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***Research Article***

# Assessment of Lymphovascular Space Invasion Extent in Endometrial Carcinoma by Serial Histological Sectioning

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**Abstract**

**Background:** The extent of lymphovascular space invasion (LVSI) is important in risk assessment for endometrial carcinoma, and pathology sampling can prove challenging. **Objective:** To assess the added value of serial histological levels in detecting LVSI, extent classification and diagnosis. **Methodology:** The study was carried out as a retrospective paired study involving 60 hysterectomy specimens from women of Basrah, Iraq. Single-level baseline and 20-level serial H&E assessment with independent review and adjudication was undertaken for Ninety blocks. Substantial LVSI > four separate distinct involvement vessels per section (not cumulative from section to section). **Results:** Of 1,800 planned levels, 1,764 (98.0%) were interpretable. Among 54 definite extent pairs, substantial LVSI increased from 8/54 (14.8%) to 15/54 (27.8%), a difference of 13.0 percentage points (95% CI, 2.6–25.2; p=0.0391). For eight cases, LVSI was reclassified as substantial; for one, to focal. There were 18 substantial cases in the full study cohort, three of which initially had indeterminate extent. Among 56 definite presence pairs, detection increased from 26/56 (46.4%) to 32/56 (57.1%; p=0.0313), with six newly positive cases. There were 21 cases where the numbers of vessels increased and three where the numbers of vessels decreased (p=0.000277). Three of four presence-indeterminate cases and four of six extent-indeterminate cases were resolved by serial assessment. In one case, secondary clarification was obtained by immunohistochemistry using ERG and CD31 as a marker, which was unavailable in the second case. **Conclusion:** Serial assessment reduced diagnostic uncertainty and serial levels improved the detection and substantial classification of LVSI. Further validation for clinical relevance and implementation is needed.

## 1. Introduction

Endometrial cancer makes a significant contribution to the global cancer burden among women [1]. While in most cases only localized diseases are encountered, it is important to be able to recognize that certain types of tumors have metastatic potential, in order to provide a prognosis. Using a clinically validated molecular classifier for Endometrial Cancer, Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), enables the identification of clinically applicable molecular classifications [2, 3]. Molecular classification should be used in conjunction with, and not in place of, morphological evaluation. An important pathological prognostic feature is lymphovascular space invasion (LVSI), which is defined as the presence of tumor cells within endothelial-lined vascular spaces outside the invasive tumor front [4]. Other than the presence or absence, lymphovascular space invasion (LVSI) conveys information on the extent. At the time of the initial investigations diffuse or multifocal involvement was linked to poor prognosis and metastasis to lymph nodes [5]. The pooled

Operative Radiation Therapy in Endometrial Carcinoma -1(PORTEC-1) and PORTEC-2 cohort analysis demonstrated that the presence of significant LVSI was an independent negative predictor for pelvic regional-relapse, distant-metastasis and overall survival while focal was not as important [6]. Later research indicated an association between significant LSVI and distant relapse in otherwise low risk endometrioid carcinomas [7], and that LSVI was prognostic in selected molecular subsets, especially in the absence of specific molecular profile [8].

This is reflected in current staging systems and management guidelines. In the 2023 International Federation of Gynecology and Obstetrics (FIGO) system, the nonaggressive histological types are classified as stage IIB when they show significant LVSI (LVSI minus 13) and appropriate molecular changes [9]. The guidelines from the 2025 ESGO–ESTRO–ESP incorporate pathological and molecular results in the risk-stratification and post-operative treatment [10]. Reliable separation of absent, focal and substantial LVSI is clinically relevant, therefore, especially around a classification threshold. Quantification remains challenging. A study that used outcomes as the basis for determining substantial LVSI suggested four or more involved vessels in a single H&E section as a threshold [11], while FIGO 2023 recommended the five-vessel threshold [9]. Explicit scoring criteria, anatomic localization and exclusion of 'diagnostic mimics' are all emphasized in practical guidance [12]. Vascular pseudoinvasion, mechanically displaced tumor fragments and retraction artifact may appear to be true LVSI [13, 14]. Detecting LVSI and classifying its extent is also complicated by a disagreement among observers [15].

