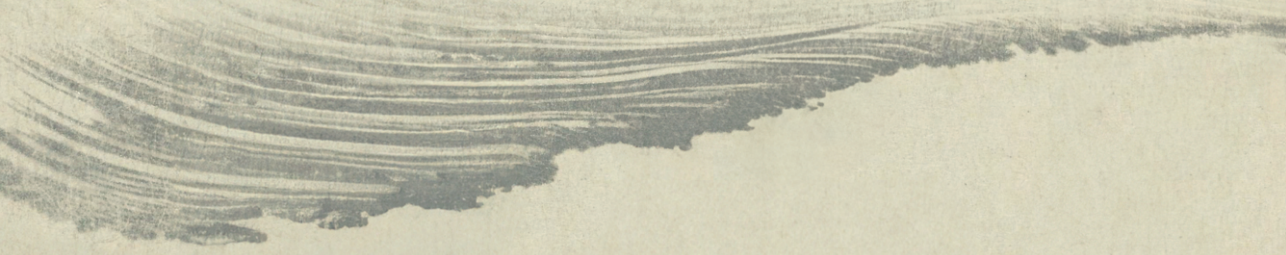
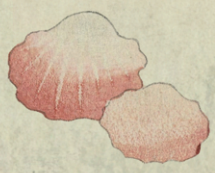
Lymphovascular space invasion in endometrial carcinoma

Peters, E.E.M.





CHAPTER 2

EXPERT OPINION: PRACTICAL GUIDANCE FOR ASSESSMENT OF LYMPHOVASCULAR SPACE INVASION IN ENDOMETRIAL CANCER

Elke E.M. Peters, Blake Gilks, W. Glenn McCluggage, Marisa R. Nucci,
Vincent T.H.B.M. Smit, Remi A. Nout, Tjalling Bosse

Submitted

Abstract

Lymphovascular space invasion (LVSI) is an important prognostic histological parameter in endometrial cancer (EC) and has gained increasing attention in the literature in recent years due to the expanding body of evidence on its independent prognostic value, especially when the presence of LVSI is quantified. The key strength of LVSI as a biomarker is that it can be detected on by routine microscopic examination, without additional ancillary tests, and so it can be assessed even in under-resourced regions. The weakness however is the lack of uniformly applied rules for assessment of LVSI, resulting in significant diagnostic interobserver variation. This is confounded by artefacts and other morphological features which may mimic LVSI (commonly referred to as pseudo-LVSI). Despite these factors, LVSI has been shown in multiple studies to be strongly associated with lymph node (LN) metastases and is an independent risk factor for LN recurrences and distant metastases. Consequently, the presence of substantial/ extensive LVSI has is an important factor in influencing adjuvant treatment recommendations. Herein, we review the current literature on LVSI in EC, discussing its role as a prognostic marker, the reproducibility of LVSI assessment and provide guidance on what represents true-LVSI as opposed to mimics and pseudo-LVSI. We provide illustrations and also discuss the current widely used three-tiered system of LVSI classification. This work is intended to provide guidance to practicing pathologists and unify the approach towards LVSI assessment in EC.

Introduction

Lymphovascular space invasion (LVSI) is an important histological prognostic parameter in many tumors, including endometrial cancer (EC), and can be visualized on routinely stained H&E- slides. LVSI is defined as the presence of tumor cells in a vascular space lined by endothelial cells [1]. Although this definition seems straightforward, LVSI assessment can be challenging due to a variety of factors, including scenarios that can mimic true LVSI. The incidence of LVSI in stage I ECs reported in the literature varies widely (3.2% to 35%) [2,3] suggesting there are local differences in LVSI assessment, for example overdiagnosis due to misinterpretation of "pseudoinvasion", or underrecognition through failure to recognize the sometimes focal nature or subtle features.

The presence of LVSI confirms that the tumor has gained critical capacities for tumor progression[4]. Consistent with this, the presence of LVSI is strongly associated with lymph node (LN) metastases in EC, and several studies have shown LVSI to be an important risk factor for LN recurrence and distant metastasis in early-stage disease. Since LVSI is an independent risk-factor it has earned a prominent position in the current risk stratification systems used for the clinical management of early-stage EC [5].

In recent years, a considerable body of evidence has shown that LVSI becomes an even more powerful prognostic factor in EC when it is quantified. This has resulted in the implementation of a semi-quantitative methodology, in which the presence of LVSI is categorized as either focal or substantial/ extensive (hereafter referred to as substantial). This approach strengthens the position of LVSI as an independent prognostic factor that should be acted upon in the adjuvant setting of early-stage disease [5]. Following on these observations, it has recently been proposed to integrate the presence of substantial LVSI into the new FIGO staging system for EC, resulting in upstaging of some cases of early-stage disease. Consequently, accurate and uniform identification of LVSI and quantification of this has become critical in clinical management of EC patients.

