. Such an approach would not only enhance the quality and consistency of tissue processing but also minimize the occurrence of fixation-related problems that may compromise diagnostic accuracy and reproducibility.

The assessment of LVSI in this study was based on evaluation at the tumor infiltration front, in accordance with recently published practical guidelines and the recommendations outlined in checklist<sup>14</sup> (also included as *Supplementary Material*). This study sought to determine the role of an optimal sampling strategy and the required sample number in accurately

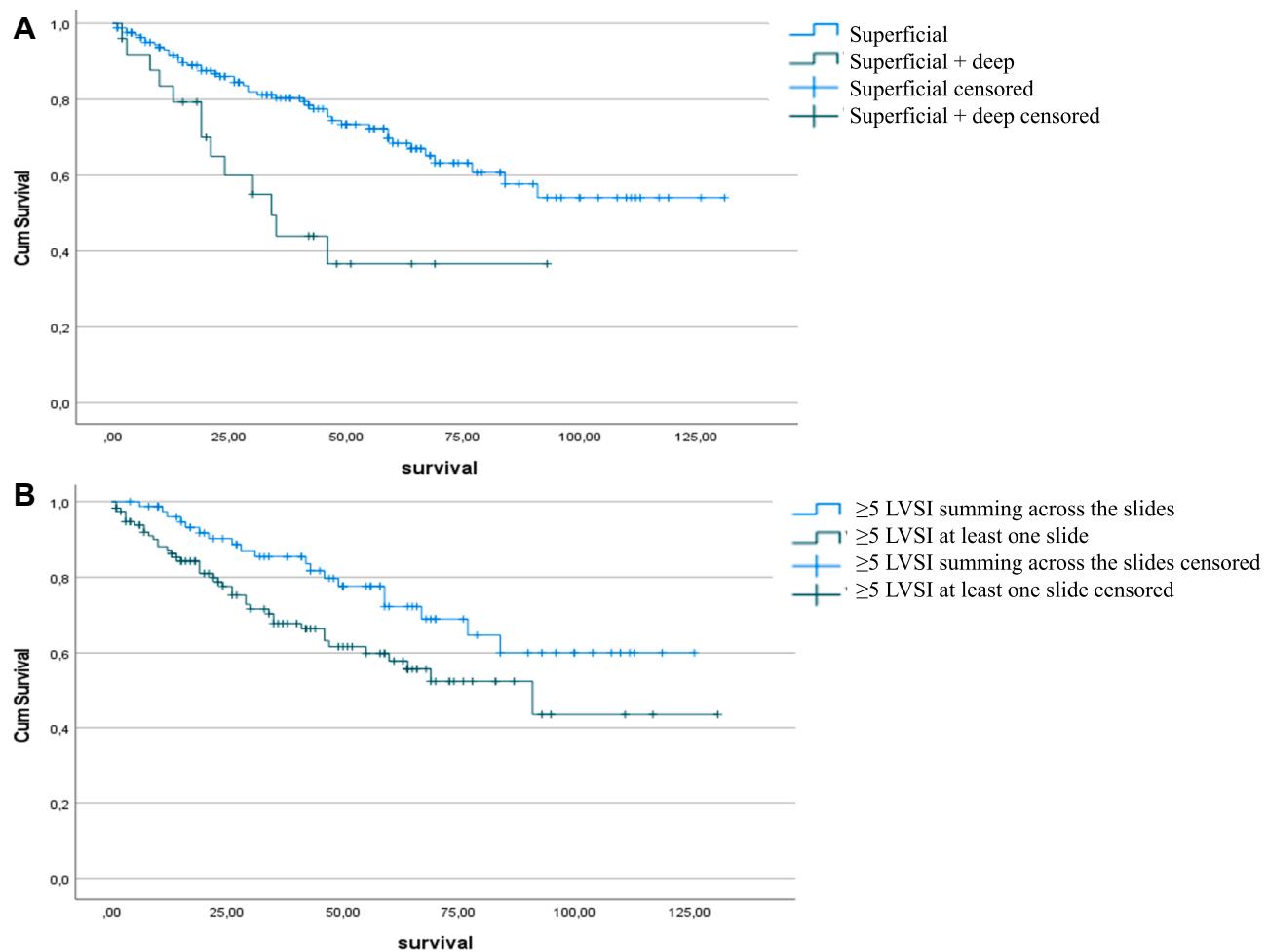
assessing substantial LVSI through a comparison of focal and substantial LVSI. We demonstrated that the number of tumor sections submitted is a relevant factor. As the number of samples containing the tumor infiltration front increased, the detection rate of LVSI also rose ( $P < .001$  correlation coefficient, 0.212). Moreover, substantial LVSI ( $\geq 5$  foci) was more frequently identified in cases with a greater number of submitted samples ( $P < .001$ ). ROC curve analysis was used to assess how many samples are necessary to meet the  $\geq 5$  and  $< 5$  cutoffs and to determine the threshold beyond which additional sampling becomes redundant. It was shown that submitting  $> 7$  tumor infiltration front samples did not further improve the detection of substantial LVSI. In practical terms, this suggests that submitting up to 7 samples is sufficient for accurate LVSI evaluation, and fewer than 7 sections—particularly in large tumors—may be inadequate. In the authors' experience, a few cases of endometrial cancer initially showed borderline lymphovascular invasion—such as 2 or 3 involved spaces—due to a limited number of submitted samples. However, upon returning to the gross room and submitting additional samples, further foci were