The use of serial sections can be useful for evaluating equivocal areas, including visualization in sequential tissue planes to help clarify vascular continuity or whether the findings are artifact or invasion or identify unexpected vessels that are involved. Repeated appearances of one vascular channel should not, however, be included as an additional vascular channel and profiles from different levels should not be added together to allow a single-section threshold. Therefore, this study aimed to incremental value of serial H&E examination for LVSI detection, extent classification and resolving diagnostic uncertainty was assessed by using a uniform block selection procedure and well-defined rules for vessel tracking.

## 2. Methodology

### 2.1. Study Design and Case Selection

This is a paired histomorphological study of 60 cases of hysterectomy specimens with primary endometrial carcinoma documented at the Oncology Center, Basrah/ Iraq from 2024-2025. Exclusion criteria included neoadjuvant treatment, presence of metastatic carcinoma, (carcino)fibrosarcomas and insufficient tissue to evaluate the tumor–myometrium interface. The original LVSI reports were not used to decide which cases to include or which blocks to select for invasive front. The unit of analysis was the case and not the block, the slide or vascular profile. No formal power calculation was performed; the target sample size was 60 cases. The following tumor characteristics were documented: age, histotype, grade, tumor size, depth of myometrial invasion and nodal findings. All blocks containing invasive front with the adjoining myometrium were selected for analysis. The cases were separated from the cases with histologically negative sampled nodes.

### 2.2. Sectioning and H&E Examination

Each block was oriented to preserve the tumor–myometrium interface. The sections were cut to the thickness of 4 $\mu$ and were numbered and mounted on charged slides. Two hundred levels of hematoxylin and eosin (H&E) stained sections were obtained every 8 $\mu$m (odd-numbered sections) and 20 sections were retained unstained for targeted immunohistochemistry (even-numbered sections). Slides were assessed by case, block, section, and evaluated for any folds, section loss and contamination. The laboratory protocol of deparaffinization, rehydration, staining with hematoxylin, differentiation, bluing, counterstaining with eosin, dehydration, clearing and coverslipping was followed with H&E staining. Slides were screened using $\times$4 and $\times$10 objectives while suspicious profiles were evaluated with $\times$20 and $\times$40 objectives. Definite LVSI was defined as viable tumor in a vascular lumen lined by endothelium in the myometrium outside of the invasive component of the primary tumor, viewed in the context of its morphology. Mechanically displaced tumor fragments, the MELF (microcystic, elongated, fragmented) pattern of invasion without definite vascular involvement and retraction spaces were excluded [16].

### 2.3. Baseline and Serial Comparators

The baseline comparator (otherwise known as routine assessment in the tables) was the initial newly cut H&E level from each selected block. Levels were considered "interpretable" only if they appeared on a single block and the serial assessment included all these levels including the baseline level for that block. This design extracted out the increases due to additional levels to additional levels within a common block-selection strategy. It was a single level assessment per block and not a reconstruction of the original diagnostic slide set. Baseline scores were also taken and not adjusted for results from subsequent levels [17].

### 2.4. Vessel Tracking and Extent Classification

All suspicious and involved vascular profiles were photographed and matching vascular profiles were made on a level-to-level basis, based on adjacent glands, stromal landmarks, and vascular walls. All sections were scored separately for definitely involved vessels. If there were several emboli in the same lumen it was counted as one vessel. Re-identification of the same vessel at another level was not counted as an additional vessel. Anatomical independence was not assumed in the vicinity of other profiles and these were not considered separate vessels. The number of different vessels involved (case density) varied across the different blocks within each reading format; the highest number seen on any interpretable H&E section was reported for each format. No level or block summing of counts was performed. LVSI was categorized as none (count of 0 vessels), focal (count of 1–3 vessels) and substantial ($\geq$4 vessels). The four-vessel threshold was the study's operational definition, it was not used interchangeably with the FIGO five vessel definition, as an outcome derived criterion. A secondary comparison was made using a cut-off of $\geq$5 vessels but with the same number per section limit. A zero count was classified as indeterminate when suspicious findings remained unresolved. Presence and extent uncertainties were separated. In a case the LVSI presence can be definite, although the focal-substantial distinction may be indeterminate. Unresolved profiles were not automatically coded as either positive or negative [18].