A relatively recent survey of members of The International Society of Gynecological Pathologists (ISGyP) showed that LVSI is reported on hysterectomy specimens by 90% of respondents, but is quantified by only 50%. We have noted significant practice differences in the interpretation of LVSI and pseudo-LVSI in our routine in-house and consultation practices and to provide guidance and attempt to unify the the assessment of LVSI in EC we herein provide guidance and outline a set of practical rules that we apply when assessing LVSI. We also provide illustrations of what represents true LVSI and of its histological mimics.

Definition of LVSI

Several terms (and abbreviations) to denote vascular invasion are used in the literature, depending on the organ and field of research. The term "intravasation" is common in basic science, whereas a multitude of terms is used in clinically orientated literature. The preferred

terms and abbreviations vary between organ systems and the lack of Medical Subject Headings (MeSH terms) does not contribute to harmonization.

LVSI is the most widely used and preferred terminology in EC and is defined as the presence of tumor cells in an endothelial-lined vascular space; in EC, it is recommended that this should be within the myometrium beyond the advancing front of the tumor since most recent publications looking at the prognostic impact of LVSI in EC have used this definition (figure 1). Several clues are helpful to recognize a lymphatic vessel. A lymphatic vessel is lined by a single layer of endothelial cells and the presence of venous and/or arterial vessels in close proximity is supportive. As is the case with most tumors, there is little evidence to support the distinction between lymphatic and blood vessel invasion and this distinction is not routinely performed. Herein we also discuss pseudo-LVSI, which is a collective term for artefacts mimickng LVSI (see *Clues to pseudo-LVSI, below*).

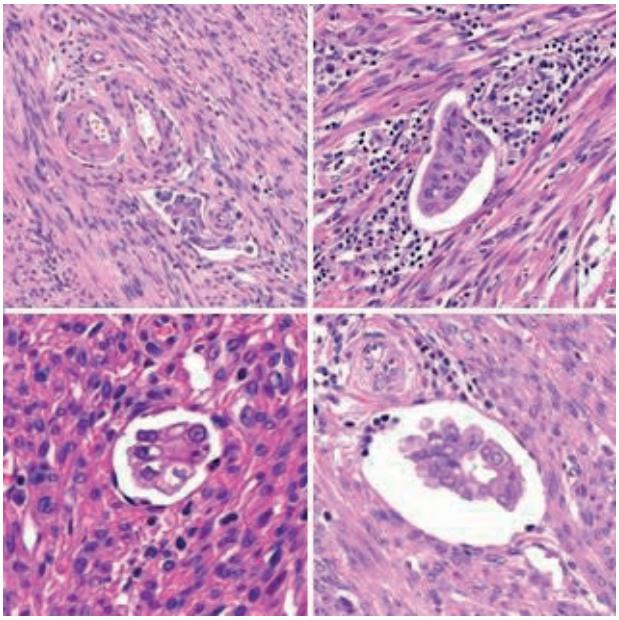


Figure 1. Examples of LVSI in EC. Cohesive clusters of tumor cells in a vascular space lined by endothelial cells. The vessels involved are situated in the myometrium beyond the invasive tumor border. Often perivascular lymphoid aggregates are found, or arterial and/or venous branches are close to the lymphatic vessel.

Features supportive for true LVSI

LVSI in EC is most commonly seen involving small vascular channels close to the invasive border of the tumor, just beyond the interface of tumor stroma and unaffected myometrium. LVSI in large blood vessels is usually not found in isolation, but in tumors with substantial LVSI involving small vascular channels, and the identification of tumor in large vessels only should result in consideration of pseudo-LVSI (see below). Tumors with broad pushing borders within the myoinvasive component are less likely to be positive for LVSI [6, 7], and with multiple patterns of myometrial invasion, LVSI is most likely to be seen where the advancing front is associated with a desmoplastic stromal response. Some clustering of LVSI is sometimes

observed, with a “spray-like” pattern (figure 2A). Clustering of LVSI makes it easier to recognize the intravascular component at low power. However, clustering may also be a result of a tortuous vessel which is cut at multiple different planes (figure 2B). Clues to this are that the vessels are of the same caliber lying in line with each other and this needs to be considered when defining the extent of LVSI (see section on *Extent of LVSI*). Because LVSI can be limited to a certain part of the tumor, extensive sampling of the tumor and surrounding myometrium may be necessary to avoid underestimating LVSI status. In accordance with most current guidelines, one section of tumor (some with adjacent myometrium) per centimeter of largest tumor diameter is required, and a minimum of four sections should be taken if possible. Submitting the entire tumor can be considered when it measures ≤ 3 cm [8].

