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ORIGINAL RESEARCH

Lymphovascular invasion has a significant prognostic impact in patients with early breast cancer, results from a large, national, multicenter, retrospective cohort study

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Background: We determined the prognostic impact of lymphovascular invasion (LVI) in a large, national, multicenter, retrospective cohort of patients with early breast cancer (BC) according to numerous factors.

Patients and methods: We collected data on 17 322 early BC patients treated in 13 French cancer centers from 1991 to 2013. Survival functions were calculated using the Kaplan—Meier method and multivariate survival analyses were carried out using the Cox proportional hazards regression model adjusted for significant variables associated with LVI or not. Two propensity score-based matching approaches were used to balance differences in known prognostic variables associated with LVI status and to assess the impact of adjuvant chemotherapy (AC) in LVI-positive luminal A-like patients.

Results: LVI was present in 24.3% (4205) of patients. LVI was significantly and independently associated with all clinical and pathological characteristics analyzed in the entire population and according to endocrine receptor (ER) status except for the time period in binary logistic regression. According to multivariate analyses including ER status, AC, grade, and tumor subtypes, the presence of LVI was significantly associated with a negative prognostic impact on overall (OS), disease-free (DFS), and metastasis-free survival (MFS) in all patients [hazard ratio (HR) = 1.345, HR = 1.312, and HR = 1.415, respectively; $P < 0.0001$], which was also observed in the propensity score-based analysis in addition to the association of AC with a significant increase in both OS and DFS in LVI-positive luminal A-like patients. LVI did not have a significant impact in either patients with ER-positive grade 3 tumors or those with AC-treated luminal A-like tumors.

Conclusion: The presence of LVI has an independent negative prognostic impact on OS, DFS, and MFS in early BC patients, except in ER-positive grade 3 tumors and in those with luminal A-like tumors treated with AC. Therefore, LVI may indicate the existence of a subset of luminal A-like patients who may still benefit from adjuvant therapy.

Key words: lymphovascular invasion, luminal A subtype, breast cancer, multicenter study

INTRODUCTION

Breast cancer (BC) accounts for almost 12% of all new cancer cases worldwide each year and is the first most common cause of cancer death in women.[1] However, patient management appears to have improved over the past decade as disease mortality has steadily decreased during the same period.[2] Indeed, at the 16th St. Gallen International Breast Cancer Conference, the panel specifically acknowledged the potential impact of adjuvant therapy on the risk of BC recurrence or overall survival (OS) and highlighted the importance of prognostic factors in prescribing individualized treatments with regard to the magnitude of clinical benefit.[3] The most commonly accepted prognostic factors for proposing adjuvant therapy include patient age, tumor size,

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axillary lymph node status, tumor pathology including grade, endocrine receptor (ER) (estrogen and progesterone) status, human epidermal growth factor receptor 2 (HER2) status, and proliferation assays such as the Ki67 labeling index.[3,4] Lymphovascular invasion (LVI) is also a known negative prognostic factor and is defined as tumor cells present within a definite endothelial-lined space (either lymphatic or blood vessels) in the area surrounding the invasive carcinoma.[5] LVI is an early indicator of the potential for metastatic dissemination.[6-8] However, its use when recommending adjuvant therapy is very limited or not recognized in most guidelines.[3,9-12] The lack of consensus on the importance of LVI in the decision for adjuvant therapy has been further emphasized by the recent and steadily increasing utilization of gene expression signature (genomic) assay results in adjuvant decision making, particularly for patients with ER-positive/HER2-negative tumors.[8] Yet LVI could be considered as an independent prognostic factor that extends beyond the contentious position of a high-risk indicator in early, ER-positive/HER2-negative or lymph node-negative tumors,[13-15] and may, in fact, have a wider prognostic impact that has been overlooked in the modern era of genomic assays. This supposition is further supported by recent studies whose findings indicate that the detection of LVI adds significant prognostic information to the 21-gene recurrence score (RS).[16,17] However, to our knowledge, no other study with a large sample population has investigated the association of LVI with survival according to both adjuvant chemotherapy (AC) and molecular BC subtypes. Therefore, we have retrospectively analyzed a large national multicenter cohort of 17 322 early BC patients for the independent prognostic impact of LVI in both the entire population and in subgroups of clinical interest including molecular BC subtypes and treatment.

PATIENTS AND METHODS

The medical records of 23 000 patients who were treated from January 1991 to December 2013 were retrieved from the clinical databases of 13 cancer centers in France for retrospective analysis. Of this initial cohort, 17 322 patients were included based on tumor size ≥2 mm and known LVI, lymph node, and ER status, while 5678 patients with unknown status were excluded. Data were collected on patient and tumor characteristics, treatments received, time periods, and clinical outcomes (Table 1) with a follow-up of 5 or more years. Patients without events were censored as of the date of last follow-up. Informed consent was waived since all data were de-identified and collected retrospectively from each center.