### 2.5. Independent Review and Adjudication

Both pathologists independently reviewed baseline section and then the serial sections after the 4-week washout. Case was recorded and presented in an order different from the one in which they had previously occurred, with scores and original reports kept hidden as well as clinical outcomes. The third pathologist independently reviewed the disagreements for each of the two reading formats, and the original two independent scores were based on the original readings. The H&E classifications used for the paired comparisons are final adjudicated. Inter observer agreement was checked using pre-adjudication readings [19]. Potential recall bias was addressed through recoding and the washout period and readers were able to identify the single level format or multilevel format.

### 2.6. Targeted Immunohistochemistry

For equivocal profiles, ERG or CD31 immunostaining was performed to demonstrate endothelial lining, and D2-40 if lymphatic differentiation was needed to be clarified, and pan-cytokeratin if epithelial identity needed to be evaluated. Staining was performed according to standard laboratory procedures and positive and negative controls. Immunostained profiles were compared to the adjacent H and E levels. If there was no target profile found at the Immunostained level, it was considered unevaluable and not negative. The mere endothelial positivity was not seen as genuine LVSI. Immunohistochemistry did not retrospectively supplant the independently made H&E classifications [20].

### 2.7. Statistical Analysis

Analyses were performed using IBM SPSS Statistics version 25, interpretable results were used for each endpoint; indeterminate results were not coded as negative. Two-sided exact McNemar tests were used to evaluate paired proportion differences which were reported with Tango 95% confidence intervals. The exact sign test was applied for the evaluation of changes in vessel-counts and the categorical transitions were described. Raw interobserver agreement was reported with Wilson 95% confidence intervals. In the paired cases, sensitivity analyses evaluated the 5-vessel threshold and extreme-case bounds for none of the classifications. Secondary analyses were exploratory, without adjustment for multiple comparisons; statistical significance was defined at $p<0.05$.

## 3. Results

### 3.1. Patient Characteristics and Histological Material

In the study 60 cases of endometrial carcinoma were included. Of these, 49 (81.7%) were endometrioid carcinoma, 7 (11.7%) serous carcinoma and 4 (6.7%) clear cell carcinoma. Of the endometrioid carcinomas, there were 19 grade 1, 22 grade 2 and eight grade 3. There was myometrial invasion of less than half of the myometrial thickness in 38 and at least half in 22 cases. Nodal assessment was available in 40 cases (8 with metastases, and 32 without metastases in the assessed nodes). Nodal assessment was not performed in 20 cases. Clinical and histological features are shown in Table 1. Percentages are based on (n=60) for all except endometrioid grade (n=49). Of the 40 patients in which there was nodal evaluation, 8 (20.0%) had metastases and 32 (80.0%) had negative nodes evaluated. We examined ninety tumor–myometrium interface blocks one in each of 30 cases and two in each of 30 cases. Of 1,800 planned H&E levels, 1,764 (98.0%) were interpretable. A total of 36 levels were excluded due to folds, tissue loss and/or contamination. All baseline H&E levels were interpretable.

**Table 1:** Clinical and histological characteristics of the 60-case cohort

| Variable | Category | N | % |
|---|---|---|---|
| Age (years) | <50 | 12 | 20.0 |
| | 50–59 | 18 | 30.0 |
| | 60–69 | 21 | 35.0 |
| | ≥70 | 9 | 15.0 |
| Histotype | Endometrioid | 49 | 81.7 |
| | Serous | 7 | 11.7 |
| | Clear cell | 4 | 6.7 |
| Endometrioid grade | Grade 1 | 19 | 38.8 |
| | Grade 2 | 22 | 44.9 |
| | Grade 3 | 8 | 16.3 |
| Tumor size | ≤2 cm | 14 | 23.3 |
| | >2 to ≤5 cm | 34 | 56.7 |
| | >5 cm | 12 | 20.0 |
| Myometrial invasion | <50% of thickness | 38 | 63.3 |
| | ≥50% of thickness | 22 | 36.7 |
| Nodal status | Metastases in evaluated nodes | 8 | 13.3 |
| | No metastases in evaluated nodes | 32 | 53.3 |
| | Not assessed | 20 | 33.3 |