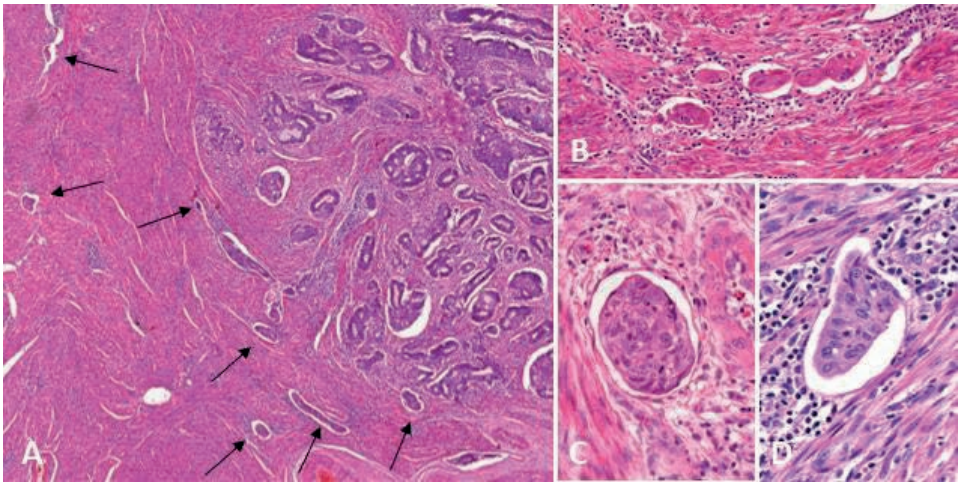


Figure 2. *2A:* LVSI is most likely to be seen near the advancing tumour front associated with a desmoplastic stromal response. Clustering of multiple vessels is often seen, appearing as a spray-like distribution of LVSI. *2B:* Example of a tortuous vessel with LVSI; due to a tortuous course it appears multiple times during sectioning. Note the same calibre and shape of both the embolus as well as the vessel. *2C:* a typical embolus has somewhat rounded shape, similar to the vessel. Tumor cells form a cohesive embolus with centrally located, rounded nuclei. *2D:* a perivascular infiltrate is often seen around involved vessels. Perivascular lymphoid infiltrates should trigger the pathologist to look for LVSI is this area.

The morphology of the tumor cells themselves may also help to distinguish between true LVSI and pseudo-LVSI. The tumor embolus in true-LVSI tends to have a smooth and rounded shape, which resembles the vessel it lies in and may sometimes be attached to the vessel wall. A true tumor embolus usually lacks any stromal elements and the tumor cells are cohesive (figure 2C). The tumor cells are “vital” with a clearly recognizable cell nucleus that is intact, surrounded by cytoplasms and shows no signs of karyorrhexis. The cells have a rounded shape and generally centrally located nucleus, which may resemble cell clusters in tissue culture. Lastly, vessels containing a true embolus are sometimes have a surrounding perivascular lymphocytic infiltrate (figure 2D). The presence of inflammatory cells (mainly lymphocytes) cuffing vessels with tumor emboli can often be appreciated at low power and can direct the attention of the

pathologist to higher power examination of foci suspicious for LVSI [9-11]; it is important to note that perivascular inflammatory cell infiltrates in the absence of LVSI are not of clinical significance [9].

Clues in support of a diagnosis of pseudo-LVSI

Awareness of the many pitfalls in LVSI assessment helps to enhance the quality and reliability of LVSI assessment. In EC, there are several frequently encountered artefacts and other factors which can mimic LVSI Artefacts can result from poor fixation and tissue preservation. To reduce factors related to poor fixation, the specimen should be transferred to the pathology laboratory as soon as possible and should be opened in the coronal plane and placed in ample formalin [8]. This reduces the likelihood of retraction artefacts and tumor displacement at grossing (figure 3A). Like necrotic tumor, autolytic tumor fragments get "transported" easily during grossing, commonly being displaced to cut surfaces, onto the serosa, and into large vessels and slit-like spaces in the myometrium. If the pathologist is aware of these artefacts, they are usually not mistaken for LVSI since the "lytic" cell changes, disorganized and fragmented clusters look like they have been wiped together.

