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Original Research

Prognostic significance of lymphovascular space invasion extent across molecular classes in early-stage endometrial cancer: A large retrospective analysis

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ABSTRACT

Background: The prognostic role of lymphovascular space invasion (LVSI) in endometrial cancer (EC) remains poorly defined across molecular subgroups. We evaluated the impact of LVSI extent in a large cohort of stage I–II EC classified according to molecular profiling.

Methods: Patients with stage I–II EC who underwent complete primary surgical staging were retrospectively included. According to WHO criteria, cases were classified as LVSI-negative, focal LVSI, or substantial LVSI. Molecular classification was performed using next-generation sequencing and immunohistochemistry, identifying the following groups: POLE-mutated (POLEmut), mismatch repair-deficient (MMRd), p53-abnormal (p53abn), low-risk no specific molecular profile (NSMP-LR), and high-risk NSMP (NSMP-HR).

Results: Among 2374 patients, LVSI was absent in 73.7% (n = 1750), focal in 11.5% (n = 273), and substantial in 14.8% (n = 351). Any LVSI was associated with larger tumor size, deeper myometrial invasion, and cervical involvement (all $p < 0.001$). Substantial LVSI was additionally associated with non-endometrioid histology, grade 3 disease, ER < 10%, and higher prevalence of p53abn and NSMP-HR tumors (all $p < 0.001$). On multivariable Cox analysis, substantial LVSI independently predicted worse disease-free survival in NSMP-LR, MMRd, and p53abn subgroups, whereas focal LVSI did not affect disease-free survival. No significant survival association was found in POLEmut or NSMP-HR tumors. Nevertheless, substantial LVSI was consistently associated with higher recurrence risk across all molecular classes (RR 2.5–2.8).

Conclusions: In stage I–II EC, focal LVSI does not worsen oncologic outcomes across molecular subgroups. In contrast, substantial LVSI associates with aggressive clinicopathologic features and independently predicts poorer disease-free survival in selected molecular groups, increasing recurrence risk by approximately 2.5–2.8-fold.

1. Introduction

Lymphovascular Space Invasion (LVSI) refers to the histologic identification of tumor cell clusters or emboli within lymphatic or vascular channels lined by endothelial cells in the myometrium or peritumoral stroma [1]. In endometrial carcinoma (EC), LVSI has

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consistently been associated with lymph node metastasis and decreased survival, establishing it as one of the most robust independent prognostic factors in this malignancy [2,3].

Beyond its mere presence, increasing attention has been directed toward the semiquantitative assessment of LVSI, based on the number of involved vessels identified at pathological evaluation. Although a universally accepted definition of LVSI extent is lacking, substantial/massive LVSI (commonly defined as ≥5 involved vessels according to World Health Organization [WHO] criteria [1]) has been reproducibly linked to adverse histopathologic features and unfavorable oncologic outcomes across multiple studies [2,4,5]. In contrast, the prognostic significance of focal LVSI (<5 involved vessels) remains less clear. The inconsistency observed in the published literature regarding the prognostic significance of focal LVSI likely reflects interobserver variability, susceptibility to histopathological artefacts, and heterogeneity in the cut-off definitions adopted across different studies [6].

Despite these limitations, accumulating evidence supports the indolent prognostic value of focal LVSI in early-stage EC, whereas substantial LVSI identifies a subgroup at significantly higher risk of recurrence [7]. Accordingly, LVSI extent (negative/focal vs substantial) has been incorporated into the 2023 FIGO staging system and into the ESGO-ESTRO-ESP risk classification since 2021, with confirmation in the 2025 update [8–10].

Concurrently, molecular classification has completely reshaped EC risk stratification over the past decade and is now fully integrated into international guidelines. The 2025 ESGO-ESTRO-ESP classification identifies five molecular classes with distinct prognostic behavior: POLE-mutated (POLEmut), characterized by excellent survival irrespective of concurrent unfavorable pathological features; mismatch repair-deficient (MMRd), associated with an intermediate prognosis and marked sensitivity to immunotherapy; p53-aberrant (p53abn), linked to aggressive clinicopathologic features and a high risk of recurrence; low-risk no specific molecular profile (NSMP-LR), typically displaying indolent pathological characteristics (grade 1–2 and positive estrogen receptor [ER] expression) and favorable outcomes; and high-risk NSMP (NSMP-HR), defined by either grade 3 histology or ER expression < 10%, and associated with poor survival outcomes resembling those of p53abn tumors [10–15]. This risk classification system integrates molecular features with tumor stage and histopathologic parameters, including LVSI extent [10].

However, the prognostic role of semiquantitative LVSI across distinct molecular subtypes remains poorly defined. Limited retrospective data suggest that LVSI may retain independent prognostic value primarily in tumors lacking dominant molecular drivers, such as NSMP carcinomas, whereas its impact may be attenuated in molecularly defined high- or ultra-low-risk groups [16,17]. Whether LVSI extent differentially influences outcomes within specific molecular classes - particularly in early-stage disease - remains uncertain.

The present study aims to evaluate the prognostic significance of LVSI status (negative, focal, substantial) in a large cohort of stage I–II ECs stratified according to the five molecular subgroups defined by the 2025 ESGO-ESTRO-ESP classification.

2. Methods

This retrospective, single-centre study was conducted at Fondazione Policlinico Universitario Agostino Gemelli IRCCS (Rome, Italy). We included patients with primary 2009 FIGO stage I–II endometrial cancer who underwent complete surgical staging at our institution between February 2017 and January 2025.

The primary objective was to evaluate potential differences in 3-year disease-free survival (DFS) across three LVSI categories (negative, focal, and substantial) in the overall population and within molecular subgroups.