### 3.2. LVSI Extent on Baseline and Serial H&E Assessment

The single level protocol defined baseline evaluation of the block from which the blocks were selected was the routine comparator and not the original diagnostic slide set. Separate baseline/serial H&E readings were produced and the results are shown below. LVSI was rated as absent, focal or substantial in 30, 16 or 8 cases on baseline assessment. Extent was indeterminate in 6 cases: 4 were indeterminate as

to presence of LVSI, and for two, there was definite LVSI, but indeterminate extent. Of those 25, 20 evaluated as absent, 15 as focal and 18 as substantial on serial assessments. Two cases were indeterminate to an extent: one was indeterminate of presence and the other was definite of presence but unknown extent. Distributions in the framework of all the 60 cases are given in Table 2. Significant LVSI: ≥4 individual vessels with involvement in a single H&E section. Percentages use (n=60). Routine refers to a single level baseline assessment as specified in the protocol. These H&E results are adjudicated and represent the classifications. The morphological appearances of LVSI and its retraction-artifact mimic are in Figure 1 and single- and multivessel involvement of LVSI also are in Figure 2, respectively.

**Table 2:** Distribution of LVSI extent on routine and serial H&E assessment

| Locked H&E extent | Routine, n (%) | Serial, n (%) |
|---|---|---|
| Absent | 30 (50.0) | 25 (41.7) |
| Focal: 1–3 vessels | 16 (26.7) | 15 (25.0) |
| Substantial: ≥4 vessels | 8 (13.3) | 18 (30.0) |
| Indeterminate for extent | 6 (10.0) | 2 (3.3) |
| Total | 60 (100.0) | 60 (100.0) |

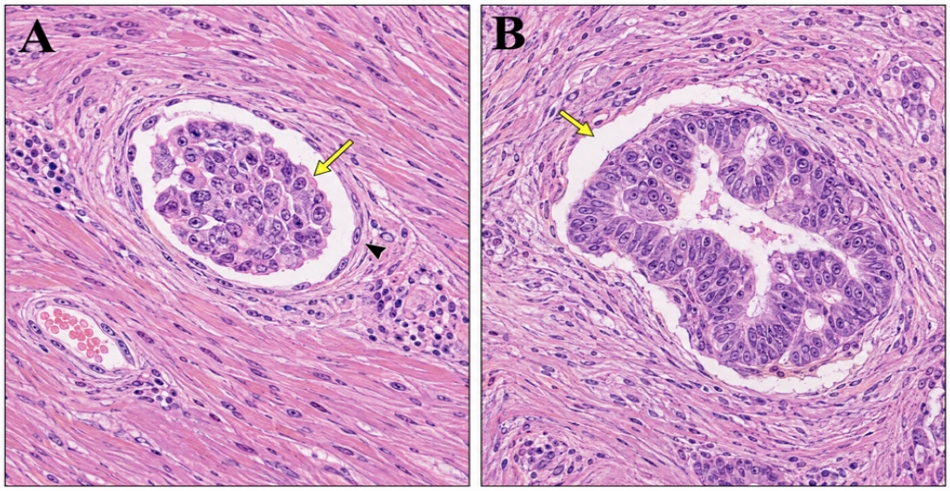



**Figure 1:** Panel A shows a tumor embolus within a vascular lumen (yellow arrow); the peripheral endothelial boundary is indicated by the arrowhead. Panel B shows a retraction space surrounding a tumor focus (yellow arrow). H&E (40X)