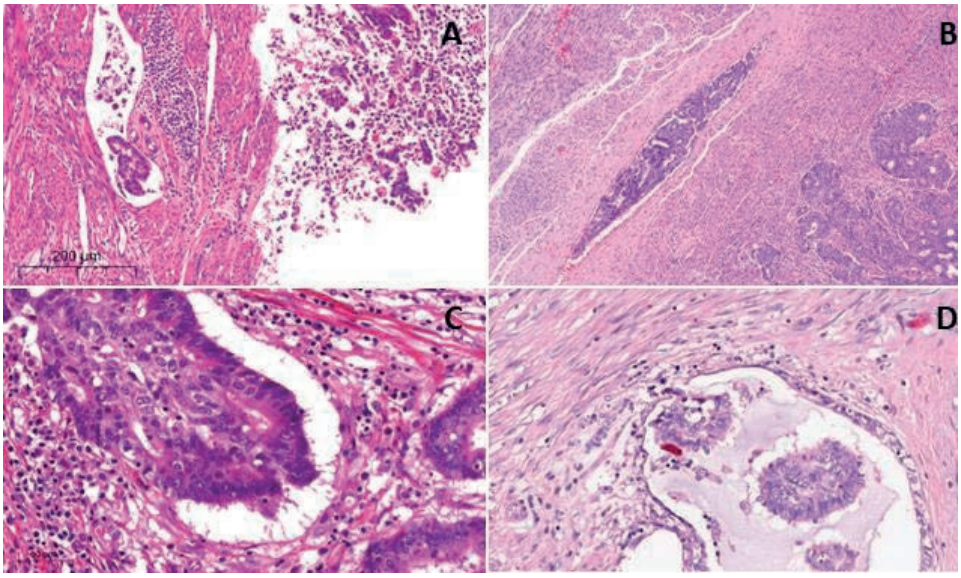


Figure 3. *3A:* lytic tumor fragments often become displaced to cut surfaces during grossing, and may also be displaced into vascular spaces. *3B:* tumor fragments pushed into vessels and in slit-like myometrial spaces. *3C:* retraction artefact shows little strands of cytoplasm between the embolus and the putative vessel wall. Also note the preserved glandular shape. **3D**: MELF type gland with partially flattened epithelial lining simulating endothelial cells. Note the delicate desmoplastic stromal response around the gland.

Another reason for tumor displacement is the use of an intrauterine manipulator during surgery. These devices are transvaginally inserted in the uterine cavity and they allow the surgeon to manipulate the uterus to improve access in the pelvis and identify anatomic structures.

However, when using the manipulator, tumor cells can be displaced into small and large vascular channels, slit-like artefactual spaces within the myometrium (which are caused by the manipulator), the lumina of the fallopian tubes or beyond (figure 3B) [12, 13]. Clues to the presence of pseudo-LVSI are that the degree of vascular involvement is often greater than that expected from the grade and stage of the tumor (for example, in a low-grade, stage IA endometrioid carcinoma, substantial LVSI would not be expected) and that the tumor emboli often preferentially involve large caliber blood vessels.

Retraction artefacts can be seen in the myometrium, forming slit-like spaces around tumor nests. These slit-like spaces can resemble a vessel and this can be challenging to distinguish from LVSI, especially since flattened myocytes can resemble endothelial cells. The following features should be considered in distinguishing between LVSI and retraction: first, be cautious about diagnosing LVSI if there are widespread obvious retraction artifacts in the tumor and the suspected focus looks similar. Second, view the embolus at high magnification and assess the border of the embolus; little strands of cytoplasm attaching the embolus to the putative vessel wall are a feature of retraction while a smooth surface suggests LVSI (figure 3C). In addition, assess the cytological features and architecture of the tumor cells; a well-retained glandular configuration with cylindrical cells and basally located nuclei support retraction.

The microcystic elongated and fragmented (MELF) pattern of myometrial invasion is associated with LVSI but can also result in mimicry of true LVSI. In MELF-pattern myometrial invasion, tumor glands may focally form microcysts lined by tumor cells which can resemble endothelial cells when they are extremely flattened and therefore become mistaken for a small vessel. Remaining glandular tumor fragments in the lumen may subsequently be interpreted as LVSI. The lining of these spaces, at least focally, typically shows obvious epithelial differentiation in the form of cuboidal cells and an epithelial, rather than endothelial lining, can be confirmed by positive staining with cytokeratin markers (figure 3D).

Use of immunohistochemistry in LVSI assessment

Routine examination of H&E-stained sections is usually sufficient to make a diagnosis of LVSI but when there is genuine doubt as to whether tumor is present within a vascular channel or not, immunohistochemistry may assist. However, as just stated this is typically not needed and will not distinguish between true LVSI and artefactual displacement of tumor emboli into vascular channels. A true vascular lining can be identified with the use of podoplanin (D2-40) or CD31 immunohistochemistry, although interpretation of these stains can be problematic, as discussed below. In one study, the detection rate of LVSI was increased threefold using a dual stain (cytokeratin AE1/AE3 and CD31) compared to evaluation of conventional H&E slides [14]. However, in another study, conventional detection of LVSI proved to be superior to immunohistochemical detection when the results were correlated with clinical outcome [15]. In addition, cancer associated fibroblasts surrounding tumors with a brisk stromal reaction often express podoplanin [16, 17] thereby making lymph vessel detection problematic. Taking

everything in consideration, the usefulness of podoplanin is limited to uncommon situations in which a diagnosis cannot be made based upon the features described above and in the absence of an intense stromal reaction. It is also important to stress that most studies that have shown LVSI to be an independent prognostic factor in EC did not use immunohistochemistry. This is important since LVSI can be adequately assessed even in under-resourced regions where immunohistochemistry is not widely available.

When the differential diagnosis is between MELF and LVSI, cytokeratin staining can confirm the presence of epithelial cells lining the space and support a diagnosis of MELF. Finally, cytokeratin immunohistochemical staining can help to discriminate between tumor cells and macrophages in rare case where there is doubt regarding the nature of the cells within a vascular space.

