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Focal lymphovascular space invasion: Friend or foe? A large retrospective analysis on stage I endometrioid endometrial carcinomas

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ABSTRACT

Background: Literature is inconsistent with respect to clinical value of lymphovascular space invasion (LVSI) semiquantitative assessment. We aim to investigate the prognostic role of LVSI extent in stage I endometrioid endometrial carcinomas (ECs) classified by immunohistochemistry (IHC) analysis.
Methods: Patients with stage I endometrioid EC undergone primary surgery were retrospectively included. Following World Health Organization definition for LVSI pathologic evaluation, subjects were divided into: LVSI-negative; LVSI-focal; LVSI-substantial. An IHC-based model was utilized to classify patients into: p53-aberrant (p53abn); mismatch repair deficient (MMRd); mismatch repair proficient with positive estrogen receptors (MMRp-ERpos); and mismatch repair proficient with negative estrogen receptors (MMRp-ERneg).
Results: 2091 subjects were included and divided into: 78.0 % (n:1631) LVSI-negative, 10.6 % (n:221) LVSI-focal, and 11.4 % (n:239) LVSI-substantial. Presence of LVSI (any extent) was associated with older age, larger tumor size and deeper myometrial infiltration. Patients with LVSI-substantial presented with higher incidence of grade 3 tumors, p53abn and MMRd status. Conversely, most LVSI-negative and LVSI-focal cases were MMRp-ERpos. At multivariable regression, LVSI-substantial was independently associated with reduced 5-year disease-free survival (DFS) and overall-survival (OS). LVSI-negative and LVSI-focal groups had similar DFS ($p = 0.42$) and OS ($p = 0.09$), whereas comparison with LVSI-substantial demonstrated significantly poorer outcomes for patients with substantial invasion. These findings were confirmed in sub-analyses of cases with grade 1–2 endometrioid and myometrial infiltration, and in the MMRp-ERpos cohort.
Conclusions: In stage I endometrioid ECs, LVSI-focal was not associated with reduced oncologic outcomes compared to LVSI-negative. In contrast, LVSI-substantial was associated with aggressive clinicopathologic and molecular features and behaved as an independent prognostic factor for reduced survival. Our results were further confirmed in two low-risk EC settings: grade 1–2 with myometrial infiltration, and the MMRp-ERpos group.

1. Introduction

Lymphovascular space invasion (LVSI) is defined as the presence of a tumor embolus or cells in an endothelium-lined space within the myometrium beyond the invasive front of the tumor [1]. In endometrial cancer (EC), robust evidence has associated presence of LVSI with lymph node metastasis and poorer survival [2,3], making LVSI one of the most relevant stand-alone prognostic factors in this malignancy. In the last

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decade, the concept of LVSI semiquantitative assessment, in terms of number of involved vessels, has been introduced. A growing body of studies have shown that ECs with focal LVSI have similar risk of recurrence and prognosis than tumors with negative LVSI [2,4,5]. On these bases, LVSI semiquantitative evaluation has been routinely performed and thoroughly integrated into the ESGO-ESTRO-ESP endometrial cancer risk classification system [6,7]. Indeed, in uterine-confined endometrioid ECs, along with 2009-FIGO stage and tumor grade, the extent of lymphovascular invasion allows the allocation of patients into low, intermediate and high-intermediate risk classes [7]. Notably, since 2016 and according to ESGO-ESTRO-ESP consensus, negative and focal LVSI have been grouped together, in contrast to substantial LVSI, which is related to unfavorable outcomes and often drives risk class and adjuvant treatment escalation [6,7]. In contrast, a recently published study from Memorial Sloan Kettering has observed distinct survivals between patients with negative and focal LVSI in a population of stage I endometrioid ECs [8]; therefore, raising questions whether focal LVSI should be really considered at low risk of adverse outcomes. However, some heterogeneity in cut-offs definitions for substantial LVSI among scientific societies challenges the comparison of studies [1,9]. Despite these differences, the reproducibility of LVSI assessment among different pathologists has been demonstrated overall acceptable both in terms of LVSI mimics (pathologic features resembling LVSI) and extent of invasion; therefore, supporting the widespread adoption of LVSI analysis in decision algorithms for adjuvant management [10]. In parallel, the introduction of molecular features into the management of patients with EC and in 2023-FIGO staging system is adding complexity to the treatment algorithm. Notably, the new 2023-FIGO staging has not only been integrated with molecular information but also emphasizes lymphovascular invasion status as a crucial biomarker for allocating patients with uterine-confined disease into specific stage groups [11]. While it is reasonable that the prognostic impact of LVSI might be more relevant in the subset of ECs typically considered at lower risk of recurrence (i.e., early-stage, endometrioid histology, and p53 wild type), there remains a distinct gap in knowledge regarding the prognostic role of semiquantitative analysis of LVSI (negative vs focal vs substantial) in patients expressing different molecular biomarkers. The present study aims to analyze the prognostic value of LVSI status in a selected population with stage I endometrioid EC. Additionally, within this specific cohort, we aim to assess the clinical value of LVSI status in a subgroup of patients classified by IHC analysis.