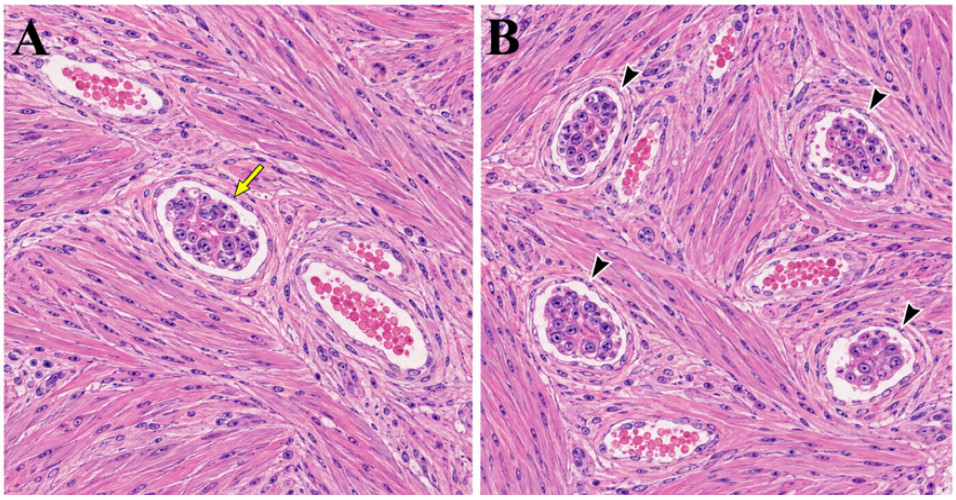



**Figure 2:** Panel A shows one involved vascular profile (yellow arrow). Panel B shows four distinct tumor-containing vascular profiles (arrowheads) within a single tissue section. H&E (40X)

### 3.3. Paired Changes in LVSI Extent

The paired analysis was restricted to the 54 cases that had definite extent classifications both reading formats. There was substantial LVSI in 8/54 (14.8%) at baseline assessment, and 15/54 (27.8%) at serial assessment. The magnitude of the serial-minus-baseline difference was 13.0 percentage points (paired 95% CI 2.6–25.2, two-sided exact McNemar p=0.0391). In the six cases in which the baseline extent was indeterminate, serial H&E agreed with it in three cases, categorized as substantial, absent, or indeterminate in one case each, respectively. There are three significant classifications that make up the difference between the paired serial total and the full cohort serial total of 18. The

reclassification of eight cases from non-substantial to substantial LVSI was seen across the separately adjudicated reading formats (two from absent, and six from focal). One case was classified as substantial at baseline and focal on serial assessment. Four cases were changed from absent to focal. There were remaining 24 cases, 10 focal cases and 7 substantial cases. These transitions refer to differences in the FSI Table 3 at the end. Figure 3 shows an example of cross level correlation of a vascular profile that contains a tumor.

**Table 3:** Paired transitions in LVSI extent among 54 definite pairs

| Routine extent | Serial: absent | Serial: focal | Serial: substantial | Total |
|---|---|---|---|---|
| Absent | 24 | 4 | 2 | 30 |
| Focal | 0 | 10 | 6 | 16 |
| Substantial | 0 | 1 | 7 | 8 |
| Total | 24 | 15 | 15 | 54 |

Rows: routine assessment; columns: serial assessment. (n=54) definite extent pairs.

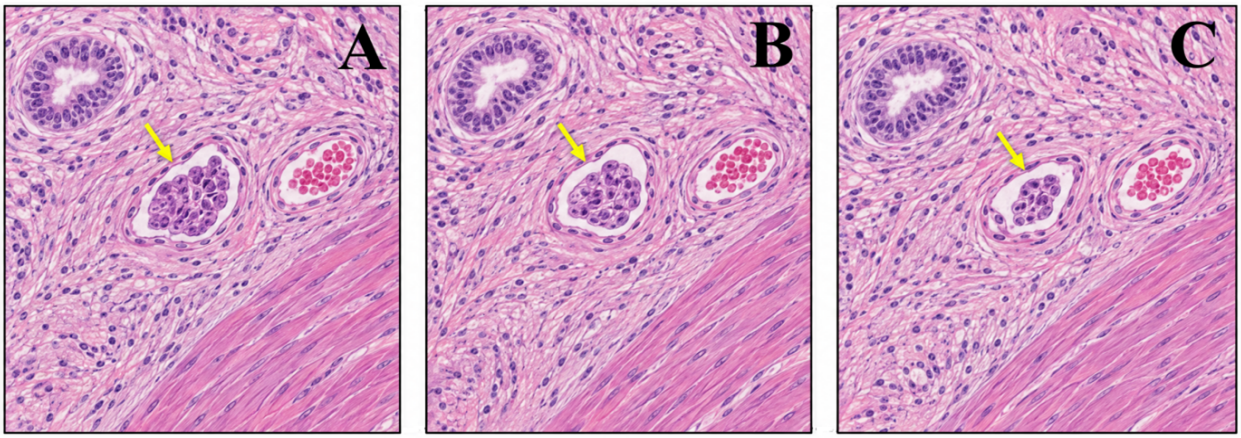



**Figure 3:** Panels A–C show a tumor-containing vascular profile (yellow arrows), with changes in its cross-sectional appearance in relation to adjacent glands and stromal landmarks. H&E (40X)