LVSI was classified according to the World Health Organization (WHO) criteria [1]: LVSI-negative (no evidence of vascular invasion), LVSI-focal (involvement of 1–4 vessels on at least one histological slide), and LVSI-substantial (involvement of ≥5 vessels on at least one histological slide). All pathological specimens showing evidence of LVSI (focal or substantial) were reviewed by two expert gynecologic pathologists (GFZ and AS). For patients who underwent surgery before the introduction of the WHO definition, pathological slides were retrospectively re-evaluated by the same pathologists (GFZ and AS). In cases of discordance, consensus was reached through case-by-case joint review.

Molecular classification was performed using immunohistochemistry (IHC) to assess mismatch repair (MMR) status, p53 expression, and hormone receptor status (estrogen receptor [ER] and progesterone receptor [PR]), as detailed in the Supplementary material. POLE mutational status was assessed using next-generation sequencing (NGS). Given the retrospective nature of this study, POLE testing was available in approximately half of the study population. However, previous evidence has shown that IHC-based classification models provide risk stratification and survival prediction largely comparable to sequencing-based approaches such as ProMisE [18]. Furthermore, given the rarity of multiple-classifier cases described in the literature - particularly those harboring a pathogenic POLE mutation (POLEmut–MMRd, POLEmut–p53abn, POLEmut–MMRd–p53abn), which account for only 0.3–0.9% of the endometrial cancer population [19] - the potential presence of POLEmut multiple-classifiers was considered negligible. Importantly, in patients without POLE sequencing, tumors classified as NSMP should be interpreted as NSMP-like tumors rather than fully ProMisE-defined NSMP tumors. This acknowledgment is particularly relevant because a small proportion of tumors classified as NSMP-like may represent undetected POLEmut tumors. Therefore, while acknowledging that the POLEmut subgroup number might be partially underestimated, patients were classified based on 2025 ESGO-ESGO-ESP nomenclature: (1) POLE-mutated (POLEmut); (2) MMR-deficient (MMRd); (3) p53-abnormal (p53abn); (4) low-risk no specific molecular profile (NSMP-LR; G1–G2 and ER ≥10%); and (5) high-risk NSMP (NSMP-HR; G3 and/or ER <10%). Tumors with multiple molecular classifiers were assigned according to the most recent ESGO 2025 recommendations [10].

In accordance with international guidelines [9,10], surgical staging consisted of total hysterectomy with bilateral salpingectomy, with or without bilateral oophorectomy, combined with lymph node staging performed either by sentinel lymph node mapping or systematic pelvic lymphadenectomy.

Patients with incomplete clinical or pathological data, incomplete surgical staging, non-epithelial histology, synchronous malignancies, or receipt of neoadjuvant therapy were excluded.

The study was approved by the FPG Ethics Committee (IRB ID: 7130). All patients provided authorization for the use of clinical and pathological data for research purposes. A dedicated electronic case report form (eCRF) was developed using REDCap to collect study variables. Follow-up data were obtained through review of clinical records and, when necessary, direct contact with patients or closer relative in the event of death.

Standard descriptive statistics were used to summarize the distribution of study variables. Continuous variables were presented as median and interquartile range (IQR), whereas categorical variables were reported as absolute frequencies and percentages. Baseline clinicopathologic and molecular characteristics were compared across LVSI groups using the χ^2 test or Fisher's exact test for categorical variables and the Kruskal–Wallis test or Mann–Whitney U test for continuous variables, as appropriate.

Disease-free survival (DFS) was estimated using the Kaplan–Meier method, and survival curves were compared using the log-rank test. DFS was defined as the time from primary surgery to the date of disease recurrence or progression. Univariable and multivariable Cox proportional hazards models were fitted to assess the prognostic impact of LVSI extent and other clinicopathologic variables on DFS. Patients with

missing data for variables included in a specific multivariable model were excluded from that analysis using a complete-case approach. Multivariable Cox models included a priori clinically relevant covariates and variables associated with DFS in univariable analysis. In the overall study population, the multivariable model included LVSI category, molecular class, age (continuous, per 1-year increase), tumor grade, depth of myometrial invasion, and adjuvant treatment. Variables showing strong collinearity with molecular classification or other pathological parameters were not simultaneously included in the multivariable model. To evaluate whether the prognostic effect of LVSI differed across molecular classes, an interaction term between LVSI and molecular class was introduced into the multivariable Cox model and formally tested using the likelihood ratio test. Subgroup-specific multivariable Cox models were also fitted within the most prevalent molecular classes. Absolute recurrence risk at 36 months according to LVSI extent and molecular class was estimated from the multivariable Cox model using model-based survival predictions derived from the fitted model. All reported p-values were two-sided, and p-values < 0.05 were considered statistically significant. Statistical analyses were performed using R software version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria). Survival analyses were conducted using the survival and survminer packages.

3. Results

A total of 2374 patients were retrospectively included: 73.7% (n = 1750) were LVSI-negative, 11.5% (n = 273) LVSI-focal, and 14.8% (n = 351) LVSI-substantial. Interobserver disagreement in LVSI scoring between the two dedicated gynecologic pathologists occurred in 4.0% of LVSI-positive cases (Supplementary Table 9). Molecular classification was distributed as follows: POLEmut 4.4% (n = 105), MMRd 29.2% (n = 694), p53abn 10.3% (n = 245), NSMP-LR 48.1% (n = 1141), and NSMP-HR 8.0% (n = 189). Presence of LVSI (focal or substantial) was associated with larger tumor size, deeper myometrial invasion, and cervical involvement (all $p < 0.001$). LVSI-substantial was further associated with non-endometrioid histology, grade 3 disease, ER < 10%, and higher rates of p53abn and NSMP-HR molecular subgroups (all $p < 0.001$). MMRd tumors were more frequent in LVSI-focal and LVSI-substantial groups compared with LVSI-negative, whereas NSMP-LR predominated in LVSI-negative and LVSI-focal tumors. Among tumors with available POLE testing, POLEmut tumors were evenly distributed across LVSI categories. Adjuvant treatment was administered in 92.9%

Table 1
Characteristics of the study population.