2. Methods

This is a retrospective, monocentric analysis performed at Fondazione Policlinico Universitario Agostino Gemelli IRCCS (Rome, Italy). We included patients with 2009-FIGO stage I endometrioid ECs (primary diagnosis) that underwent complete surgical staging in our institution between May 1996 and January 2024.

Our primary outcome was analyzing potential differences in 5-year disease-free survival (DFS) among three groups of patients with different LVSI status (negative, focal, and substantial). Secondary endpoints comprised: 1) differences in 5-year overall survival (OS) based on LVSI status; 2) differences in DFS and OS based on LVSI status in patients presenting with grade 1–2 endometrioid EC with myometrial invasion; and 3) differences in DFS based on LVSI status in tumors with no specific molecular signature upon IHC-based classification (mismatch repair proficient, p53 wild type, with positive expression of estrogen receptors). This latter subset, representing the largest group of EC patients, was selected as it is characterized by the absence of specific molecular alterations that could potentially influence the impact of LVSI on survival outcomes.

Included subjects were required to have their pathologic specimen analyzed by two pathologists with expertise in gynecologic malignancies (GFZ e AS). LVSI was classified according to World Health Organization (WHO) definition[1]: negative (LVSI-negative) if no focus of invasion was detected; focal (LVSI-focal) if 1–4 vessels were involved on at least one pathology slide; and substantial (LVSI-substantial) if $\geq$ 5 vessels were involved on at least one pathology slide. For those patients undergone surgery before WHO definition was introduced, retrospective re-evaluation of pathologic slides was performed by the same pathologists involved in the study. In the event of disagreement between the two pathologists, a case-by-case discussion was held until a unique decision was reached. Molecular status was assessed by immunohistochemistry (IHC) to analyze the status of mismatch repair (MMR), p53, and estrogen and progesterone receptor (ER and PR), as detailed in Supplementary material. Acknowledging that POLE mutational status assessment was limited to a small subset of our population, patients were classified according to a recently published IHC-based model, which demonstrated non-inferior risk-classification performance and prognostic prediction compared to the standard molecular classification model [12], into: 1) MMR proficient, p53 wild type, ER positive (MMRp-ERpos); 2) MMR proficient, p53 wild type, ER negative (MMRp-ERneg); 3) MMR deficient (MMRd); and 4) p53 aberrant (p53abn).

According to international guidelines [7], primary surgical staging consisted of total hysterectomy with bilateral salpingectomy, with/-without bilateral oophorectomy, and lymph-nodal staging, conducted either by sentinel lymph nodes mapping or systematic pelvic lymphadenectomy.

Decisions regarding adjuvant management were based on the guidelines available at the time of surgery [6,7,13,14].

Subjects with incomplete clinical/pathological records or surgical staging were excluded, along with those diagnosed with tumors of non-epithelial histology or synchronous cancers, and patients who have received neoadjuvant treatment.

This study received approval from FPG Ethics Committee (IRB ID: 7130), and all the subjects granted research authorization to the use of clinical and pathologic data for research purposes. A customized electronic Case Report Form (eCRF) was developed using RedCap to collect and manage variables of interest. Follow-ups were retrieved by review of clinical records and/or phone calls to patients or closer caregivers in case of death.

Relapses were classified as local failure (including vaginal, rectal, and pelvic wall), lymph node failure, and distant failure.

Standard descriptive statistics were used to illustrate the distribution of each factor. Continuous variables were presented as medians and InterQuartile Range (IQR) and categorical variables were presented as absolute frequencies and percentages. Baseline clinicopathologic and molecular characteristics were compared between the cohorts using the $\chi 2$ test or Fisher exact-test (categorical variables) and the Kruskal-Wallis-test or Mann-Whitney test (continuous variables), as appropriate. OS and DFS were 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 until the date of disease recurrence or progression. OS was defined as the time from primary surgery until the date of last available patient contact or death due to disease. A Cox Proportional Hazards model was fitted to evaluate the prognostic value of different variables in survival outcomes. Variables associated with a p-value of < 0.05 in simple regression were included in multiple regression, a stepwise forward selection based on Wald statistics was used to identify uncorrelated factors. All reported p-values are 2-sided, and p-values < 0.05 were considered statistically significant.