### 3.4. LVSI Presence and Changes in Vessel Count

In the total population, baseline evaluation revealed definite LVSI in 26 and definite absence of LVSI in 30 cases while 4 were indeterminate for LVSI presence. A definite diagnosis LVSI was made on serial assessment in 34 cases and definite negative in 25 cases, while one was indeterminate for LVSI. The presence-paired analysis comprised 56 cases with definite classification in both formats, including the two with definite baseline presence, but indeterminate baseline extent. LVSI was seen in 26/56 (46.4%) on baseline assessment and 32/56 (57.1%) on serial assessment. The 'serial minus baseline' difference was 10.7 percentage points (95% CI 3.6-21.5; two-sided exact McNemar p=0.0313). Table 4 shows that six cases moved from absent to present, and no cases moved from present to absent. Of the 54 definite extent pairs, 21 pairs revealed higher number of vessels assessed under the serial system, 30 had an equal number of vessels assessed and three had fewer vessels assessed. They are not additional to an unaltered base vessel count and are simply a fluency and standardized language interpretation of the differences between independently interpreted reading modes. The baseline section was added to the serial slides set but, for pairing the previously set interpretation was kept and was not used as a fixed numerical lower bound for the separate serial interpretation. Of the 24 cases with different counts, 21 had a higher number of serial and 3 had a lower number of serial counts (two-tailed exact sign test p=0.000277).

**Table 4:** Paired LVSI presence classifications among 56 definite pairs

| Routine presence | Serial: absent | Serial: present | Total |
|---|---|---|---|
| Absent | 24 | 6 | 30 |
| Present | 0 | 26 | 26 |
| Total | 24 | 32 | 56 |

(n=56) definite presence pairs. Two-sided exact McNemar p= 0.0313.

### 3.5. Pre-adjudication Interobserver Agreement

Of the 47/54 definite baseline assessments, the two pathologists agreed before adjudication for presence of LVSI in 87.0%, and of the 55/58 definite serial assessments, before adjudication, 94.8% were agreed for presence of LVSI. Agreement occurred 41 out of the 50 baseline evaluations (82.0%) and 51 out of 56 serial evaluations (91.1%) for the 3 extent categories. They disagreed over the size of the area, as reflected in the presence count (disagreement =7) and extent count (disagreement =9). Raw agreement estimates and their Wilson 95% confidence intervals are presented in Table 5. While estimates of agreement were descriptively improved with serial assessment, these estimates are based on different definite-case subsets and do not represent a paired statistical demonstration of an improvement in agreement. Levels of extent are absent, focal and substantial. Excluded (n) are cases that were not definite before adjudication for both pathologists. The

subset of cases for each value of each reading format/endpoint was retinaefic initially. These reader-agreement denominators differ from the post-adjudication paired-analysis denominators. The Wilson method is used to calculate confidence intervals.

**Table 5:** Pre-adjudication interobserver agreement

| Format / endpoint | Definite n | Agreed n | Disagreed n | Excluded n | Agreement %, 95% CI |
|---|---|---|---|---|---|
| Routine / presence | 54 | 47 | 7 | 6 | 87.0 (75.6–93.6) |
| Serial / presence | 58 | 55 | 3 | 2 | 94.8 (85.9–98.2) |
| Routine / extent | 50 | 41 | 9 | 10 | 82.0 (69.2–90.2) |
| Serial / extent | 56 | 51 | 5 | 4 | 91.1 (80.7–96.1) |