Reproducibility of LVSI assessment

Given the significance of substantial LVSI for individual adjuvant treatment recommendations (discussed in detail below), the diagnosis of LVSI must be reliable and reproducible. Implementation of the semi-quantitative LVSI reporting system in a series of EC cases meeting PORTEC-1 inclusion criteria [18], found substantial LVSI in 16.0% of tumors [19], around three times as many as reported in the combined PORTEC-1-2 cohorts (4.8%)[9]. Given the marked difference in the proportion of cases exhibiting substantial LVSI in the two cohorts despite similar composition, it is fair to conclude the definitions for extensive/ substantial LVSI should be improved to lead to reproducible results. The same criteria for LVSI were applied in two other studies and less divergent results were obtained for substantial LVSI (11.1% among 941 FIGO stage I EC (including endometrioid and non-endometrioid types)[20] and 4.2% among 524 FIGO stage I grade 1 and 2 endometrioid carcinomas [21]).

Reproducibility of LVSI assessment in EC was assessed in an interobserver variation study among a team of expert gynecological pathologists. The level of agreement in discriminating true LVSI from pseudo-LVSI and in assessing extent of LVSI assessment were studied. Although cases were enriched for difficult LVSI mimics, there was substantial agreement among participants in discriminating true LVSI from LVSI mimics. Assessment of the extent of LVSI resulted in substantial agreement, illustrating the definitions can be applied in daily practice for reliable semi-quantitative LVSI assessment [22].

Extent and quantification of LVSI

Review of PORTEC-1 and -2 prospective study cohorts for LVSI showed that a two-tier assessment of LVSI had the strongest prognostic value and for the first time, extent of LVSI was shown to be associated with prognosis [9]. This tow-tier system tool was adopted from a study by Fujimoto et al. and categorizes LVSI as none, focal or substantial LVSI [23]. Focal LVSI was originally defined as a single focus of LVSI beyond the invasive front while substantial LVSI was defined as diffuse or multifocal (figure 4). However, clearly this classification did not cover all

possible scenarios. The prognostic value of the two-tier system was subsequently confirmed in several other studies [20, 21, 24]. Previous retrospective studies had also shown that the risk of LN and distant metastases increased with the extent of LVSI [23, 25].

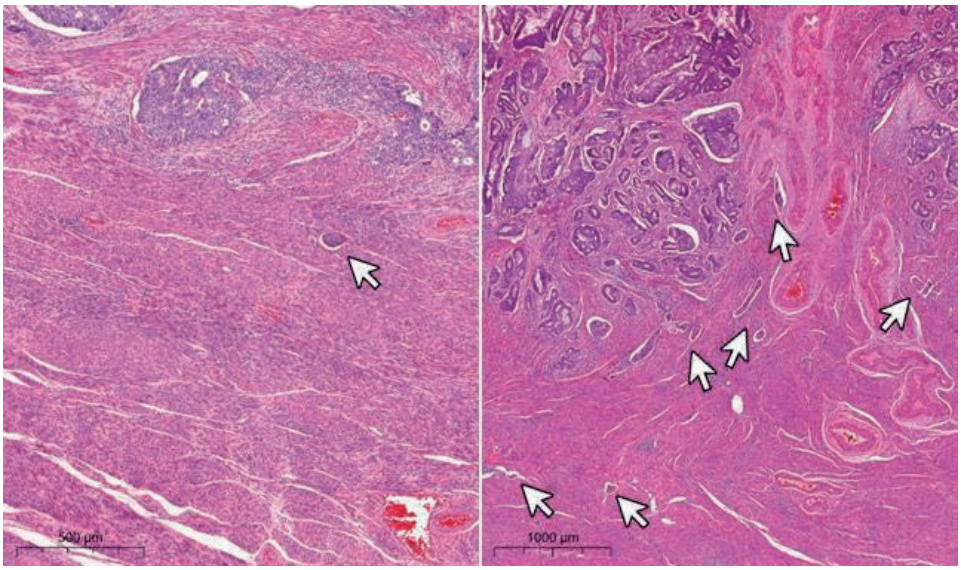


Figure 4. *Left:* in focal LVSI there are up to three foci of LVSI in a single H&E beyond the invasive front of a tumor. *Right:* substantial LVSI is charactarized by multiple foci of LVSI, usually easy to recognize and is diagnosed then at least four foci are present in a single H&E.