Pathological assessment

ER and HER2 status were determined according to French guidelines [immunohistochemistry (IHC) detection of estrogen and/or progesterone receptors with a 10% threshold for ER positivity; HER2 positivity with a 3+ IHC score and/or HER2 amplification identified by *in situ* hybridization]. Patients with unknown HER2 status were included in the

Table 1. Association of LVI with clinical and pathological characteristics

| | Lymphovascular invasion status | | | | χ^2 |
| | Negative | | Positive | | |
	n	%	n	%	P
All patients	13 117	75.7	4205	24.3	
Age, years					<0.0001
≤40	673	57.6	495	42.38	
40.1-50	2724	70.8	1123	29.19	
50.1-74.9	8621	79.0	2295	21.02	
≥75	1094	79.0	291	21.01	
T size, mm					<0.0001
≤5	1016	94.3	61	5.66	
5.1-10	4105	89.0	505	10.95	
10.1-19.9	4909	75.8	1569	24.22	
20-50	3087	59.9	2070	40.14	
T histology					<0.0001
Ductal	10 049	72.8	3757	27.21	
Lobular	1896	88.6	244	11.40	
Mixed	229	73.4	83	26.60	
Others	943	88.6	121	11.37	
Grade					<0.0001
1	5317	88.6	681	11.35	
2	5893	73.8	2091	26.19	
3	1907	57.1	1433	42.90	
pN status					<0.0001
pN0	9785	85.3	1686	14.70	
pN0 (i+)	376	66.1	193	33.92	
pN1mi	994	70.5	415	29.45	
pN1 macro	1962	50.7	1911	49.34	
Breast surgery					<0.0001
Conservative	11 138	77.8	3171	22.16	
Mastectomy	1710	64.1	958	35.91	
Unknown	269	78.0	76	22.03	
Axillary surgery					<0.0001
SLNB	7888	87.8	1092	12.16	
SLNB + ALND	3046	67.4	1476	32.64	
ALND	2181	57.1	1637	42.88	
Periods					<0.0001
<2005	6346	71.7	2499	28.25	
≥2005	6771	79.9	1705	20.12	
T location					<0.0001
Outer/equatorial	6282	73.7	2241	26.29	
Inner	2906	74.7	982	25.26	
Unknown	3929	80.0	982	20.00	
Endocrine therapy					<0.0001
No	3082	72.5	1168	27.48	
Yes	10 029	76.8	3034	23.23	
Chemotherapy					<0.0001
No	8917	86.2	1427	13.80	
Yes	4200	60.2	2778	39.81	
RNI					<0.0001
No	7285	84.2	1367	15.80	
Yes	3379	61.1	2153	38.92	
Postmastectomy Radiotherapy					<0.0001
No	699	83.7	136	16.29	
Yes	1009	55.1	821	44.86	
Subtypes					<0.0001
Luminal A	7127	84.0	1362	16.04	
Luminal B HER2− G3	611	56.4	473	43.63	
Luminal B HER2+	448	66.7	224	33.33	
HER2	231	64.2	129	35.83	
Triple negative	737	74.4	254	25.63	

ALND, axillary lymph node dissection; HER2, human epidermal growth factor receptor 2; LVI, lymphovascular invasion; RNI, regional node irradiation; SLNB, sentinel lymph node biopsy; T, tumor.

overall analyses of the entire patient population but excluded in analyses of BC subtypes defined as surrogates for intrinsic BC molecular subtypes based on tumor grade,

ER and HER2: triple negative (HER2−/ER−), HER2 positive (non-luminal, HER2+/ER−), luminal A like (ER+/HER2−/ grade 1 or 2), luminal B like/HER2 negative (ER+/HER2−/ grade 3), and luminal B like/HER2 positive (ER+/HER2+/all grades).[18] LVI was determined to be present when lympho-vascular emboli, defined as tumor cells present in an endothelium-lined space within the peritumoral area, were detected by trained pathologists via examination of hema-toxylin-eosin-saffron (HES) slides in accordance with the policies/guidelines of each individual center. Only specimens with definite LVI were classified as LVI positive. When LVI was rare, uncertain, or detected by IHC only, this was considered as negative to account for variability in reporting concordance between pathologists, since BC cases either without LVI or those involving the invasion of multiple vessels have report-edly had the highest LVI detection rates and concordance.[19]

Statistical analysis

Analyses were carried out separately for all patients or patients with ER-positive or -negative tumors on factors associated with the presence or not of LVI according to patient, disease, and clinical characteristics such as age; tumor size, histology, Scarff−Bloom−Richardson grade, and subtypes (triple negative, HER2 positive, luminal A like, luminal B like/HER2 negative, and luminal B like/HER2 positive; pN status [four categories: pN0, pN0(i+), pN1mi, and pNmacro (any pN+ >2 mm)]; breast and axillary sur-gery, endocrine therapy (ET), AC, and radiotherapy; and periods before and after 2005 (according to availability of trastuzumab therapy). OS, disease-free survival (DFS), and metastasis-free survival (MFS) were defined as the time interval from the date of surgery to death or last follow-up, to an event (recurrence, metastasis, or death) or last follow-up, and to the date of a distant recurrence as a first event or last follow-up, respectively. Patients lost to follow-up were considered as alive as of the date of last contact.