### 3.6. Resolution of Indeterminate Findings and Immunohistochemical Assessment

On routine assessment, four cases were indeterminate for LVSI, with three of which being definite on serial H&E (absent in one and present in two). None was "indeterminate" of presence. There were six indeterminate cases on routine assessment; out of these, four had definite serial classifications (one 'absent' and three 'substantial'). There were two indeterminate extents. Included in the six extent-indeterminate cases were the four cases in which the presence was indeterminate. The sequence of diagnostic resolution is shown in Table 6. IHC= immunohistochemistry. The extent-indeterminate group is the combination of the presence-indeterminate and indeterminate cases. IHC are reported independently from the locked H&E classifications. After locking the HE classification, the extent was assessed using targeted immunohistochemistry in two cases. With definite LVSI presence, but unresolved extent, the secondary interpretation was focal following clarification of endothelial boundaries. In the case where a target vascular profile was not seen on the Immunostained level, the stain was marked as unevaluable (PINALT) and this is what was recorded. The locked H&E data cases maintained their indeterminate extent status and the primary paired denominator was 54 in both cases. Characteristic H&E morphology and CD31 staining is provided in Figure 4.

**Table 6:** Resolution of indeterminate LVSI findings

| Outcome | Routine indeterminate | Resolved by serial H&E | H&E unresolved | Additional IHC resolution | Still unresolved |
|---|---|---|---|---|---|
| LVSI presence | 4 | 3 | 1 | 0 | 1 |
| LVSI extent | 6 | 4 | 2 | 1 | 1 |

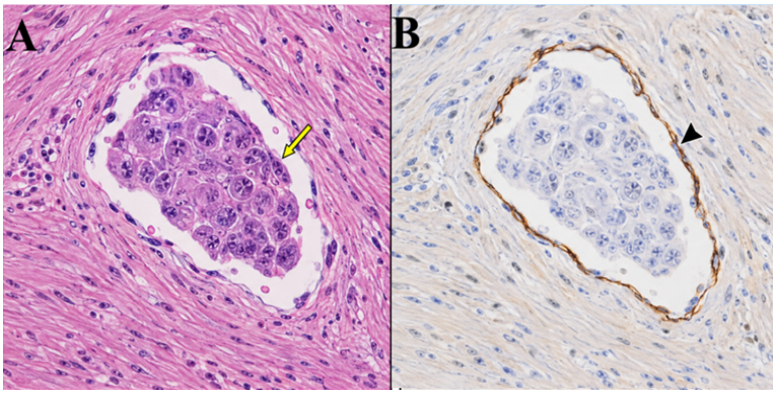



**Figure 4:** Panel A shows a tumor embolus within a vascular space (yellow arrow). Panel B shows brown CD31 staining outlining the endothelial boundary (arrowhead) (40X)

## 4. Discussion

The results of this study showed that serial levels of histology were useful in the evaluation of lymphovascular space invasion (LVSI) of endometrial carcinoma, especially in the extent of LVSI. For definite pairs, there was a significant difference of 13.0 percentage points (95% CI: 2.6-25.2, p=0.0391) for substantial LVSI between the two groups. Local invasion might thus be only partially captured by a single level. The finding is clinically relevant because there are reports showing association between LVSI and recurrence and survival [21], and between LVSI extent and nodal metastasis, with different findings for extent definition in the case of uterine serous carcinoma [22]. This observed increase may be accounted for by better delineation of the spatial relationship of tumor emboli and the accompanying vascular walls than repetitive counting of the same vascular profiles. The construction of identical blocks and the recording of tracking vessels, which were the same regardless of depths, using the number of different vessels involved in one section (not summed over different depths) favored this interpretation. Evaluating the tumor–myometrium interface accords with appropriate sampling recommendations [23]. It is important to exclude retraction clefts as well as displaced fragments as surgical artifacts can be confused with genuine vascular invasion [24].

Six cases switched from absent to present LVSI, with no reclassification from present to absent, which is reflected in the increased paired detection from 46.4% to 57.1% (p=0.0313). However, there were also changes in LVSI extent classification which did more than expose invasion, with eight determined as a substantial extent and one case was reclassified from substantial to focal LVSI. These reclassifications reflect independent interpretation rather than automatic upgrading. Separate calculation of distillation effect for the 15 main cases in the paired analysis from the 18 in the complete cohort is needed to avoid over-stating the beneficial effect. The three presence-indeterminate

and four extent-indeterminate cases were also resolved. Immunohistochemistry provided a second opinion in one of the one remaining case in which the target profile was not found. Absence from an immunostained level should therefore not be considered a negative result. Combined dual immunostaining has in the past improved on LVSI detection without any definite correlation with recurrence or survival to date [25], lending support to the selective use of dual staining in the context of morphology, rather than substituting for histological criteria.