The definitions of focal and substantial LVSI used in the initial studies were useful but the lack of an explicit threshold for distinction between focal and substantial LVSI left room for confusion and differing interpretations in borderline cases. Both Tortorella and Winer et al. defined extensive LVSI as ≥3 vessels containing tumor emboli [21, 26] but the definitions used in those studies still lacked a sufficient level of detail. For instance, should substantial LVSI be diagnosed if the threshold of 3 vessels is met in at least one histological section, in every section with tumor, as an average per section, or cumulative of all sections? In an attempt to determine a threshold for substantial LVSI, additional analyses were done in the PORTEC-1-2 cohort and an independent validation cohort. The risk of pelvic LN recurrence was correlated with the number of emboli per histological section. Toxicity of adjuvant treatment was also taken into consideration to determine a threshold for "clinically-relevant" LVSI; the threshold should be set low enough to prevent patients at risk for regional recurrence being denied effective treatment. At the same time, the threshold should be high enough to prevent patients from being exposed to the toxicity of adjuvant treatment with limited reduction of risk of disease recurrence. In that study, it was concluded that substantial LVSI should be diagnosed when ≥4 positive vessels were present in at least one histological section [27]. To further complicate this issue, the latest World Health Organization (WHO) chapter on EC sets the threshold for substantial LVSI at ≥5 positive vessels [28]. Meanwhile, the College of American Pathologists (CAP) uses a threshold of

≥3 positive vessels in the cancer protocol template for examination of hysterectomies with EC. The International Society of Gynecological Pathologists recommends a threshold of 3 vessels while ESGO/ESTRO/ESP recommends 5, as per the WHO (5th edition, p.255 [29]). In these guidelines, it is not clear whether these figures are based on the number of vessels involved in all slides examined or in a single slide. In addition, the International Collaboration on Cancer Reporting (ICCR) recommends the semi-quantitative approach (no/focal/substantial) for LVSI reporting [1].

It is important to note that relatively few cases present any difficulty in classification as "none", "focal", or "substantial" LVSI. After publication of the paper by Bosse et al. [9] the Vancouver group adopted the semi-quantitative approach for LVSI assessment and data of LVSI scores were prospectively collected. LVSI scores of 3200 EC's were collected between 2016 and 2022 and show LVSI status was obvious (present or not) in 98% but it was inconclusive in 2% (figure 5A). Extent of LVSI was determined in 722 EC's (23%) and 71% had substantial and 29% had focal LVSI (figure 5B). The number of LVSI-positive vessels was counted in 58 tumors (figure 6), the number of cases with 3, 4 and 5 vessels containing tumor emboli, was 1, 2 and 1, respectively. This presents a challenge, however, as it means it will be difficult or impossible to generate data critically comparing a cut-off of 3 versus 4 versus 5 vessels, as the most clinically appropriate threshold. In contrast to the pragmatically choses thresholds of 3 and 5 vessels, the threshold of ≥4 positive vessels in at least one single H&E was based on an optimal balance between risk of disease recurrence versus toxicity of adjuvant treatment [27]. Given the small range these thresholds are in, none of them can be wrong, however the ≥4 vessel threshold also supports pathologists in the application of the threshold and therefore contributes to reproducibility. Therefore, substantial LVSI should be diagnosed when ≥4 positive vessels are present in at least one histological section from the uterus. and we recommend that the various protocols should be harmonized to reflect this.

Summary and future directions

The presence of and extent of LVSI has prognostic and therapeutic impact in EC depending on the stage of disease and can consequently affect patient management recommendations. We acknowledge LVSI assessment is not always straightforward, and provide an "LVSI checklist" (table 1) which we hope will be helpful in problematic cases. In some cases, deeper levelling or additional sampling may be of value and in general, we recommend in cases of uncertainty as to whether an embolus is present within a vessel to not count these foci.

The criteria we propose will reduce confusion and allow for uniform criteria to be used by all pathologists. While ongoing and future studies may refine the cut-off for substantial LVSI, there are practical considerations that will make such studies difficult, specifically the identification of sufficient numbers of cases near the diagnostic threshold between focal and substantial LVSI.