The associations between categorical values were evalu-ated via χ^2 tests. Factors significantly associated with LVI were determined by binary logistic regression adjusted for all significant variables determined by univariate analysis. Sur-vival functions were calculated using the Kaplan−Meier method with differences assessed via the log-rank test. Multivariate survival analyses were carried out using the Cox proportional hazards regression model adjusted for signifi-cant variables associated with LVI. To balance differences in prognostic variables associated with LVI status, we generated 1 : 1 LVI-positive and LVI-negative matched cohorts. Co-efficients of a logistic regression adjusted on age, tumor size, ERs, HER2, pN status, AC, ET, and tumor grade were used to compute a propensity score. LVI-positive patients were then matched on this score to LVI-negative patients using nearest-neighbor matching without replacement.[20,21] The impact of LVI on DFS, MFS, and OS was assessed on this matched population by log-rank tests stratified on the pairs.[22] A sec-ond propensity score-based matching approach was designed to assess the impact of AC specifically in LVI-positive luminal A-like patients. To reduce confounding

biases associated with AC prescription, coefficients of a lo-gistic regression adjusted on age, tumor size, pN status, and ET were used to compute a second propensity score. LVI-positive luminal A-like patients with AC were then matched on this score to LVI-positive luminal A-like patients without AC using nearest-neighbor matching without replacement. The impact of AC on DFS, MFS, and OS of LVI-positive luminal A patients was then assessed in this matched pop-ulation by log-rank tests stratified on the pairs.[22] Further details of both propensity score matching approaches have been provided. (Supplementary Figures S1 and S2, available at https://doi.org/10.1016/j.esmoop.2021.100316). Statisti-cal significance was set as $P \leq 0.05$. Analyses were carried out with SPSS 16.0 (SPSS Inc., Chicago, IL) and R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Association of LVI with other clinical and pathological features

LVI was present in 24% (4205/17 322) of patients overall (Table 1), was significantly associated with all clinical and pathological characteristics analyzed in the entire popula-tion, and was most clearly prevalent in the following cate-gories: patient age ≤40 years (42%); tumor size 20-50 mm (40%); grade 3 (G3, 43%); pN1macro status (49%); patients treated before 2005 (28%); and patients treated via mas-tectomy (36%), axillary lymph node dissection (ALND) (43%), chemotherapy (40%), regional nodal irradiation (RNI) (39%), and postmastectomy radiotherapy (45%), but not ET (no = 28%). Additionally, LVI was most prevalent in patients presenting the luminal B-like subtype, specifically HER2-negative G3 (44%). When analyzed according to ER-positive or -negative status (Table 2), where 22% (3279/ 14 655) and 35% (926/2667) of patients were LVI positive, respectively, LVI was most prevalent in the following cate-gories: patient age ≤40 years (41%, 45%); tumor size 20-50 mm (39%, 46%); grade 3 (G3; 46%, 38%); pN1macro status (47%, 62%); patients treated before 2005 (26%, 38%); and patients treated via mastectomy (35%, 44%), ALND (42%, 45%), ET (23%, 44%), chemotherapy (40%, 39%), RNI (37%, 46%), and postmastectomy radiotherapy (43%, 55%), respectively. Additionally, LVI was most prevalent in ER-negative patients presenting HER2+ BC and in ER-positive patients presenting the luminal B-like subtype, specifically HER2-negative G3 (44%). The prevalence of LVI according to tumor subtypes and AC was also analyzed (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop. 2021.100316). Binary logistic regression according to ER status revealed that LVI was significantly associated with all variables except time period (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2021.100316), where the difference before/after 2005 was not significant, with increases alongside both grade and tumor size. Multivariate analysis of LVI alongside other significant characteristics provided results that were consistent with the literature (Supplementary Table S3, available at https:// doi.org/10.1016/j.esmoop.2021.100316).

Table 2. Association of LVI with clinical and pathological characteristics according to ER status