Observed interobserver agreement for presence improved from 87.0% to 94.8% and for extent improved from 82.0% to 91.1%. This could be attributed to improved delineation and continuity of vascular profiles. But the definite-case subsets and denominators are different with the exception of a paired demonstration of improved agreement. Adopting raw agreement does not also control for chance agreement; further analyses using an appropriate agreement coefficient and confidence intervals would be more informative [26]. Best comparisons are to compare similar cases in both types of reading. There is a minimum staging threshold of 5 involved vessels, however, a minimum operational threshold of at least 4 involved vessels in one section must be maintained. However, because the threshold was not considered an alternative, it cannot be taken for granted that the observed trend will continue. Moreover, with no independent reference test, these results show an increase in detection and reclassification, and not sensitivity or specificity, in keeping with the principles of reporting diagnostic accuracy [27]. However, the prognostic implications of reclassification remain unclear. One node-negative cohort was associated with poorer survival rates with LVSI [28], while another study of LVSI in uterine-confined cases reported low rates of recurrence (and no LVSI-negative comparison group) [29]. It was associated with recurrence as well, depending on methods for assessment of nodes [30]. Twenty cases in the present study do not have a nodal evaluation and cannot therefore be classified as node negative or as increased histological detection be correlated with results of metastasis.

LVSI has to be interpreted in conjunction with other tumor characteristics. Molecular and clinicopathological factors led to an improvement in risk assessment with clinical cohorts [31]. However, the presence of prognosis and treatment differences was shown between molecular groups in adjuvant trial analyses [32], and also for radiotherapy response in early-stage endometrial carcinoma [33]. Hence, histological reclassification of this molecularly unclassified group cannot directly be used as a basis for the treatment recommendations. Within the no specific molecular profile category, there is in fact additional risk refinement that highlights its heterogeneity [34]. Treatment indications are also based on a evaluation of LVSI, stage, histotype, grade and molecular characteristics [35]. There may then be a mis-match between the improved histological information of decisions made, and the demonstration of the benefit of those decisions made, at serial levels. Only 98.0% of the levels can be interpreted as technically feasible, but not for economic utility. Examining 20 levels per block may increase workload and tissue consumption, which was not measured. Decreases in the number of vessels found in three successive assessments also need to be interpreted as standalone, rather than as accumulative, decreases from a static baseline assessment.

Advantages include a within case comparison, standardized block selection, independent interpretation and adjudication and the separation of the immunohistochemical interpretation from the primary findings. Generalizability is limited due to retrospective design, single center, limited sample size, and a lack of clinical follow-up and prospective power calculations. Importantly, serial assessment of a protocol-defined first histological level in the chosen blocks was performed and did not try to duplicate the original set of diagnostic slides. These results support further evaluation of target levels for cases of equivocation or borderline levels. When routine intensive sectioning is used, future studies are needed to determine optimal level numbers, costs, and relationship to clinical outcomes.

## 5. Conclusion

For the endometrial carcinoma, the precise pathological evaluation of invasive lymphovascular space (LVSI) is fundamental and essential factor in the effective risk stratification. Diagnostic considerations should include diagnostic certainty, distinguishing vascular invasion from mimics and standardization of terminology. In addition, pathological features should not be the only basis for treatment but along with the type of tumor, its pathological grade and stage, molecular characteristics of the tumor and the overall clinical scenario. Further, realistic performance standards and clinical significance need to be set using prospective validation studies prior to widespread use of the practice in other clinical settings and institutions.

### Article Information

**Ethical Approval:** Ethical approval was obtained from the Basra Health Directorate (reference number 642, dated April 21, 2024) prior to the commencement of the study.

**Informed Consent:** Not applicable.

**Data Availability:** The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Conflict of Interest:** The author declare no conflict of interest.

**Funding:** No external funding was received for this study.

**AI Use Disclosure:** No generative artificial intelligence tools were used in the preparation of this manuscript.

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