3. Results

Out of 3772 patients with primary EC undergoing surgery in the study period, 2091 subjects diagnosed with 2009-FIGO stage I endometrioid tumor were included. Study population was divided into: 78.0 % (n:1631) LVSI-negative, 10.6 % (n:221) LVSI-focal, and 11.4 % (n:239) LVSI-substantial. Out of the 2091 patients enrolled, we observed 441 cases (21.1 %) for which an intrauterine manipulator has been used during primary surgical staging. Of these subjects, 342/441 (77.6 %)

were LVSI-negative, 55/441 (12.5 %) were LVSI-focal, and 44/441 (10,0 %) were LVSI-substantial. Clinicopathologic and molecular features distribution among study groups are reported in Table 1. Patients with LVSI-negative were the youngest ($p < 0.0001$). As expected, compared to LVSI-negative cases, ECs presenting with LVSI (any extent of invasion) were associated with larger tumor size ($p < 0.0001$) and deeper myometrial infiltration ($p < 0.0001$). Consequently, incidence of stage IA in LVSI-negative patients were almost double compared to both LVSI-focal and LVSI-substantial ($p < 0.0001$). Importantly, grade 3 tumors were mostly represented in LVSI-substantial compared to LVSI-focal and LVSI-negative ($p < 0.0001$). In LVSI-substantial, higher rates of p53abn and MMRd tumors were detected compared to the other LVSI groups ($p < 0.0001$). Contrariwise, the vast majority of LVSI-negative (69.3 %) and LVSI-focal (58.7 %) cases were MMRp-ERpos.

Median follow-up was 35 months (IQR: 18–62).

Univariate and multivariable Cox regression models (Table 2) were fitted, and we found that LVSI-substantial, the age, and having received adjuvant treatment were independently associated with DFS at multivariable regression.

In the overall population, 5-year DFS was 91.3 % (95 % CI 89.3–93.3) in LVSI-negative, 88.1 % (95 % CI 82.6–93.6) in LVSI-focal, and 73.8 % (95 % CI 65.6–82.0) in LVSI-substantial, $p < 0.001$. Importantly, when comparing LVSI-negative and LVSI-focal, no difference in 5-year DFS was detected ($p = 0.42$), whereas comparison between LVSI-negative and LVSI-focal to LVSI-substantial showed significantly worse DFS for LVSI-substantial ($p < 0.001$ and $p = 0.003$, respectively), Fig. 1.

Overall-survival analyses are reported in Supplementary material.

In our population, 1472 (70.4 %) presented with grade 1–2 disease with myometrial invasion. Of these, 1153 (78.3 %) were LVSI-negative, 172 (11.7 %) LVSI-focal, and 147 (10.0 %) LVSI-substantial. Five-year DFS was 91.7 % (95 % CI 89.7–93.7) in LVSI-negative, 88.2 % (95 % CI 81.7–94.7) in LVSI-focal, and 73.3 % (95 % CI 61.5–85.1) in LVSI-substantial, $p < 0.001$. DFS in LVSI-negative and LVSI-focal was similar ($p = 0.88$), whereas comparison of LVSI-negative and LVSI-focal to LVSI-substantial showed worse DFS for LVSI-substantial ($p < 0.001$ and $p = 0.009$, respectively), Fig. 2.

Of 2091 subjects, 1330 (63.6 %) underwent complete IHC analysis. These patients were classified into: 65.5 % (n:871) MMRp-ERpos, 29.9 % (n:398) MMRd, 3.4 % (n:45) MMRp-ERneg, and 1.2 % (n:16) p53abn. Five-year DFS was 93.3 % in MMRp-ERpos, 89.3 % in MMRd, 84.6 % in MMRp-ERneg, and 77.8 % in p53abn. In MMRp-ERpos endometrial carcinomas, 5-year DFS was 95.7 % in LVSI-negative, 93.7 % in LVSI-focal, and 57.7 % in LVSI-substantial ($p < 0.001$).

Table 1
Study population characteristics.