	ER >0 patients					ER <0 patients				
	Negative		Positive		χ²	Negative		Positive		χ²
	n	%	n	%	P	n	%	n	%	P
	11 376	77.6	3279	22.4		1741	65.3	926	34.7	
Age, years										<0.0001
≤40	481	4.2	340	10.4	<0.0001	192	11.0	155	16.8	
40.1-50	2305	20.3	882	28.9		419	24.1	241	26.1	
50.1-74.9	7604	66.9	1810	55.2		1017	58.4	485	52.4	
≥75	982	8.6	247	7.5		112	6.4	44	4.8	
T size, mm										<0.0001
≤5	854	7.5	42	1.3	<0.0001	162	9.3	19	2.1	
5.1-10	3671	32.3	389	11.9		434	24.9	116	12.5	
10.1-19.9	4340	38.2	1274	38.9		569	32.7	295	31.9	
20-50	2511	22.1	1574	48.0		576	33.1	496	53.6	
T histology										<0.0001
Ductal	8545	75.1	2904	88.6	<0.0001	1504	86.4	853	92.1	
Lobular	1780	15.6	201	6.1		116	6.7	43	4.6	
Mixed	216	1.9	72	2.2		13	0.7	11	1.2	
Others	835	7.3	102	3.1		108	6.2	19	2.1	
Grade										<0.0001
1	5024	44.2	623	19.0	<0.0001	293	16.8	58	6.3	
2	5307	46.7	1749	53.3		586	33.7	342	36.9	
3	1045	9.2	907	27.7		862	49.5	526	56.8	
pN status										<0.0001
pN0	8385	73.7	1245	38.0	<0.0001	1400	80.4	441	47.6	
pN0 (i+)	353	3.1	177	5.4		23	1.3	16	1.7	
pN1mi	938	8.2	379	11.6		56	3.2	36	3.9	
pN1macro	1700	14.9	1478	45.1		262	15.0	433	46.8	
Breast surgery										<0.0001
Conservative	9642	84.8	2426	74.0	<0.0001	1496	85.9	745	80.5	
Mastectomy	1489	13.1	786	24.0		221	12.7	172	18.6	
Unknown	245	2.2	67	2.0		24	1.4	9	1.0	
Axillary surgery										<0.0001
SLNB	7125	62.6	938	28.6	<0.0001	763	43.8	154	16.6	
SLNB + ALND	2786	24.5	1293	39.4		260	14.9	183	19.8	
ALND	1463	12.9	1048	32.0		718	41.2	589	63.6	
Periods										<0.0001
<2005	5221	45.9	1803	55.0	<0.0001	1125	64.6	696	75.2	
≥2005	6155	54.1	1475	45.0		616	35.4	230	24.8	
T location										<0.0001
Outer/equatorial	5416	47.6	1716	52.3	<0.0001	866	49.7	525	56.7	
Inner	2488	21.9	749	22.8		418	24.0	234	25.3	
Unknown	3472	30.5	815	24.9		457	26.2	167	18.0	
Endocrine therapy										0.007
No	1428	12.6	311	9.5	<0.0001	1654	95.0	857	92.5	
Yes	9942	87.4	2965	90.5		87	5.0	69	7.5	
Chemotherapy										<0.0001
No	8189	72.0	1158	35.3	<0.0001	728	41.8	269	29.0	
Yes	3187	28.0	2121	64.7		1013	58.2	657	71.0	
RNI										<0.0001
No	6471	69.7	1133	40.4	<0.0001	814	58.9	234	32.7	
Yes	2810	30.3	1671	59.6		569	41.1	482	67.3	
Postmastectomy Radiotherapy										<0.0001
No	607	40.8	123	15.7	<0.0001	92	41.6	13	7.6	
Yes	880	59.2	662	84.3		129	58.4	159	92.4	
Subtypes										
Luminal A	7127	87.1	1382	66.1	<0.0001					
Luminal B HER2− G3	611	7.5	473	23.0						
Luminal B HER2+	448	5.5	224	10.9						
HER2						231	23.9	129	33.7	<0.0001
Triple negative						737	76.1	254	66.3	

ALND, axillary lymph node dissection; ER, endocrine receptor; HER2, human epidermal growth factor receptor 2; LVI, lymphovascular invasion; RNI, regional node irradiation; SLNB, sentinel lymph node biopsy; T, tumor.

Prognostic impact of LVI on OS, DFS, and MFS in the entire population: univariate analysis

Univariate analyses were carried out on 17 292 patients. Median follow-up for all patients was 63.9 months [range: 0.1-436 months, 95% confidence interval (CI) 70.5-71.8 months]. In total, there were 727, 1086, and 628 events and 808, 1516, and 577 events for OS, DFS, and MFS in LVI-positive and LVI-negative populations, respectively. The
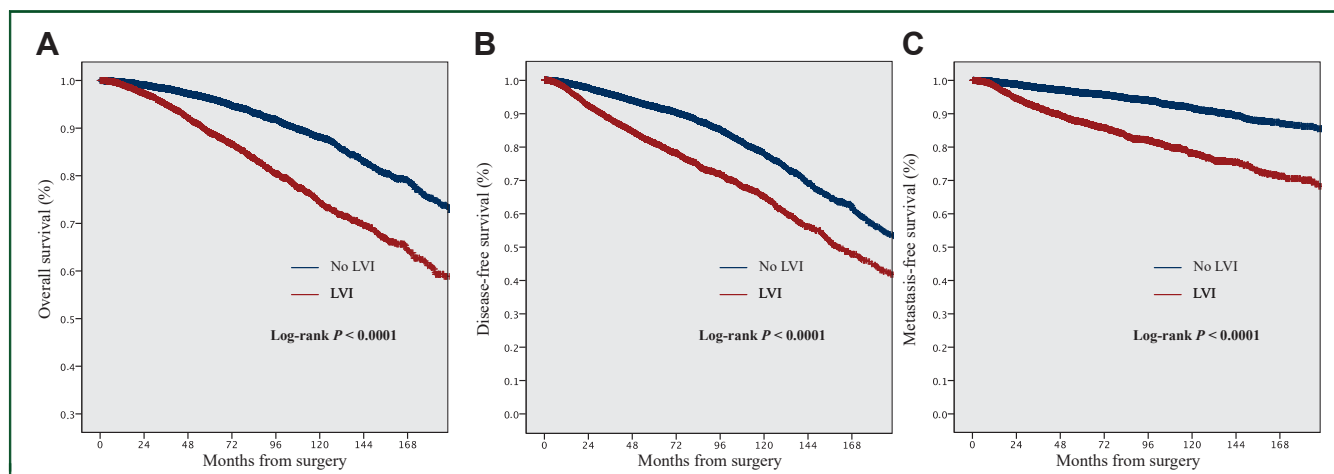
Figure 1. Kaplan−Meier curves demonstrating (A) disease-free survival, (B) metastasis-free survival, and (C) overall survival, according to the presence of lymphovascular invasion (LVI).

presence of LVI was significantly associated with decreased survival: the 5-year OS, DFS, and MFS rates were 89%, 81%, and 87% in LVI-positive versus 96%, 92%, and 96% in LVI-negative populations, respectively (Figure 1A-C).