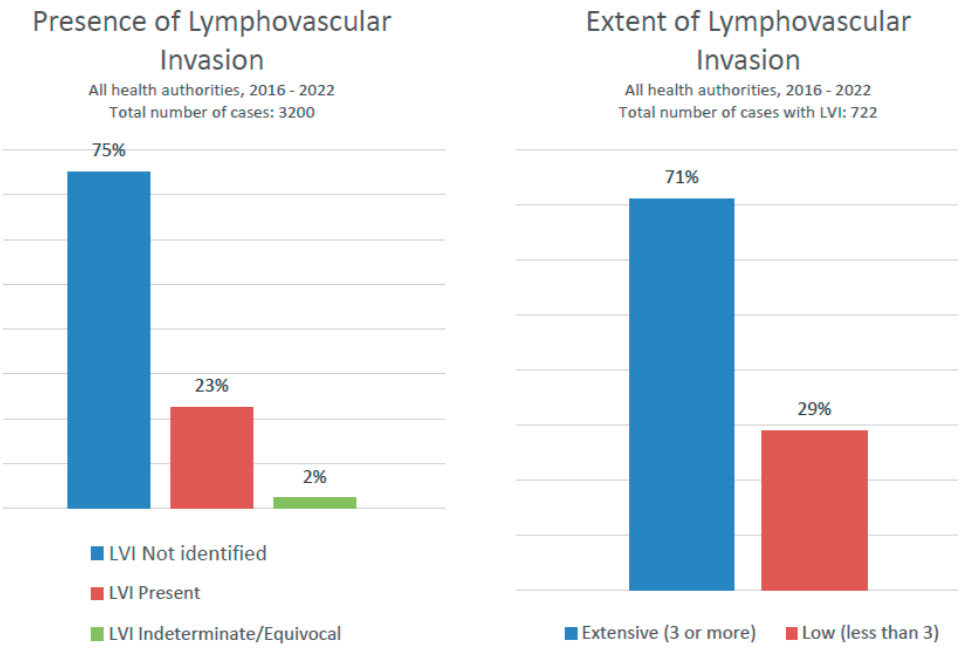


Figure 5. *5A* LVSI assessment of 3200 EC's according to LVSI status (not present, present or indeterminate). *5B*: semiquantitative LVSI assessment of 722 LVSI positive EC's.

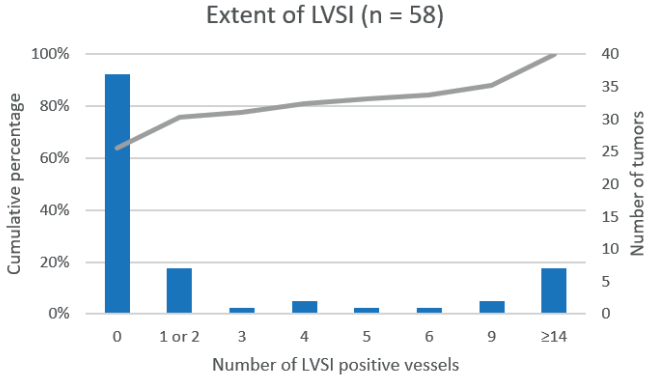


Figure 6. Quantitative LVSI assessment of 58 EC's. The number on emboli counted is on the X-axis. The right Y-axis shows the absolute count and the left Y-axis shows the cumulative percentage.

Table 1. LVSI Checklist

Clues for true LVSI	Features favoring true LVSI
Clues for artefactual LVSI	Located near invasive front
Consider IHC	Perivascular infiltrate
CD31/D2-40	Close to venous and/or arterial vessel
Pancytokeratin	Cohesive tumor embolus
Representative sampling	Molding of tumor embolus
One sample per cm tumor	Altered cell cytology
Focal LVSI	Close to infiltrative or MELF growth pattern
1, 2 or 3 emboli	More than one vessel involved
1 or 2 H&E's	
Small emboli	Features favoring pseudo-invasion
Substantial LVSI	Signs of poor fixation (autolysis) and/or spill
≥4 vessels in 1 H&E	Embolus with admixed inflammatory cells and debris
Often multiple H&E's	Close to retraction artefacts
Larger emboli	Surrounded by stromal reaction
Downgrade if in doubt	Preserved glandular structure and stroma

Summary of features in favor of LVSI and characteristics of pseudo-LVSI.