Characteristic	Total2374	No LVSI1750 (73.7)	Focal LVSI273 (11.5)	Substantial LVSI351 (14.8)	p-value
Age at diagnosis (years)	62.0(54.0–70.0)	61.0(53.0–69.0)	62.0(57.0–70.0)	64.0(58.0–72.0)	< 0.001
BMI (kg/m²) Missing	28.1(23.9–33.7)7	28.1(23.9–34.0)6	28.5(24.1–33.3)0	27.5 (23.6–32.4)1	0.281
Tumor dimension (mm) Missing	30.0 (20.0–40.0)165	25.0(17.0–38.0)154	35.0 (28.0–50.0)6	40.0(30.0–52.0)5	< 0.001
Histology					
Endometrioid	1947 (87.0)	1467 (88.4)	227 (87.3%)	253 (79.3%)	< 0.001
Non-endometrioid	292 (13.0%)	193 (11.6%)	33 (12.7%)	66 (20.7%)	
Missing	135	90	13	32	
FIGO grade					
Grade – 1	394 (16.6%)	385 (22.0%)	5 (1.8%)	4 (1.1%)	< 0.001
Grade – 2	1324 (55.8%)	1000 (57.2%)	173 (63.4%)	151 (43.0%)	
Grade – 3	655 (27.6%)	364 (20.8%)	95 (34.8%)	196 (55.9%)	
Missing	1	1	0	0	
Myometrial invasion					
none	325 (13.7%)	325 (18.6%)	0 (0.0%)	0 (0.0%)	< 0.001
< 50%	1205 (50.8%)	1026 (58.7%)	87 (31.9%)	92 (26.2%)	
≥ 50%	841 (35.5%)	396 (22.7%)	186 (68.1%)	259 (73.8%)	
Missing	3	3	0	0	
Cervical stroma invasion					
no	2155 (90.8%)	1644 (93.9%)	229 (83.9%)	282 (80.3%)	< 0.001
yes	219 (9.2%)	106 (6.1%)	44 (16.1%)	69 (19.7%)	
ER status					
< 10%	244 (10.6%)	147 (8.6%)	37 (13.9%)	60 (17.9%)	< 0.001
≥ 10%	2059 (89.4%)	1554 (91.4%)	229 (86.1%)	276 (82.1%)	
Missing	71	49	7	15	
Molecular class					
POLEmut	105 (4.4%)	73 (4.2%)	10 (3.6%)	22 (6.3%)	< 0.001
MMRd	694 (29.2%)	460 (26.2%)	102 (37.4%)	132 (37.6%)	
p53abn	245 (10.3%)	161 (9.2%)	28 (10.3%)	56 (16.0%)	
NSMP-LR	1141 (48.1%)	943 (53.9%)	108 (39.5%)	90 (25.6%)	
NSMP-HR	189 (8.0%)	113 (6.5%)	25 (9.2%)	51 (14.5%)	
Lymph node staging					< 0.001
SLN only	2100 (88.5%)	1577 (90.1%)	224 (82.1%)	299 (85.2%)	
SLN plus pelvic	101 (4.3%)	63 (3.6%)	21 (7.7%)	17 (4.8%)	
SLN plus pelvic plus aortic	3 (0.1%)	2 (0.1%)	-	1 (0.3%)	
Pelvic only	157 (6.6%)	103 (5.9%)	24 (8.8%)	30 (8.5%)	
Pelvic plus aortic	13 (0.5%)	5 (0.3%)	4 (1.5%)	4 (1.1%)	
Postoperative management					
follow-up	1186 (50.0%)	1106 (63.2%)	55 (20.1%)	25 (7.1%)	< 0.001
BRT alone	407 (17.1%)	288 (16.5%)	87 (31.9%)	32 (9.1%)	
EBRT w/out BRT	373 (15.7%)	124 (7.1%)	70 (25.6%)	179 (51.0%)	
CT + EBRT w/out BRT	290 (12.2%)	146 (8.3%)	54 (19.8%)	90 (25.6%)	
CT	118 (5.0%)	86 (4.9%)	7 (2.6%)	25 (7.2%)	

Abbreviations: LVSI, lymphovascular space invasion; BMI, body mass index; ER, estrogen receptor; POLEmut, POLE-mutated; MMRd, mismatch repair deficient; p53abn, p53-aberrant; NSMP-LR, low-risk No Specific Molecular Profile; NSMP-HR, high-risk No Specific Molecular Profile; BRT, vaginal brachytherapy; EBRT, external beam radiotherapy; CT; chemotherapy; SLN, sentinel lymph node. Missing values are explicitly reported only for variables with incomplete information. Variables without a corresponding "Missing" row had complete data available. P-values for categorical variables with sparse expected counts were calculated using Fisher's exact test with Monte Carlo simulation.

of LVSI-substantial, 80.6% of LVSI-focal, and 37% of LVSI-negative patients. Details on study population characteristics are reported in Table 1. Median follow-up was 34.1 months (reverse Kaplan–Meier method).