Characteristic	Total (N = 2091)	No LVSI (N = 1631)	Focal LVSI (N = 221)	Substantial LVSI (N = 239)	*p*-value
Age	61 (54–69)	60 (53–68)	63 (57–70)	63 (57–71)	**< 0.0001**
BMI	28.6 (24.2–34.6)	28.6 (24.2–34.6)	29.3 (25.0–35.4)	27.9 (23.7–33.1)	**0.048**
Grading					
G1	477 (22.8 %)	468 (28.7 %)	5 (2.3 %)	4 (1.7 %)	**< 0.001**
G2	1332 (63.7 %)	1022 (62.7 %)	167 (75.6 %)	4 (1.7 %) 143 (59.8 %)	
G3	282 (13.5 %)	141 (8.6 %)	49 (22.2 %)	92 (38.5 %)	
Tumor dimension					**< 0.001**
≤ 20 mm	722 (34.9 %)	671 (41.8 %)	28 (12.7 %)	23 (9.6 %)	
> 20 mm	1344 (65.1 %)	936 (58.2 %)	192 (87.3 %)	216 (90.4 %)	
Myometrial invasion					**< 0.001**
no invasion	348 (16.7 %)	348 (21.4 %)	0	0	
< 50 %	1121 (53.7 %)	955 (58.7 %)	92 (41.6 %)	74 (31.0 %)	
≥ 50 %	619 (29.6 %)	325 (20.0 %)	129 (58.4 %)	165 (69.0 %)	
FIGO stage 2009					**< 0.001**
IA	1475 (70.5 %)	1310 (80.3 %)	93 (42.1 %)	72 (30.1 %)	
IB	617 (29.5 %)	322 (19.7 %)	128 (57.9 %)	167 (69.9 %)	
ER status					**< 0.001**
< 10 %	48 (3.4 %)	26 (2.4 %)	10 (7.0 %)	12 (6.8 %)	
≥ 10 %	1350 (96.6 %)	1053 (97.6 %)	133 (93.0 %)	164 (93.2 %)	
PR status					**0.008**
< 10 %	192 (13.6 %)	134 (12.3 %)	20 (14.1 %)	38 (20.8 %)	
≥ 10 %	1222 (86.4 %)	955 (87.7 %)	122 (85.9 %)	145 (79.2 %)	
Molecular class					**< 0.001**
p53abn	16 (1.2 %)	9 (0.9 %)	2 (1.4 %)	5 (2.9 %)	
MMRd	398 (29.9 %)	272 (26.6 %)	49 (35.5 %)	77 (45.0 %)	
MMRp-ERpos	871 (65.5 %)	708 (69.3 %)	81 (58.7 %)	82 (48.0 %)	
MMRp-ERneg	45 (3.4 %)	32 (3.1 %)	6 (4.3 %)	7 (4.1 %)	
SURGICAL APPROACH					0.14
LPS	1183 (56.8 %)	903 (55.6 %)	128 (57.9 %)	152 (63.9 %)	
Robotic	734 (35.3 %)	585 (36.0 %)	75 (33.9 %)	74 (31.1 %)	
LPT	165 (7.9 %)	135 (8.3 %)	18 (8.1 %)	12 (5.0 %)	
Adjuvant Treatment					**< 0.001**
CT +/- BRT	73 (3.8 %)	56 (3.8 %)	7 (3.3 %)	10 (4.2 %)	
EBRT +/- BRT	328 (17.3 %)	126 (8.7 %)	67 (32.1 %)	135 (57.0 %)	
CT + EBRT +/- BRT	88 (4.6 %)	29 (2.0 %)	19 (9.1 %)	40 (16.9 %)	
BRT alone	343 (18.0 %)	234 (16.1 %)	73 (34.9 %)	36 (15.2 %)	
No treatment	1069 (56.2 %)	1010 (69.4 %)	43 (20.6 %)	16 (6.8 %)	
Recurrence location					0,99
local only	47 (29.2)	31 (30.1)	5 (26.3)	11 (28.2)	
lymph nodes +/- local	35 (21.7)	23 (22.3)	4 (21.1)	8 (20.5)	
distant +/- other	53 (32.9)	32 (31.1)	7 (36.8)	14 (35.9)	
Unknown	26 (16.1)	17 (16.5)	3 (15.8)	6 (15.4)	

Abbreviations: LVSI, lymphovascular space invasion; BMI, body mass index; FIGO, International Federation of Gynecology and Ostetrics; ER, estrogen receptor status; PR, progesterone receptor status; p53abn, p53-aberrant; MMRd, mismatch repair deficient; MMRp-ERpos, mismatch repair proficient with positive estrogen receptor expression; MMRp-ERneg, mismatch repair proficient with negative estrogen receptor expression; LPS, laparoscopy; LPT, laparotomy; CT, chemotherapy; BRT, brachytherapy; EBRT, external beam radiotherapy.