Prognostic impact of LVI on OS, DFS, and MFS: multivariate analyses

Multivariate analyses were conducted on the entire population (all patients) and the following subgroups of interest: patients with ER-positive, ER-positive/G1 or 2, ER-positive/G3, luminal A-like (ER+/HER2−/G1 or 2), ER-negative tumors, or pN0. The impact of LVI was further analyzed according to exposure to AC in the subgroups with available data (Table 3). The presence of LVI was significantly associated with a negative impact on OS, DFS, and MFS in all patients [hazard ratio (HR) = 1.345, 95% CI 1.202-1.505; HR = 1.312, 95% CI 1.201-1.432; and HR = 1.415, 95% CI 1.247-1.605, respectively; $P < 0.0001$], both with AC (HR = 1.439, 95% CI 1.227-1.687; HR = 1.321, 95% CI 1.170-1.491; and HR = 1.480, 95% CI 1.259-1.740, respectively; $P < 0.0001$) and without (HR = 1.230, 95% CI 1.043-1.450; $P = 0.014$; HR = 1.293, 95% CI 1.136-1.471; $P < 0.0001$; HR = 1.271, 95% CI 1.033-1.563; $P = 0.024$, respectively). This adverse prognostic impact was also observed in a propensity score-based analysis. In the matched cohort (Supplementary Figure S1 and Table S4, available at https://doi.org/10.1016/j.esmoop.2021.100316), tumors associated with LVI had significantly reduced OS, DFS, and MFS (HR = 1.574, 95% CI 1.164-1.816; HR = 1.488, 95% CI 1.330-1.666; and HR = 1.609, 95% CI 1.415-1.831, respectively; $P < 0.0001$) (Figure 2).

The negative impact of LVI on OS, DFS, and MFS was also significant in all patients with ER-positive tumors (14 627) and pN0 (11 470). Similar results were observed for those with ER-positive/G1-2 tumors, either overall (12 703) or without AC (8809). However, in patients with AC (3876), only DFS (HR = 1.288, 95% CI 1.070-1.550; $P = 0.008$) and MFS (HR = 1.331, 95% CI 1.029-1.721; $P = 0.029$) were associated with a negative impact of LVI, while OS did not

reach statistical significance (HR = 1.279, 95% CI 0.990-1.651; $P = 0.06$). Furthermore, there was no significant difference concerning the presence of LVI or not regarding the survival of patients with ER-positive/G3 tumors (1948), regardless of HER2 expression.

In all patients presenting the luminal A-like subtype (8471), the presence of LVI was only significantly associated with a negative impact on DFS (HR = 1.230, 95% CI 1.020-1.483; $P = 0.030$). In patients without AC (5946), LVI was negatively associated with both OS (HR = 1.501, 95% CI 1.050-2.146; $P = 0.026$) and DFS (HR = 1.464, 95% CI 1.134-1.891; $P = 0.003$), but not MFS (HR = 1.379, 95% CI 0.878-2.165; $P = 0.163$). There was no significant association between LVI and any survival outcome in patients with luminal A-like tumors who underwent AC. Furthermore, in the propensity score-matched cohort of luminal A-like tumors (Supplementary Table S5 and Figure S2, available at https://doi.org/10.1016/j.esmoop.2021.100316), AC was associated with a significant increase in both DFS and OS (HR = 0.580, 95% CI 0.363-0.927; $P = 0.021$ and HR = 0.469, 95% CI 0.232-0.947; $P = 0.03$, respectively), but statistical significance was not reached for MFS (HR = 0.689, 95% CI 0.383-1.241; $P = 0.215$) (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2021.100316).

Cox regression analysis further demonstrated the significant association of the presence of LVI with a negative impact on OS, DFS, and MFS in patients with ER-negative tumors (2665; HR = 1.548, 95% CI 1.280-1.871; HR = 1.425, 95% CI 1.221-1.663; HR = 1.543, 95% CI 1.244-1.915, respectively; $P < 0.0001$). In all patients with ER-negative tumors (2667), as well as those with HER2-positive/ER-negative (360), triple-negative (1351), and pN0 tumors (11 471), LVI had an independent adverse prognostic impact on OS, DFS, and MFS (Table 3).