In the overall cohort, 3-year DFS was 94.5% (95% CI 93.3–95.8) in LVSI-negative, 92.6% (95% CI 89.1–96.3) in LVSI-focal, and 76.7% (95% CI 71.7–82.1) in LVSI-substantial patients. DFS did not differ significantly between LVSI-negative and LVSI-focal ($p = 0.082$), whereas LVSI-substantial was associated with worse DFS ($p < 0.001$), Fig. 1A. Across specific molecular settings, LVSI-substantial was significantly associated with poorer DFS in the NSMP-LR, MMRd, and p53abn subgroups, while LVSI-focal had no prognostic impact, with only a trend toward reduced DFS in the p53abn ($p = 0.054$), Fig. 1**B**−**1D**. No significant DFS differences were observed across LVSI categories in POLEmut and NSMP-HR tumors, Fig. 1**E-1F**.

At multivariable Cox analysis of the overall study population (Table 2), LVSI-substantial independently predicted worse DFS (HR 3.37, 95% CI 2.28–5.00), whereas LVSI-focal was not prognostic. Molecular classification retained independent prognostic value, with poorer outcomes observed in p53abn (HR 9.10), NSMP-HR (HR 4.44), and MMRd (HR 2.05) compared to NSMP-LR. Myometrial invasion and adjuvant chemoradiotherapy were also independent predictors. Interaction analysis between LVSI and molecular class (Supplementary Table 1) showed no statistically significant interaction, either when modelling LVSI as three-tiered (negative/focal/substantial; $p = 0.19$) or using a binary definition (negative+focal vs substantial; $p = 0.39$), supporting a consistent adverse prognostic effect of LVSI-substantial across subgroups. In the NSMP-LR, MMRd, and p53abn molecular subgroups, subgroup-specific multivariable Cox models confirmed the independent adverse prognostic impact of substantial LVSI, whereas focal LVSI showed no significant association with survival (Supplementary Tables 2–4). An exploratory multivariable Cox analysis was performed within the NSMP-HR subgroup. After adjustment for age and depth of myometrial invasion, no statistically significant association between LVSI extent and DFS was observed in this subgroup (Supplementary Table 5).

A separate subgroup analysis restricted to patients with stage I–II low-grade endometrioid endometrial carcinoma showed that substantial LVSI remained independently associated with worse DFS after adjustment for age and depth of myometrial invasion, whereas focal LVSI was not associated with increased recurrence risk (Supplementary Table 7 and Supplementary Figure 1). Three-year DFS rates were 97.2% for LVSI-negative tumors, 96.0% for focal LVSI, and 88.1% for substantial LVSI.

Model-derived absolute 36-month recurrence risk confirmed a higher predicted risk associated with substantial LVSI across all molecular classes, with a relative risk of recurrence 2.5–2.8-fold higher. (Table 3).

In Table 4, we reported the distribution of risk classes according to 2025 ESGO-ESTRO-ESP recommendations [10] and postoperative management across molecular classes.

In addition, the distribution of recurrence patterns - classified as local, lymph nodal, distant, and multifocal – has been presented in Supplementary Table 8. No statistically significant differences in recurrence patterns according to LVSI extent were observed in the present cohort.

4. Discussion

4.1. Summary of the main results

In our large series of surgically staged stage I-II ECs (pN0), presence of LVSI (any extent) was associated with larger tumor size, deeper myometrial invasion, and cervical involvement; while LVSI-substantial was further associated with non-endometrioid histology, grade 3 disease, ER < 10%, and higher rates of p53abn and NSMP-HR molecular subgroups. In multivariable analysis, substantial LVSI independently predicted worse DFS in the overall cohort and in the NSMP-LR, MMRd, and p53abn subgroups, whereas focal LVSI was not associated with DFS impairment. No significant association between LVSI extent and survival was observed in POLEmut and NSMP-HR groups. However, substantial LVSI was consistently associated with increased recurrence risk across all molecular classes (RR 2.5–2.8).

4.2. Results in the context of published literature

Our findings are consistent with and expand the current evidence supporting the prognostic relevance of LVSI extent in early-stage EC. The adverse prognostic impact of substantial LVSI has been widely demonstrated in several landmark analyses. In particular, the pooled PORTEC−1 and PORTEC−2 trials established substantial LVSI as one of the strongest predictors of distant relapse and poor survival outcomes in early-stage EC [2], leading to its incorporation into modern risk stratification systems [9,10] and the 2023 FIGO staging revision [8]. Subsequent retrospective studies have confirmed that extensive lymphovascular invasion correlates with aggressive tumor biology and independently predicts recurrence and survival outcomes[4,5,20].

In contrast, the clinical significance of focal LVSI has remained debated. Earlier studies produced conflicting results, partly due to heterogeneous pathological definitions, limited cohort sizes, and interobserver variability in focal LVSI detection [21,22]. However, more recent large retrospective series have increasingly suggested that focal LVSI behaves similarly to LVSI-negative disease, whereas substantial LVSI remained an independent predictor of recurrence and survival impairment [7].

Our results strongly confirm these findings in a large population including all histologic subtypes and stage I-II disease, further reinforcing the concept that limited lymphovascular involvement does not carry the same biological and prognostic significance as extensive/massive lymphovascular dissemination.