Table 2
Univariate and multivariable cox regression models for 5-year disease-free survival in the overall study population.

	N (events)	5-year DFS	Univariate regression HR (95 % CI)	Multivariable regression aHR (95 % CI)
Cohort	2091 (161)	88.7 %		
LVSI			P < 0.001	P = 0.008
Negative	1631 (103)	91.1 %	Ref.	Ref.
Focal	221 (19)	88.1 %	1.18 (0.73–1.93) p = 0.50	0.87 (0.50–1.50) p = 0.61
Substantial	239 (39)	73.8 %	2.84 (1.96–4.11) p < 0.001	1.84 (1.19–2.86) p = 0.006
AGE AT SURGERY			1.03 (1.02–1.05) p < 0.001	1.03 (1.01–1.04) p = 0.002
BMI			0.99 (0.97–1.01) p = 0.44	–
SURGICAL APPROACH				–
MIS (LPS or Robotic)	1916 (131)	89.7 %	Ref.	
LPT	165 (29)	81.6 %	1.84 (1.21–2.80) p = 0.004	
ER				–
< 10 %	48 (6)	65.5 %	Ref.	
≥ 10 %	1350 (54)	92.1 %	0.26 (0.11–0.61) p = 0.002	
2009-FIGO STAGE				–
IA	1474 (87)	91.7 %	Ref.	
IB	617 (74)	82.5 %	2.07 (1.52–2.82) p < 0.001	
GRADING			P < 0.001	–
G1	477 (15)	94.1 %	Ref.	
G2	1332 (101)	89.4 %	2.19 (1.27–3.77) p = 0.005	
G3	282 (45)	77.4 %	5.12 (2.85–9.19) p < 0.001	
MYOMETRIAL INVASION			P < 0.001	–
None	347 (7)	96.5 %	Ref.	
< 50 %	1121 (78)	90.4 %	2.69 (1.24–5.84) p = 0.012	
≥ 50 %	619 (74)	82.6 %	4.95 (2.28–10.74) p < 0.001	
ADJUVANT TREATMENT				
No	1069 (69)	92.1 %	Ref.	Ref.
Yes	764 (89)	83.1 %	2.41 (1.70–3.42) p < 0.001	1.89 (1.26–2.81) p = 0.002

Abbreviations: DFS, disease-free survival; LVSI, lymphovascular space invasion; BMI, body mass index; MIS, minimally invasive surgery; LPS, laparoscopy; LPT, laparotomy; ER, estrogen receptor status; FIGO, International Federation of Gynecology and Ostetrics.

Within the MMRp-ERpos group, DFS was similar between patients with LVSI-negative and LVSI-focal (p = 0.16), whereas LVSI-substantial had poorer DFS, Fig. 3.

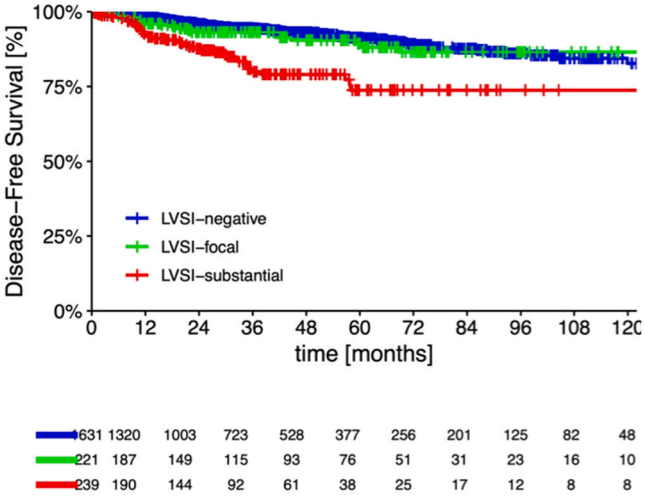

Fig. 1. Kaplan-Meier survival curves for 5-year disease-free survival in the overall study population (n:2091). *Overall p < 0.001; LVSI-negative vs LVSI-focal p = 0.42; LVSI-negative vs LVSI-substantial p < 0.001; LVSI-focal vs LVSI-substantial p = 0.003.*