References

1. Matias-Guiu, X., C.I. Selinger, L. Anderson, et al., *Data Set for the Reporting of Endometrial Cancer: Recommendations From the International Collaboration on Cancer Reporting (ICCR).* Int J Gynecol Pathol, 2022. **41**(Suppl 1): p. S90-S118.
2. Geels, Y.P., J.M. Pijnenborg, S.H. van den Berg-van Erp, et al., *Endometrioid endometrial carcinoma with atrophic endometrium and poor prognosis.* Obstet Gynecol, 2012. **120**(5): p. 1124-31.
3. Rasool, N., A.N. Fader, L. Seamon, et al., *Stage I, grade 3 endometrioid adenocarcinoma of the endometrium: an analysis of clinical outcomes and patterns of recurrence.* Gynecol Oncol, 2010. **116**(1): p. 10-4.
4. Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* Cell, 2011. **144**(5): p. 646-74.
5. Concin, N., X. Matias-Guiu, I. Vergote, et al., *ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma.* Int J Gynecol Cancer, 2021. **31**(1): p. 12-39.
6. Stewart, C.J., B.A. Brennan, Y.C. Leung, et al., *MELF pattern invasion in endometrial carcinoma: association with low grade, myoinvasive endometrioid tumours, focal mucinous differentiation and vascular invasion.* Pathology, 2009. **41**(5): p. 454-9.
7. Wei, S., M.G. Conner, K. Zhang, et al., *Juxtatumoral stromal reactions in uterine endometrioid adenocarcinoma and their prognostic significance.* Int J Gynecol Pathol, 2010. **29**(6): p. 562-7.
8. Malpica, A., E.D. Euscher, J.L. Hecht, et al., *Endometrial Carcinoma, Grossing and Processing Issues: Recommendations of the International Society of Gynecologic Pathologists.* Int J Gynecol Pathol, 2019. **38 Suppl 1**(Iss 1 Suppl 1): p. S9-S24.
9. Bosse, T., E.E.M Peters, C.L. Creutzberg, et al., *Substantial lymph-vascular space invasion (LVSI) is a significant risk factor for recurrence in endometrial cancer--A pooled analysis of PORTEC 1 and 2 trials.* Eur J Cancer, 2015. **51**(13): p. 1742-50.
10. Yamazawa, K., K. Seki, H. Matsui, et al., *Significance of perivascular lymphocytic infiltrates in endometrial carcinoma.* Cancer, 2001. **91**(9): p. 1777-84.
11. Ambros, R.A. and R.J. Kurman, *Combined assessment of vascular and myometrial invasion as a model to predict prognosis in stage I endometrioid adenocarcinoma of the uterine corpus.* Cancer, 1992. **69**(6): p. 1424-31.
12. Delair, D., R.A. Soslow, G.J. Gardner, et al., *Tumoral displacement into fallopian tubes in patients undergoing robotically assisted hysterectomy for newly diagnosed endometrial cancer.* Int J Gynecol Pathol, 2013. **32**(2): p. 188-92.
13. Krizova, A., B.A. Clarke, M.Q. Bernardini, et al., *Histologic artifacts in abdominal, vaginal, laparoscopic, and robotic hysterectomy specimens: a blinded, retrospective review.* Am J Surg Pathol, 2011. **35**(1): p. 115-26.
14. Alexander-Sefre, F., N. Singh, A. Ayhan, et al., *Detection of tumour lymphovascular space invasion using dual cytokeratin and CD31 immunohistochemistry.* J Clin Pathol, 2003. **56**(10): p. 786-8.
15. Alexander-Sefre, F., N. Singh, A. Ayhan, et al., *Clinical value of immunohistochemically detected lymphovascular invasion in endometrioid endometrial cancer.* Gynecol Oncol, 2004. **92**(2): p. 653-9.
16. Hoshino, A., G. Ishii, T. Ito, et al., *Podoplanin-positive fibroblasts enhance lung adenocarcinoma tumor formation: podoplanin in fibroblast functions for tumor progression.* Cancer Res, 2011. **71**(14): p. 4769-79.
17. Pula, B., W. Witkiewicz, P. Dziegiel, et al., *Significance of podoplanin expression in cancer-associated fibroblasts: a comprehensive review.* Int J Oncol, 2013. **42**(6): p. 1849-57.
18. Creutzberg, C.L., W.L. van Putten, P.C. Koper, et al., *Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma.* Lancet, 2000. **355**(9213): p. 1404-11.

19. Pifer, P.M., R. Bhargava, A.K. Patel, et al., *Is the risk of substantial LVSI in stage I endometrial cancer similar to PORTEC in the North American population? - A single-institution study.* Gynecol Oncol, 2020. **159**(1): p. 23-29.
20. Restaino, S., L. Tortorella, G. Dinoi, et al., *Semiquantitative evaluation of lymph-vascular space invasion in patients affected by endometrial cancer: Prognostic and clinical implications.* Eur J Cancer, 2021. **142**: p. 29-37.
21. Tortorella, L., S. Restaino, G.F. Zannoni, et al., *Substantial lymph-vascular space invasion (LVSI) as predictor of distant relapse and poor prognosis in low-risk early-stage endometrial cancer.* J Gynecol Oncol, 2021. **32**(2): p. e11.
22. Peters, E.E.M., C. Bartosch, W.G. McCluggage, et al., *Reproducibility of lymphovascular space invasion (LVSI) assessment in endometrial cancer.* Histopathology, 2019. **75**(1): p. 128-136.
23. Fujimoto, T., H. Nanjyo, J. Fukuda, et al., *Endometrioid uterine cancer: histopathological risk factors of local and distant recurrence.* Gynecol Oncol, 2009. **112**(2): p. 342-7.
24. Barnes, E.A., K. Martell, C. Parra-Herran, et al., *Substantial lymphovascular space invasion predicts worse outcomes in early-stage endometrioid endometrial cancer.* Brachytherapy, 2021. **20**(3): p. 527-535.
25. Hachisuga, T., T. Kaku, K. Fukuda, et al., *The grading of lymphovascular space invasion in endometrial carcinoma.* Cancer, 1999. **86**(10): p. 2090-7.
26. Winer, I., Q.F. Ahmed, I. Mert, et al., *Significance of lymphovascular space invasion in uterine serous carcinoma: what matters more; extent or presence?* Int J Gynecol Pathol, 2015. **34**(1): p. 47-56.
27. Peters, E.E.M., A. Leon-Castillo, V. Smit, et al., *Defining Substantial Lymphovascular Space Invasion in Endometrial Cancer.* Int J Gynecol Pathol, 2022. **41**(3): p. 220-226.
28. *Female Genital Tumours*. 5 ed. WHO Classification of Tumours, ed. R.J. Kurman, et al. Vol. 4. 2020.