The integration of molecular classification into EC risk stratification has raised the question of whether traditional pathological factors such as LVSI retain prognostic value within molecularly defined subgroups. Evidence addressing this issue remains limited. In the KimBer cohort, Siegenthaler et al. showed that presence of LVSI was associated with recurrence in MMRd, p53abn, and NSMP tumors, but remained an independent prognostic factor only in NSMP cancers [16]. However, the analysis by Siegenthaler et al. was likely underpowered to adequately assess the prognostic impact of LVSI extent across molecular subgroups, as only approximately 40% of the cohort underwent semiquantitative LVSI assessment. Loukovaara et al. recently evaluated the molecular subgroup-specific prognostic value of semiquantitative LVSI in stage I–II endometrioid endometrial carcinoma. In that study, the prognostic impact of LVSI varied across molecular subgroups: both focal and substantial LVSI were associated with worse outcomes in MMRd tumors, only substantial LVSI was prognostic in NSMP tumors, and neither focal nor substantial LVSI reached prognostic significance in p53abn tumors. Our findings partly differ from those results, particularly in the MMRd subgroup, where substantial LVSI retained an independent adverse prognostic impact, whereas focal LVSI was not associated with worse DFS. This discrepancy may be explained by differences in cohort composition, event rates, follow-up duration, and postoperative management. In our MMRd population, the event rate was relatively low, particularly among patients with focal LVSI, which may have limited the statistical power to detect a more modest prognostic effect of focal LVSI. Moreover, differences in adjuvant treatment allocation, including a higher use of EBRT and combined chemoradiation in our cohort, may have contributed to the more favorable outcomes observed in this subgroup [17]. Of note, as both Siegenthaler and Loukovaara studies were conducted prior to the publication of the 2025 ESGO–ESTRO–ESP guidelines, the NSMP subgroup had not yet been further stratified according to ER expression and tumor grade.