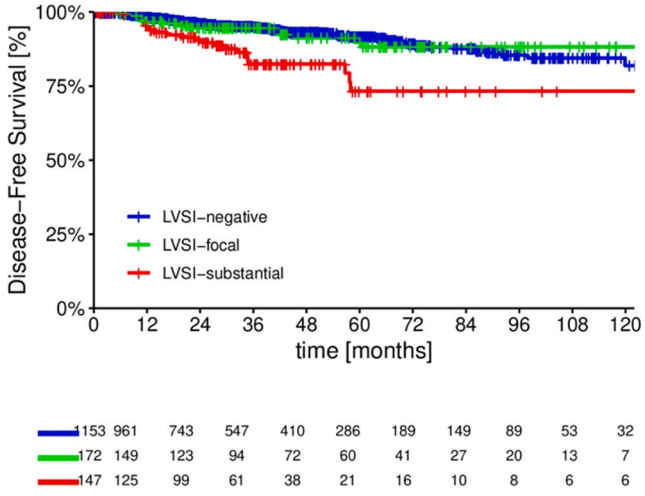

Fig. 2. Kaplan-Meier survival curves for 5-year disease-free survival in the subset of grade 1–2 with myometrial infiltration endometrial carcinoma (n:1472). *Overall p < 0.001; LVSI-negative vs LVSI-focal p = 0.88; LVSI-negative vs LVSI-substantial p < 0.001; LVSI-focal vs LVSI-substantial p = 0.009.*

4. Discussion

4.1. Summary of the main results

In our large series of stage I endometrioid ECs, substantial LVSI was associated with a higher incidence of grade 3 tumors, p53abn, and MMRd status. Conversely, the majority of cases with LVSI-negative and LVSI-focal exhibited a MMRp-ERpos profile. At multivariable regression, LVSI-substantial was independently associated with reduced 5-year DFS and OS. Notably, patients with LVSI-negative and LVSI-focal had similar survival, whereas those with LVSI-substantial demonstrated poorer outcomes. These findings were further confirmed in two sub analyses: cases with grade 1–2 endometrioid and myometrial infiltration (comprising 70.4 % of our population); and tumors with no specific molecular signature at IHC analysis (mismatch repair proficient, p53 wild type, with positive expression of estrogen receptors), representing the largest molecular entity.

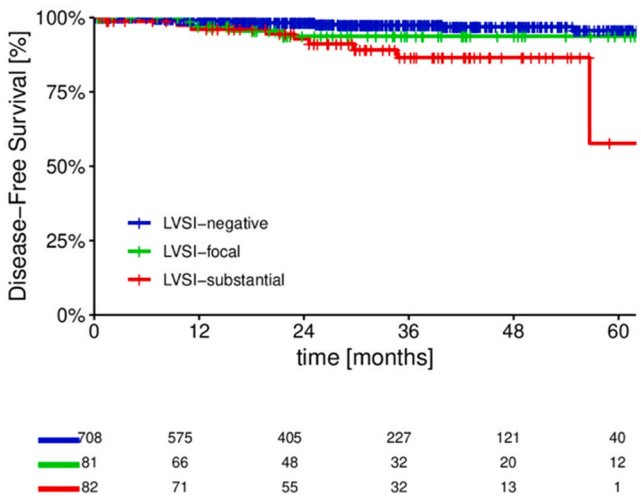

Fig. 3. Kaplan-Meier survival curves for 5-year disease-free survival in the MMRp-ERpos IHC group (n:871). *Overall $p < 0.001$; LVSI-negative vs LVSI-focal $p = 0.16$; LVSI-negative vs LVSI-substantial $p < 0.001$, LVSI-focal vs LVSI-substantial $p = 0.14$.*

4.2. Results in the context of published literature

Most EC patients present with an early-stage disease (80–90 %, [15,16]). In the context of uterine-confined ECs, LVSI has been robustly established as a stand-alone prognostic biomarker [2]. Notably, presence of LVSI has been associated with an increased risk of recurrence across all molecular subgroups of ECs; however, evidence defining its prognostic role in the context of molecular classification remains limited, with an independent prognostic role of substantial LVSI demonstrated only in stage I no specific molecular profile (NSMP) patients [17].