In patients with LVI-positive/HER2-positive/ER-negative tumors, those treated after 2005 ($n = 85$) (date of introduction of adjuvant trastuzumab in France) had significantly fewer events than those treated before 2005 ($n = 44$) (OS: HR = 0.313, 95% CI 0.127-0.775, $P = 0.012$; DFS:

Table 3. Association of LVI with overall, disease-free, and metastasis-free survival according to pN0 status and tumor subtypes with or without adjuvant chemotherapy

Patients	OS			DFS			MFS		
	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI
All patients	**<0.0001**	1.345	1.202	1.505	**<0.0001**	1.312	1.201	1.432	**<0.0001**	1.415	1.247	1.605
Without AC	**0.014**	1.230	1.043	1.450	**<0.0001**	1.293	1.136	1.471	**0.024**	1.271	1.033	1.563
With AC	**<0.0001**	1.439	1.227	1.687	**<0.0001**	1.321	1.170	1.491	**<0.0001**	1.480	1.259	1.740
ER > 0	**<0.0001**	1.301	1.131	1.498	**<0.0001**	1.309	1.176	1.457	**<0.0001**	1.322	1.130	1.547
ER > 0 G1-2	**0.002**	1.305	1.106	1.54	**<0.0001**	1.32	1.165	1.494	**0.002**	1.362	1.125	1.649
Without AC	**0.017**	1.307	1.048	1.629	**0.001**	1.331	1.124	1.576	**0.026**	1.385	1.040	1.844
With AC	0.06	1.279	0.99	1.651	**0.008**	1.288	1.07	1.55	**0.029**	1.331	1.029	1.721
ER > 0 G3	0.104	1.249	0.956	1.631	0.062	1.223	0.99	1.511	0.175	1.206	0.920	1.581
HER2−	0.854	0.964	0.652	1.426	0.281	1.175	0.876	1.576	0.878	1.030	0.876	1.493
HER2+	0.506	1.647	0.378	7.168	0.939	1.039	0.392	2.751	0.515	0.677	0.209	2.195
Luminal A	0.134	1.227	0.939	1.602	**0.030**	1.230	1.020	1.483	0.460	1.117	0.833	1.497
Without AC	**0.026**	1.501	1.050	2.146	**0.003**	1.464	1.134	1.891	0.163	1.379	0.878	2.165
With AC	0.967	1.008	0.684	1.485	0.736	1.047	0.801	1.369	0.894	1.026	0.703	1.498
ER < 0	**<0.0001**	1.548	1.28	1.871	**<0.0001**	1.425	1.221	1.663	**<0.0001**	1.543	1.244	1.915
ER < 0												
TN	**<0.0001**	1.849	1.322	2.587	**<0.0001**	1.646	1.26	2.149	**0.001**	1.853	1.294	2.653
ER < 0												
HER2+	**0.011**	2.406	1.227	4.718	**0.018**	1.791	1.103	2.907	**0.019**	2.239	1.140	4.390
>2005	**0.007**	0.383	0.191	0.767	**0.001**	0.433	0.265	0.708	**0.018**	0.447	0.229	0.273
pN0	**0.019**	1.225	1.034	1.452	**0.004**	1.210	1.064	1.375	**0.021**	1.269	1.037	1.553

Significant values are indicated in bold.
AC, adjuvant chemotherapy; CI, confidence interval; DFS, disease-free survival; ER, estrogen receptor; G, grade; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; LVI, lymphovascular invasion; MFS, metastasis-free survival; OS, overall survival; TN, triple negative.

HR = 0.371, 95% CI 0.187-0.736, P = 0.005; and MFS: HR = 0.422, 95% CI 0.198-0.897, P = 0.025). More specifically, OS, DFS, and MFS at 5 years versus 7 years before 2005 were 71.9% ± 6.9% (standard error) versus 66.3% ± 7.4%, 56.5% ± 7.5% versus 53.5% ± 7.7%, and 64.1% ± 7.5% versus 64.1% ± 7.5%, respectively, whereas OS, DFS, and MFS at 5 years versus 7 years after 2005 were 90.4% ± 3.8% versus 90.4% ± 3.8%, 79.2% ± 5.0% versus 79.2% ± 5.0%, and 82.2% ± 4.7% versus 82.2% ± 4.7%, respectively.