**Table 4**

Association of some morphological parameters with lymph node positivity and number of positive lymph nodes

Parameter	No. of cases with lymph node metastasis	no. of cases without lymph node metastasis	Comparison with the presence of lymph node metastases (P)	Comparison with the no. of metastatic lymph nodes (P)
Is there a tumor in the cervix or endocervical canal? (n = 156)				
Yes (n=57)	34 (59.6%)	23 (40.4%)	.023	<.001
No (n = 99)	39 (39.4%)	60 (60.6%)		
Is there a microcystic elongated fragmented pattern? (n = 157)				
Yes (n = 40)	18 (45%)	22 (55%)	.971	.686
No (n = 117)	55 (47%)	62 (53%)		
LVSI localization:				
Is there LVSI in the cervix/ endocervix? (n = 144)				
Yes (n= 31)	21 (67.7%)	10 (32.3%)	.017	.003
No (n = 113)	47 (41.6%)	66 (58.4%)		
LVSI depth:				
Is there LVSI deep into the myometrium? (n = 157)				
Superficial (n = 138)	59 (42.8%)	79 (57.2%)	.026	.001
Superficial + deep (n = 19)	14 (73.7%)	5 (26.3%)		

LVSI, lymphovascular space invasion.

**Figure 2.**

Kaplan-Meier curves illustrating overall survival. (A) Deep LVSI is associated with poorer survival compared with superficial LVSI. Deep myometrial LVSI is defined as LVSI located  $\geq 3$  mm beyond the tumor invasion front within the outer half of the myometrium. (B) Among cases with a total LVSI count  $\geq 5$ , survival was worse in those with  $\geq 5$  foci on at least a single slide compared with those with  $\geq 5$  foci in the overall count but distributed across multiple slides. LVSI, lymphovascular space invasion.

identified, leading to reclassification as substantial lymphovascular invasion.

This study is the first in the literature to demonstrate that the location of sampling, as well as the location and depth of LVSI, are critical factors. It was observed that when LVSI was located deep within the myometrium, the total number of LVSI foci was significantly higher. Among the 27 cases with deep LVSI, 25 (92.6%) had substantial LVSI ( $\geq 5$  foci), whereas only 2 (7.4%) had focal LVSI ( $< 5$  foci). Deep myometrial LVSI was defined as LVSI located at least 3 mm beyond the invasive tumor front in the outer half of the myometrium. This included either (1) cases where no tumor was seen in the endometrium but LVSI was detected in the outer myometrium, especially in the tumor-free endomyometrial sample adjacent to the tumor, or (2) cases with deep myometrial invasion, where LVSI was observed  $> 3$  mm beyond the deepest point of tumor infiltration into the outer myometrium (Fig. 1C, D). Tumors with deep invasion and a tumor infiltration front near the serosa were not classified as having deep LVSI. However, LVSI located near the serosa but distant from the tumor front was considered deep LVSI.

Deep LVSI was significantly associated with lymph node metastasis—both in terms of the number of metastatic lymph nodes ( $P = .001$ ) and overall lymph node positivity ( $P = .026$ ). Among the 27 patients with deep LVSI, 8 either lacked lymphadenectomy or had unknown nodal status. Of the remaining 19 patients, 14 (73.7%) had positive lymph nodes and 5 (26.3%) were negative. Although deep LVSI was also linked to poor overall survival, this association did not reach statistical significance in multivariate analysis. To our knowledge, only Matsuo et al<sup>19</sup> have previously addressed the prognostic relevance of both deep and substantial LVSI in the English-language literature. Although their study included 70 patients who were LVSI positive, our study evaluated 208 LVSI-positive hysterectomies and demonstrated the impact of deep LVSI on lymph node positivity in a larger cohort.

Similar to deep LVSI, the presence of LVSI in the cervix or endocervical canal was associated with a higher total number of LVSI foci ( $P < .001$ ). Among the 39 cases with known cervical or endocervical LVSI, 35 (89.7%) had substantial LVSI ( $\geq 5$  foci), whereas 4 had focal LVSI ( $< 5$  foci). Cervical or endocervical LVSI was also significantly associated with lymph node metastasis—both in terms of the number of metastatic lymph nodes ( $P = .003$ ) and lymph node positivity ( $P = .017$ ). Of the 31 patients with known nodal status or who had undergone lymphadenectomy, 21 (67.7%) had positive lymph nodes, whereas 10 (32.3%) had negative nodes. Nevertheless, in survival analysis, the presence of LVSI in the cervix or endocervical canal did not show a statistically significant correlation with overall survival.