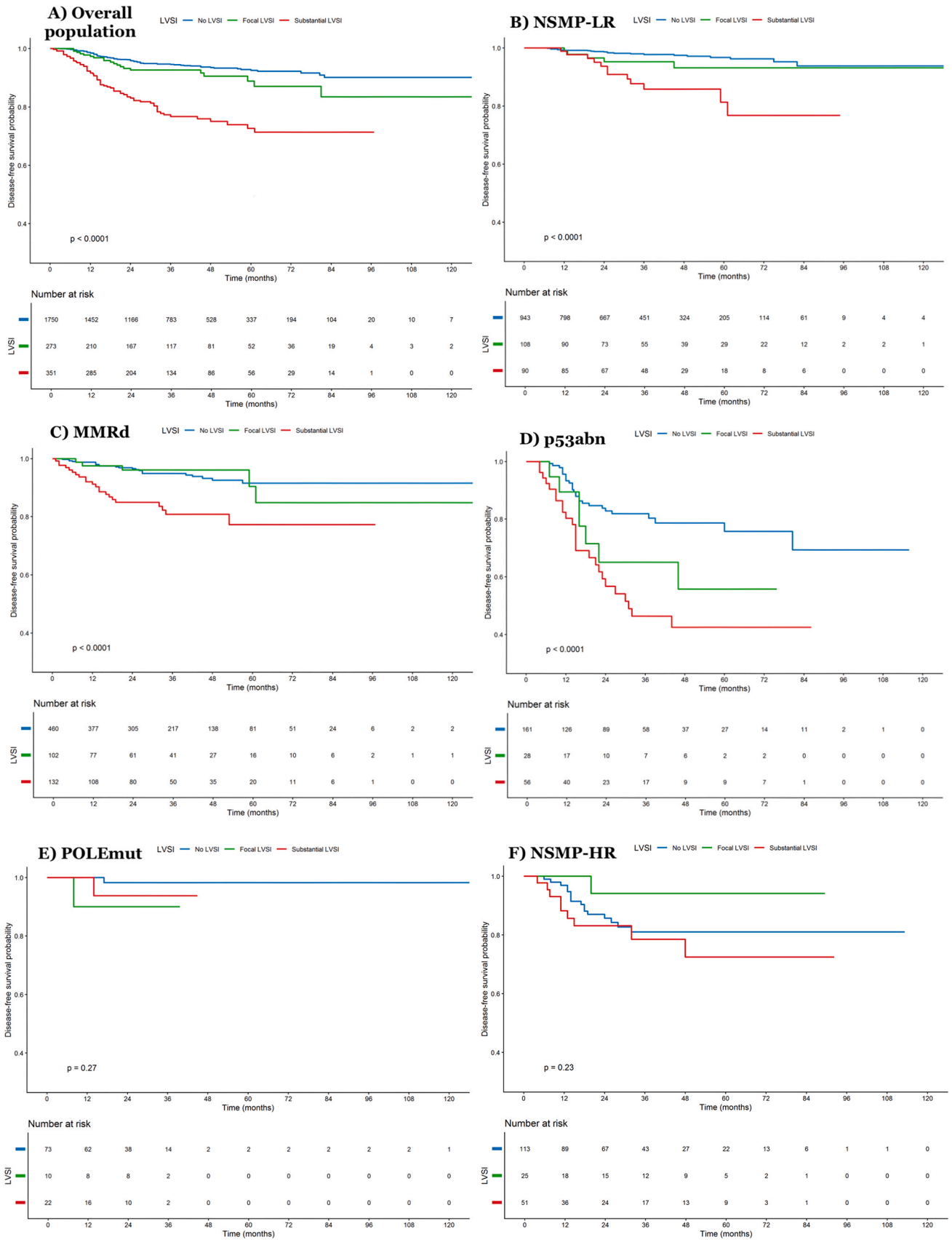

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Fig. 1. Kaplan Meier survival analysis for disease-free survival for the overall study population and molecular subgroups. A ***(Overall population)***. 3-year DFS: LVSI-negative 94.5% [95% CI 93.3–95.8], LVSI-focal 92.6% [95% CI 89.1–96.3], LVSI-substantial 76.7% [95% CI 71.7–82.1]. LVSI-negative vs LVSI-focal p: 0.082; LVSI-negative vs LVSI-substantial p: < 0.001; LVSI-focal vs LVSI-substantial p: < 0.001. B ***(NSMP-LR).*** 3-year DFS: LVSI-negative 97.7% [95% CI 96.6–98.9], LVSI-focal 95.2% [95% CI 90.7–99.9], LVSI-substantial 85.8% [95% CI 77.9–94.5]. LVSI-negative vs LVSI-focal p: 0.192; LVSI-negative vs LVSI-substantial p:< 0.001; LVSI-focal vs LVSI-substantial p: 0.101. C ***(MMRd)***: 3-year DFS: LVSI-negative 94.9% [95% CI 92.6–97.2], LVSI-focal 96.1% [95% CI 91.8–100.0], LVSI-substantial 80.8% [95% CI 73.5–88.8]. LVSI-negative vs LVSI-focal p: 0.875; LVSI-negative vs LVSI-substantial p: < 0.001; LVSI-focal vs LVSI-substantial p: 0.023. D ***(p53abn)****:* 3-year DFS: LVSI-negative 81.8% [95% CI 75.4–88.9], LVSI-focal 65.1% [95% CI 45.8–92.4], LVSI-substantial 46.4% [95% CI 33.5–64.2]. LVSI-negative vs LVSI-focal p: 0.054; LVSI-negative vs LVSI-substantial p: < 0.001; LVSI-focal vs LVSI-substantial p: 0.469. E ***(POLEmut)****:* 3-year DFS: LVSI-negative 98.3% [95% CI 95.0–100.0], LVSI-focal 90.0% [95% CI 73.2–100.0], LVSI-substantial 93.8% [95% CI 82.6–100.0]. LVSI-negative vs LVSI-focal p: 0.335; LVSI-negative vs LVSI-substantial p: 0.583; LVSI-focal vs LVSI-substantial p: 0.661. F ***(NSMP-HR)****:* 3-year DFS: LVSI-negative 81.0% [95% CI 72.9–90.0], LVSI-focal 94.1% [95% CI 83.6–100.0], LVSI-substantial 78.5% [95% CI 65.8–93.7]. LVSI-negative vs LVSI-focal p: 0.383; LVSI-negative vs LVSI-substantial p: 0.406; LVSI-focal vs LVSI-substantial p: 0.290.

Table 2
Multivariable Cox regression model for disease-free survival in the overall population.

Covariate	Hazard Ratio (95% CI)	p-value
LVSI		
No LVSI	Reference	—
Focal vs No	1.40 (0.84–2.33)	0.20
Substantial vs No	3.37 (2.28–5.00)	< 0.001
Molecular class		
NSMP-LR	Reference	—
POLEmut vs NSMP-LR	1.07 (0.32–3.63)	0.91
MMRd vs NSMP-LR	2.05 (1.31–3.19)	0.002
p53abn vs NSMP-LR	9.10 (4.80–17.27)	< 0.001
NSMP-HR vs NSMP-LR	4.44 (2.29–8.61)	< 0.001
Age (per 1-year increase)	1.01 (1.00–1.03)	0.15
Grade		
Grade 3 vs Grade 1–2	0.88 (0.52–1.50)	0.65
Myometrial invasion		
None	Reference	—
< 50%	2.22 (1.06–4.65)	0.035
≥ 50%	2.62 (1.18–5.84)	0.018
Adjuvant treatment		
Follow-up	Reference	—
BRT alone vs follow-up	0.97 (0.58–1.61)	0.90
EBRT w/out BRT vs follow-up	0.72 (0.42–1.21)	0.21
CT + EBRT w/out BRT vs follow-up	0.47 (0.26–0.85)	0.012
CT vs follow-up	1.53 (0.86–2.71)	0.15

Abbreviations: LVSI, lymphovascular space invasion; BMI, body mass index; ER, estrogen receptor; POLEmut, POLE-mutated; MMRd, mismatch repair deficient; p53abn, p53-aberrant; NSMP-LR, low-risk No Specific Molecular Profile; NSMP-HR, high-risk No Specific Molecular Profile; BRT, vaginal brachytherapy; EBRT, external beam radiotherapy; CT; chemotherapy.

Our findings are largely consistent with previous observations while extending them in several important ways. In our cohort, substantial LVSI independently predicted worse DFS in NSMP-LR, MMRd, and p53abn tumors, whereas focal LVSI was not associated with impaired outcomes in any molecular subgroup. However, in p53abn tumors, focal LVSI showed a trend toward worse DFS but did not reach statistical significance. Given the small number of focal LVSI cases and events in this subgroup, this finding should be interpreted cautiously. Our results suggest that extensive lymphovascular dissemination represents an additional layer of risk stratification beyond molecular classification in several EC molecular contexts. Conversely, no clear survival differences according to LVSI extent were observed in POLEmut tumors, likely reflecting the intrinsically favorable prognosis of this molecular subgroup and the very low number of events observed in this population. Similarly, the lack of significant survival differences in NSMP-HR tumors may be due to the small number of patients included in this subgroup or reflect the dominant adverse prognostic role of underlying molecular and histopathologic features that characterize this group.

Importantly, the apparent association between combined chemoradiation as postoperative management and improved DFS should be interpreted with caution. In this retrospective non-randomized cohort, adjuvant treatment allocation was influenced by clinicopathologic risk factors, evolving guideline recommendations, physician judgment, and patient-specific considerations, introducing a substantial risk of indication bias and residual confounding. Therefore, adjuvant treatment was included in the multivariable model primarily as an adjustment variable, and our findings should not be interpreted as evidence supporting combined chemoradiation for all patients with early-stage disease.