DISCUSSION

The decision to offer BC patients adjuvant systemic treatment is most often made for those with node-positive diseases, while prognostic factors including LVI, tumor size, tumor grade, proliferation factors, and HER2 and ER status are primarily used to identify a subset of node-negative patients with a reduced risk of recurrence without additional therapy.[23] However, the exact extent of LVI's prognostic significance remains unclear. LVI's clinical utility in adjuvant decision making is either unrecognized or limited in major guidelines.[3,9-12] Indeed, the panels of both the 14th and 15th St. Gallen International Breast Cancer Conferences advocated for the consideration of LVI regarding the implementation of either AC or RNI, respectively, only in patients with other high-risk features/molecular subtypes such as triple-negative or grade 3 cancers or with >3 positive nodes, while the 16th Conference only included LVI as part of individualized chemotherapy decisions based on numerous factors.[3,24,25] Yet, our results
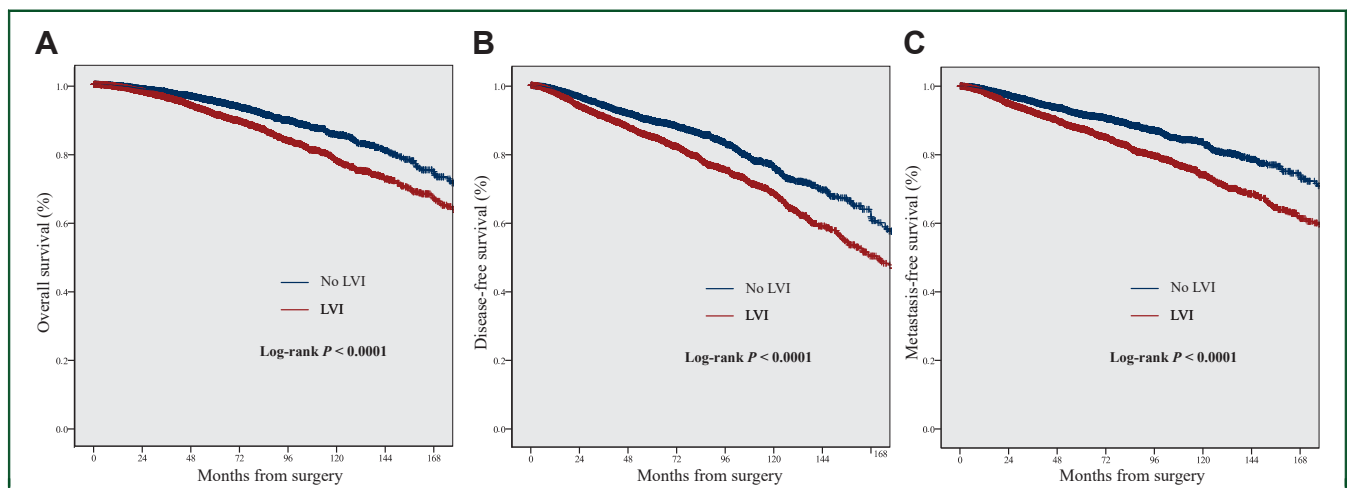

Figure 2. Kaplan−Meier curves demonstrating (A) overall survival, (B) disease-free survival, and (C) metastasis-free survival, according to the presence of lymphovascular invasion (LVI), in the matched cohort.

from a large population of patients with early BC have demonstrated that the presence of LVI may also be an important independent prognostic factor in patients without other high-risk features, and to our knowledge, no other study of this size has investigated the association of LVI in BC patients with treatment according to molecular subtypes. We found that LVI was significantly associated with not only every clinical and pathological characteristic that we examined, regardless of ER status and including both node-negative and node-positive early BC patients, but also with a negative impact on OS, DFS, and MFS in almost every subtype, with the notable exceptions of ER-positive G3 tumors and luminal A-like tumors with AC. These findings suggest that the analysis of LVI may hold greater prognostic significance than what was previously thought both in general and more particularly in patients with luminal A-like tumors, with subsequently greater relevance in the decision for adjuvant therapy. This contention is further supported by the results of our previously published studies on both the strong interaction between the presence of LVI and axillary lymph node involvement,[26] and the significance of LVI as an independent prognostic factor in patients with triple-negative tumors.[27,28]

More specifically, in our present study, Cox regression analysis indicated the significant association of the presence of LVI with a negative impact on OS, DFS, and MFS in all patients with and without AC, those with ER-positive/grade 1-2 tumors without AC, and those with pN0 status. This was also the case for DFS and MFS, but not OS, in patients with ER-positive/grade 1-2 tumors treated with AC. Interestingly, no independent significant prognostic impact was detected in the ER-positive/grade 3 subgroup, regardless of HER2 expression, with most patients having received AC (73.0%). This may indicate either that the high grade already contains adverse prognostic information associated with LVI or that AC eliminates the adverse prognostic impact of LVI in these patients.

Similarly, but of greater interest, the analysis of patients within the luminal A-like subgroup did not reveal any significant negative impact of the presence of LVI on survival in those treated with AC, whereas DFS in all patients and both DFS and OS in patients without AC were negatively affected in this subgroup. We obtained similar results in an additional survival analysis of a chemotherapy propensity score-matched cohort of patients with luminal A-like cancers treated either with or without AC, though no other study exists with which we could compare these results. Our findings therefore indicate that LVI independently identifies a subgroup of patients with poor prognosis within the luminal A-like subtype, generally considered to be rather indolent, for whom chemotherapy may reduce the adverse prognostic impact of LVI. Further study is required in order to determine the extent of LVI's association with luminal A-like cancers.

Our findings are consistent with those of other recent studies that investigated the prognostic significance of LVI,[16,17,29-38] even within the modern era of personalized

medicine with its emphasis on genomic assays. However, very few of these studies have analyzed LVI in the context of molecular BC subtypes. For instance, while investigating genes associated with LVI but neither subtypes nor treatment, Kurozumi et al. found that LVI positivity was an independent poor prognostic factor in multivariate analysis with 10-year OS significantly worse in LVI-positive versus LVI-negative BC patients in two different cohorts (Molecular Taxonomy of Breast Cancer International Consortium (METABRIC): LVI+ $n = 635$, HR = 1.70; $P < 0.0001$ and The Cancer Genome Atlas (TCGA): LVI+ $n = 295$, HR = 2.2; $P = 0.00019$).[37] In a study that did analyze the prognostic impact of LVI according to subtype, Ryu et al. demonstrated that LVI had greater prognostic significance than pathologic complete response in 187 BC patients treated with neoadjuvant chemotherapy, with LVI associated with worse RFS and OS in all patients while patients with both LVI and hormone receptor-negative cancers experienced the worst RFS and OS (hormone receptor status alone had no impact).[30]