The incidence of LVSI in stage I ECs reported in the literature ranges widely (3.2–35 %), likely reflecting the lack of globally accepted protocols for LVSI evaluation [2,18,19]. In our analysis, LVSI was detected in 22.0 % of cases (10.6 % focal and 11.4 % substantial). Notably, LVSI detection (any extent of invasion) was associated with larger tumor size and deeper myometrial invasion, in line with previous findings [2]. Furthermore, our study highlights significant differences in pathologic and molecular features distribution between tumors with substantial LVSI and those with negative/focal LVSI: higher rate of grade 3 tumors, p53abn and MMRd status in the LVSI-substantial group compared to the negative/focal counterparts, which are largely composed of MMRp-ERpos tumors. Importantly, the distribution of LVSI extent in the subgroup utilizing intrauterine manipulator (77.6 % LVSI-negative, 12.5 % LVSI-focal, and 10.0 % LVSI-substantial) was consistent with that of the overall study population (78.0 % LVSI-negative, 10.6 % LVSI-focal, and 11.4 % LVSI-substantial), likely suggesting that the use of intrauterine manipulator is not associated with presence of LVSI or extent of LVSI, in line with previous studies [20,21].

There is broad agreement in the literature regarding the detrimental prognostic role of massive/substantial LVSI; nevertheless, inconsistencies persist concerning the clinical significance of focal LVSI [2,4,5,8,22,23]. Most importantly, the heterogeneity in threshold definitions for substantial LVSI complicates the comparison of results across different studies. A previous study from Peters et al. associated the number of involved vessels to risk of recurrence in stage I ECs not receiving adjuvant treatment, observing reduced survival in patients with ≥ 4 vessels [9]. While, other studies referred to WHO guidelines [1] and defined substantial LVSI as presence of ≥ 5 involved vessels, as also recommended by international ESGO-ESTRO-ESP consensus [7]. Despite some inconsistencies among cutoffs for substantial LVSI, several studies have demonstrated a significant association between presence of substantial LVSI and reduced survival compared with absent or focal invasion, with these latter subsets having similar outcomes [2,4,5,24]. In particular, in a population of low-grade endometrioid tumors with < 50 % myoinvasion, Tortorella et al. utilized a 3-tiered system defining substantial LVSI as involvement of ≥ 3 vessels; whereas, in patients with stage I endometrioid ECs, Bosse et al. observed poorer survival in patients with more than one focus of invasion - defined as substantial -; with the same cutoff utilized in Restaino et al. analysis in a population of stage I-II ECs, with similar results and conclusions.

In our analysis, by applying the WHO 3-tiered definition, not only we confirmed that substantial LVSI is independently associated with reduced DFS and OS, but we further observed significantly poorer outcomes in patients with LVSI-substantial compared to LVSI-negative and LVSI-focal in specific low-risk subsets (grade 1–2 with myoinvasion, and MMRp-ERpos).

In contrast to these findings, other studies have observed significant survival differences between patients with negative and focal LVSI [8,22], therefore questioning the indolent prognostic value of focal invasion. By applying the WHO definition, a recently published analysis from Dagher and colleagues showed an increased risk of recurrence or death in patients with any LVSI extent (either focal or substantial) in a population with stage I endometrioid EC undergone complete nodal staging [8]. Notably, compared to our study population, which included 11.4 % of LVSI-substantial and 10.6 % LVSI-focal cases, Dagher's analysis showed a lower prevalence of LVSI detection (4.2 % substantial and 7.7 % focal). One of the key challenges to LVSI widespread applicability is interobserver agreement among pathologists. Focal LVSI requires thorough specimen inspection and may sometimes be misclassified as absent invasion, leading to high interobserver variability. Moreover, it has been reported that the number of cases with LVSI increase after re-evaluation of pathologic slides, with most false-negative cases being reclassified as focal LVSI [2,25]. Notably, in Dagher's study, which included nearly 90 % of endometrial carcinomas classified as LVSI negative, no pathological re-evaluation was performed. As a result, a proportion of focal LVSI cases may have been missed, potentially introducing biases in LVSI groups allocation and assessment of survival outcomes.

Notably, with respect to adjuvant treatment, our multivariable Cox regression shows that receipt of therapy was independently associated with reduced DFS and OS, regardless LVSI extent (Table 2 and Supplementary Table 1), likely reflecting underlying adverse prognostic factors that guided treatment selection rather than a detrimental effect of therapy itself. Furthermore, in contrast to Dagher et al. [26], and in line with previous studies [27], we did not observe statistically significant correlations between LVSI extent and sites of recurrence. This discrepancy among studies might reflect the different cutoffs utilized to classify LVSI extent, a lack of uniform nodal evaluation, and differences in the classification of recurrence locations.