This is a retrospective, single-center study in which sampling was performed in accordance with institutional guidelines. A key limitation of this study is the lack of prospective data. Due to the standardized and retrospective nature of the study, which results in low variability, it is not possible to definitively determine the minimum number of samples required to predict substantial LVSI. It also remains unclear, based on this study alone, how many samples are required from the deep myometrium or cervix/endocervix to reliably identify substantial LVSI.

In daily practice, in addition to sampling the tumor infiltration front during grossing, it is essential to include sections from the deep myometrium and cervix/endocervical canal, even if no gross tumor is visible. These samples should be carefully examined under the microscope, with particular attention to the presence

of LVSI in these regions. If LVSI is identified in the deep myometrium or cervix/endocervical canal, the tumor is likely to exhibit substantial LVSI and a higher risk of lymph node metastasis. Furthermore, the presence of deep LVSI may also indicate a poorer overall survival.

The MELF pattern has historically been considered a form of myometrial invasion associated with lymph node metastasis and aggressive tumor behavior.<sup>20,21</sup> However, current literature reflects divergent views on its prognostic significance.<sup>22,23</sup> In this study, all slides from the 208 LVSI-positive hysterectomies were re-evaluated by experienced gynecologic pathologists for the presence of the MELF pattern, based on its recently defined microscopic features and considering potential histologic mimics. The MELF pattern was not found to be significantly associated with substantial lymphovascular invasion ( $P = .536$ ) or lymph node metastasis, either in terms of the number of metastatic lymph nodes ( $P = .686$ ) or overall lymph node positivity ( $P = .971$ ).

As previously demonstrated, higher total LVSI counts and increased lymph node positivity were more frequently observed in tumors with larger diameters, higher histologic grades, and serous histology compared with endometrioid type.

The number of LVSI foci on each slide was recorded individually. The authors observed that in some hysterectomy specimens, several slides showed no LVSI, whereas 1 or 2 slides contained numerous foci, ultimately meeting the criteria for substantial lymphovascular invasion. To evaluate the effect of macroscopic sampling and the total number of slides on this distribution, a variation score was calculated to reflect interslide differences in LVSI counts. This analysis revealed that interslide variability was significantly higher in cases with greater total LVSI ( $P < .001$ ). Similarly, cases with substantial LVSI ( $\geq 5$  foci) exhibited greater variation ( $P < .001$ ). Interestingly, this variability did not increase with the number of submitted samples ( $P = .952$ ). This suggests that beyond a certain point, increasing the number of samples does not proportionally increase variability, indicating a limit to the diagnostic value of excessive sampling.

The strengths of this study include its relatively large sample size, standardized surgical sampling guided by internal protocols, and the involvement of experienced pathologists. It also addresses several controversial topics that remain underexplored in the literature. However, key limitations include its retrospective design and considerable variability in sampling among consultation cases. Moreover, the study cohort comprises only LVSI-positive cases, with comparisons limited to substantial vs focal LVSI, preventing the assessment of prognostic parameters in LVSI-negative cases.

In conclusion, this study evaluated 208 cases of endometrial cancer and underscored the importance of macroscopic sampling in the assessment of LVSI. A greater number of sections from the tumor infiltration front was associated with a higher detection rate of substantial LVSI. Although sampling beyond 7 sections did not yield significant additional benefit, submitting fewer than 7—particularly in large tumors—may be insufficient. A threshold of  $\geq 5$  vs  $< 5$  LVSI foci was significantly associated with lymph node metastasis; however, no cutoff was predictive of prognosis. Notably, identifying  $\geq 5$  foci on a single slide—rather than distributed across multiple slides—was linked to poorer overall survival. The study also recommends including sections from the deep myometrium and cervix/endocervical canal, in addition to the tumor infiltration front. The presence of LVSI in these locations may indicate both extensive lymphovascular invasion and adverse overall survival due to deep infiltration.

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## Author Contributions

A.U. and D.A. prepared the figures and tables, and wrote the manuscript and designed the study and obtained the study cohort. S.K. analyzed data. A.O. participated in obtaining the study cohort. D.A., A.U., and S.Y. participated in the study design, edited the manuscript, and prepared for submission. All authors read and approved the final paper.

## Data Availability

Original data used in this study can be requested by emailing to the corresponding author Alp Usubutun [alpusubutun@yahoo.com](mailto:alpusubutun@yahoo.com)

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## Declaration of Competing Interest

All authors have no conflict of interest.

## Ethics Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Also this study is approved by the institutional ethical committee (Approval number: GO 23/203).

## Supplementary Material

The online version contains supplementary material available at <https://pmc.ncbi.nlm.nih.gov/articles/PMC11649510/>. It is available as an LVS1 checklist.

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