Another relevant observation from our study is the consistent magnitude of recurrence risk associated with substantial LVSI across molecular classes. Model-derived estimates demonstrated a 2.5–2.8-fold increase in predicted recurrence risk at 36 months in tumors with substantial LVSI compared with negative or focal LVSI. This relatively uniform effect size across molecular backgrounds suggests that extensive lymphovascular invasion may reflect a fundamental biological mechanism of tumor dissemination that operates independently of specific genomic drivers. Such findings further support the integration of LVSI extent into contemporary molecularly informed risk stratification algorithms.

4.3. Strengths and weaknesses

The main strength of the present study lies in the large sample size of a well-defined cohort of patients with stage I–II EC. With more than 2300 cases analyzed using standardized semiquantitative LVSI assessment and contemporary molecular classification, our study represents the largest series specifically addressing the prognostic role of LVSI extent across molecular subgroups. In addition, interobserver disagreement in LVSI scoring of LVSI-positive cases occurred in only 4% of cases in the present series. This relatively low disagreement rate likely reflects centralized review by dedicated gynecologic pathologists and the use of standardized WHO criteria [1], reducing the risk of misclassification and enhancing the reliability of LVSI assessment. Given previous concerns regarding LVSI reproducibility, these findings support the feasibility of semiquantitative LVSI assessment when performed within a structured pathology workflow. Another relevant strength is the integration of multiple complementary statistical approaches, including subgroup-specific Cox models and model-based estimation of absolute

Table 3
Predicted absolute risk of recurrence and relative risk of recurrence at 36 months by molecular class and LVSI.

Molecular Class	36 m Negative/Focal LVSI	36 m Substantial LVSI	Δ36 m (Substantial−Negative/Focal)	Relative Risk (RR) of recurrence
NSMP-LR	1.7%[0.4–2.9%]	4.8%[0.8–8.6%]	+3.1%	2.8
POLEmut	2.0%[0.0–4.6%]	5.6%[0.0–13.0%]	+3.6%	2.8
MMRd	3.3%[0.8–5.8%]	9.3%[1.5–16.5%]	+6.0%	2.8
NSMP-HR	7.4%[0.8–13.5%]	19.8%[0.6–35.3%]	+12.4%	2.7
p53abn	13.7%[2.3–23.9%]	34.6%[2.5–56.1%]	+20.9%	2.5

Table 4
Distribution of risk classes, according to 2025 ESGO-ESTRO-ESP recommendations, and postoperative management across molecular classes.

	Total	POLEmut	MMRd	p53abn	NSMP-LR	NSMP-HR	p value
2025 ESGO-ESTRO-ESP risk class							
low	1188 (50.0%)	105 (100%)	301 (43.4%)	0 (0%)	782 (68.5%)	0 (0%)	< 0.001
intermediate	481 (20.3%)	0 (0%)	212 (30.5%)	0 (0%)	269 (23.6%)	0 (0%)	
high-intermediate	271 (11.4%)	0 (0%)	181 (26.1%)	0 (0%)	90 (7.9%)	0 (0%)	
high	386 (16.3%)	0 (0%)	0 (0%)	214 (87.3%)	0 (0%)	172 (91.0%)	
uncertain	48 (2.0%)	0 (0%)	0 (0%)	31 (12.7%)	0 (0%)	17 (9.0%)	
total	2374 (100%)	105 (100%)	694 (100%)	245 (100%)	1141 (100%)	189 (100%)	
Postoperative management							
follow-up	1186 (50.0%)	56 (53.3%)	296 (42.7%)	33 (13.5%)	765 (67.0%)	36 (19.0%)	< 0.001
BRT alone	407 (17.1%)	20 (19.0%)	140 (20.2%)	8 (3.3%)	203 (17.8%)	36 (19.0%)	
EBRT w/out BRT	373 (15.7%)	15 (14.3%)	153 (22.0%)	19 (7.8%)	146 (12.8%)	40 (21.2%)	
CT + EBRT w/out BRT	290 (12.2%)	11 (10.5%)	82 (11.8%)	130 (53.1%)	9 (0.8%)	58 (30.7%)	
CT	118 (5.0%)	3 (2.9%)	23 (3.3%)	55 (22.4%)	18 (1.6%)	19 (10.1%)	
total	2374 (100%)	105 (100%)	694 (100%)	245 (100%)	1141 (100%)	189 (100%)	

No patients in the present cohort were classified as advanced/metastatic according to the 2025 ESGO-ESTRO-ESP risk classification, as only FIGO stage I–II surgically staged tumors were included.
Abbreviations: POLEmut, POLE-mutated; MMRd, mismatch repair deficient; p53abn, p53-aberrant; NSMP-LR, low-risk No Specific Molecular Profile; NSMP-HR, high-risk No Specific Molecular Profile; BRT, vaginal brachytherapy; EBRT, external beam radiotherapy; CT; chemotherapy.
P-values were calculated using Fisher's exact test with Monte Carlo simulation because of sparse cells.

recurrence risk, allowing a robust evaluation of the prognostic effect of LVSI across molecular contexts.

However, several limitations should be acknowledged. First, the retrospective single-center design may introduce bias and limit the generalizability of our findings. Second, although the cohort is large overall, the number of events within some molecular subgroups - particularly POLEmut and NSMP-HR - was limited, preventing stable multivariable modeling in these populations and restricting interpretation to descriptive analyses. Third, despite standardized pathological review, LVSI evaluation remains partly susceptible to interobserver variability and potential artefacts, a limitation inherent to all studies investigating this parameter.