4.3. Strengths and weaknesses

The retrospective nature and the lack of POLE mutational status assessment for the entire study population represent major study weaknesses. Nevertheless, as demonstrated previously [12], the IHC-based classification model resulted in overlapping risk-classification performance and survival prediction compared to the standard model encompassing genomic sequencing (ProMisE)[28]. Furthermore, although the study spans a 28-year period, the median follow-up is relatively short. This reflects the fact that most patients were treated in the last decade, following a substantial increase in the number of patients operated at our institution, along with the introduction of RedCAP platform for systematic data collection and management during the last decade. Moreover, the inclusion of highly selected low-risk populations (stage I endometrioid, grade 1–2; and stage I endometrioid, MMRp-ERpos) led to an unbalanced representation of low-risk histopathologic and molecular features within our study

cohort, potentially limiting our survival analysis. However, focusing on these low-risk clinical settings may provide the most suitable cohort for assessing the real clinical impact of LVSI, minimizing the influence of other histopathologic factors (non-endometrioid histology, grade 3, cervical stromal invasion, nodal metastasis, etc.) or molecular factors (p53abn, ER-negative, etc.) that act as prognostic drivers. 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.

As major strengths, our series is one of the largest populations of completely staged patients with stage I endometrioid endometrial carcinomas described in the literature to date. Also, the retrospective pathologic assessment of cases with focal and substantial LVSI utilizing the validated WHO definition, as recommended by available guidelines, enhance the reproducibility of our methodology and results.

4.4. Clinical and research implications

Given the robust evidence associating substantial/massive LVSI with reduced survival, efforts are needed to identify globally accepted and validated protocols for LVSI pathologic evaluation and cutoffs definition. In this context, the adoption of digital pathology may further enhance diagnostic accuracy by supporting standardized image analysis, remote slide review, and second-opinion sharing. Its application could be particularly beneficial for reproducible assessment of LVSI extent and IHC markers, reducing interobserver variability and supporting multicenter diagnostic consistency. Our findings support the clinical relevance of semiquantitative LVSI classification in early-stage and low-risk EC settings. In line with current ESGO/ESTRO/ESP recommendations, our data show that LVSI-focal does not associate with significantly worse oncologic outcomes compared to LVSI-negative, suggesting that these two categories may be safely grouped together in risk stratification models. In contrast, LVSI-substantial was independently associated with reduced DFS and OS across all analyzes, even in tumors traditionally considered low-risk based on pathologic or molecular features. These results support and reinforce the current FIGO 2023 [11] staging and newly introduced ESGO-ESTRO-ESP 2025 risk classification systems [29], which incorporate LVSI-substantial as a key determinant in guiding adjuvant treatment decisions. Since our study was not designed to directly assess therapeutic benefit of escalation/descalation of adjuvant treatment based on LVSI extent, clinical decision-making regarding adjuvant therapy should continue to be guided by a combination of clinicopathologic and molecular factors, as endorsed by international guidelines [7,29]. Future prospective trials are warranted to determine whether direct incorporation of LVSI extent into treatment algorithms could further optimize patient outcomes.

5. Conclusions

In our population of stage I endometrioid ECs, focal LVSI was not associated with reduced disease-free survival and overall survival compared to the negative LVSI counterpart. In contrast, substantial LVSI was associated with aggressive clinicopathologic and molecular features and behaved as an independent prognostic factor for reduced survival. Our results were further confirmed in two specific and selected low-risk endometrial cancer settings: grade 1–2 with myometrial infiltration, and the subgroup characterized by a MMRp with ER positive IHC profile.

CRediT authorship contribution statement

Fulvia Pirrelli: Data curation. **Fabiana Salvati:** Data curation. **Angela Santoro:** Resources, Investigation. **Gian Franco Zannoni:** Resources, Investigation. **Diana Giannarelli:** Methodology, Investigation, Formal analysis. **Anna Fagotti:** Supervision. **Matteo Loverro:** Methodology, Investigation, Formal analysis. **Andrea Mariani:** Supervision. **Emanuele Perrone:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Alessia Fossatelli:** Data curation. **Ilaria Capasso:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Emilia Palmieri:** Data curation. **Giovanni Esposito:** Data curation. **Lucia Tortorella:** Writing – review & editing. **Camilla Nero:** Writing – review & editing. **Francesco Fanfani:** Supervision, Project administration.

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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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2025.115736.

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