Fourth, because POLE testing was performed in only approximately half of the cohort, the POLEmut subgroup was likely underestimated, as reflected by the lower prevalence observed in our study (4.4%) compared with the 7–10% rate reported in the literature [11,23]. Consequently, some tumors classified as NSMP-LR or NSMP-HR may represent undetected POLEmut tumors; therefore, these categories should be interpreted as NSMP-like groups rather than fully ProMisE-defined NSMP classes in patients without POLE sequencing. This may have introduced some degree of molecular misclassification and could have attenuated recurrence estimates within the NSMP groups, given the excellent prognosis of POLEmut tumors and the limited prognostic impact of traditional clinicopathological factors in this subgroup. Nevertheless, the small expected proportion of missed POLEmut cases is unlikely to fully account for the observed association between substantial LVSI and poorer DFS in the NSMP-LR group. Furthermore, the POLEmut subgroup in our cohort showed an excellent prognosis, with only three recurrences observed among 105 patients (2.9%). These events were distributed across LVSI categories, with one recurrence in LVSI-negative, one in focal LVSI, and one in substantial LVSI tumors. Therefore, even if the POLEmut subgroup had been larger, the very low number of events expected in this molecular class would likely have limited the statistical power to detect any association between LVSI extent and DFS in the POLEmut group. Therefore, to ensure clarity and consistency throughout the manuscript, we adopted a simplified molecular nomenclature in accordance with the 2025 ESGO-ESTRO-ESP recommendations, as detailed in the Methods section. Another limitation of our study is the lack of detailed data on the exact number of vessels involved in cases classified as focal LVSI. Although we applied the WHO three-tiered definition, we were unable to distinguish among cases with 1, 2, 3, or 4 involved vessels. This prevents further sub-stratification and exploration of alternative thresholds. Furthermore, by excluding patients with incomplete surgical staging, we reduced the risk of occult nodal disease and improved the homogeneity of the study population. However, this design choice may limit generalizability to patients treated without nodal assessment, including frailer patients. Finally, although a two-year time horizon generally reflects the period during which most early-stage EC recurrences typically occur, the median follow-up of 34 months may not fully capture late recurrences, particularly in molecular subgroups with more indolent behavior. Therefore, the absence of a significant association between focal LVSI and DFS in selected subgroups, especially MMRd tumors, should be interpreted cautiously and requires confirmation with longer follow-up.

4.4. Clinical and research implications

Our findings have several clinically relevant implications. First, they further support the current ESGO-ESTRO-ESP risk stratification system, which distinguishes substantial LVSI from negative or focal LVSI when defining risk categories and guiding adjuvant treatment decisions. The absence of a prognostic impact for focal LVSI across all molecular subgroups reinforces the concept that focal lymphovascular involvement should not drive treatment escalation in early-stage EC.

Second, our results highlight that substantial LVSI retains prognostic significance even in the era of molecular classification, particularly among surgically staged, node-negative patients with early-stage disease. In particular, the independent association between substantial LVSI and reduced DFS in NSMP-LR, MMRd, and p53abn tumors suggests that LVSI remains a relevant pathological marker of metastatic potential beyond genomic drivers. These observations support the continued integration of semiquantitative LVSI assessment into molecularly informed risk stratification models.

Finally, although in our population no significant association was observed between recurrence patterns and LVSI extent, the consistent relative increase in recurrence risk associated with substantial LVSI across molecular classes suggests that extensive lymphovascular dissemination may represent a biological hallmark of metastatic capability. Future research should explore the biological mechanisms underlying LVSI development and its interaction with tumor genomic and molecular architecture. Prospective studies integrating molecular features, LVSI extent, and emerging biomarkers may further refine individualized risk stratification and guide tailored adjuvant strategies.

5. Conclusions

In this large cohort of stage I–II endometrial cancer patients classified according to contemporary molecular subgroups, focal LVSI was not

associated with worse oncologic outcomes compared with LVSI-negative disease. In contrast, substantial LVSI was associated with aggressive clinicopathologic and molecular features and independently predicted reduced disease-free survival in several molecular contexts, including NSMP-LR, MMRd, and p53abn tumors. These findings reinforce the clinical relevance of semiquantitative LVSI assessment and support its continued integration into molecularly informed risk stratification algorithms for early-stage endometrial cancer.

CRediT authorship contribution statement

Pierpaolo Merola: Data curation. **Luca Palmieri:** Data curation. **Rita La Marca:** Data curation. **Vincenzo Tarantino:** Data curation. **Francesco Fanfani:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Conceptualization. **Martina Corrado:** Data curation. **Emanuele Perrone:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anna Fagotti:** Validation, Supervision, Resources. **Dario Aprea:** Data curation. **Ilaria Capasso:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Matteo Loverro:** Methodology, Investigation, Formal analysis. **Maria Consiglia Giuliano:** Data curation. **Rossella Letizia Mancusi:** Methodology, Investigation, Formal analysis. **Fabiana Salvati:** Data curation. **Emilia Palmieri:** Data curation. **Giuseppe Parisi:** Data curation. **Nicola Macellari:** Data curation. **Gian Franco Zannoni:** Methodology, Investigation, Formal analysis. **Fulvia Pirrelli:** Data curation. **Lucia Tortorella:** Data curation. **Raffaella Sardo Infirri:** Data curation. **Giovanni Esposito:** Data curation. **Alessia Fossatelli:** Data curation. **Angela Santoro:** Methodology, Investigation, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2026.116846.

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