In regard to the modern era of genomic assays, Mutai et al. analyzed the association between LVI status and the Oncotype DX RS in 657 patients with ER-positive BC. They found that the presence of LVI was significantly associated with worse DFS in all patients and worse OS in patients in an Oncotype DX intermediate-risk (RS 18-30) subgroup. Notably, they also observed that more LVI-positive patients than LVI-negative patients (42% versus 23%, respectively; $P = 0.009$) received AC regardless of the Oncotype DX risk category, further highlighting the perceived clinical significance of LVI. However, these results should be interpreted with caution as only 38 patients were LVI positive and the number of DFS events was too low for multivariate analysis.[16] In another larger study of the relationship between LVI and the 21-gene RS, Makower et al. reported that LVI was associated with poor OS in their entire cohort of 77 425 ER-positive early BC patients with an RS (9856 LVI positive, HR = 1.24; $P < 0.0001$) and with N0 (HR = 1.37; $P < 0.0001$) but not N+ status, as well as in all 119 321 patients without an RS and those with N0 and N+ (HR = 1.21; $P < 0.0001$, HR = 1.15; $P = 0.008$, and HR = 1.22; $P < 0.00001$, respectively). Among patients with both an RS and N0 status, LVI was associated with worse OS in those with RS 11-25 (HR = 1.31, 95% CI 1.09-1.57) and 26-100 (HR = 1.58, 95% CI 1.30-1.93), but not RS 0-10 (HR = 1.1, 95% CI 0.77-1.53), although there was no statistically significant interaction between LVI and RS. The authors concluded that while LVI did not predict chemotherapy benefit in those with intermediate RS (11-25), it did add prognostic information in ER-positive, HER2-negative, N0 BC with RS (11-100).[17] Both of these studies' findings further support those of the Trial Assigning Individualized Options for Treatment (TAILORX) study which, though did not evaluate LVI, similarly demonstrated that while patients with an intermediate-risk Oncotype DX score (11-25) did not receive an overall benefit from AC, certain patients (pre-menopausal with RS 16-25) could still derive some advantages, especially when high-risk clinical features such as tumor size and/or grade were present.[39,40] Taken together, the results

of our own study and those of these studies evaluating RS suggest the existence of subgroups of patients with intermediate RS that may not normally receive AC but for whom other prognostic factors, LVI in particular, may indicate clinical benefit.

It should also be noted that while the morphological assessment of LVI status in our study relied heavily on the assessments of individual pathologists across 13 centers, the prevalence of LVI in our patient population (24.3%) corresponded to the average rate (24%) of LVI detected via hematoxylin—eosin-stained slides across 32 studies in Gujam et al.'s systematic review of data published from 1964 to 2012 regarding LVI in BC. Similarly, of the 34 studies included in the review, 32 reported the association of LVI with an unfavorable outcome (reduced survival in 19), while Gujam et al. themselves concluded that LVI was a powerful prognostic factor of poorer survival whose impact was mainly seen in patients with node-negative BC.[41] However, the majority of these studies were only able to include a small number of LVI-positive low-risk or luminal A-like patients, which left the prognostic significance of LVI undecided. Even the study conducted by Ejlertsen et al., one of the largest multicenter studies to date with the inclusion of 16 172 (2453 LVI positive) patients with operable BC, only included 54 low-risk LVI-positive patients, though they reported that while LVI was consistently significantly associated with reduced OS and DFS in patients with other high-risk features, this was not the case for low-risk disease. Additionally, the authors found that the negative prognostic influence of LVI was not statistically significantly different between patients with or without adjuvant therapy, though HER2 status was not assessed, aromatase inhibitors were not fully implemented at the time of the study, and only 38 patients received adjuvant trastuzumab while none received taxanes.[13] In our study, in LVI-positive/HER2-positive/ER-negative tumors, patients treated after 2005 (date of introduction of adjuvant trastuzumab in France) had fewer events than those treated before 2005, suggesting that trastuzumab was effective at improving survival outcomes in this subset of patients. However, our binary logistic regression analysis according to ER status found that the difference before/after 2005 was not significantly associated with the presence of LVI. Of note, and as previously reported,[27] we found that LVI had also an independent adverse prognostic impact in triple-negative BC. In this subtype, recent data indicate that tumor-infiltrating lymphocytes (TILs) rather than tumor tissue/stromal features such as LVI[42] or grade may have an important prognostic impact. Yet, TILs were not available in this large retrospective cohort, and we could not assess the impact of LVI with regard to this variable. Our study has several other limitations. Most notably, LVI was mainly assessed via HES, and while this has been shown to be less accurate than IHC for lymphatic endothelial markers, such as D2-40,[5,41,43] IHC more often identifies invasive emboli in lymphatic rather than blood vessels.[37,44] The limited use of IHC was mostly due to the inherent difficulty in applying an identification method such as IHC to a large and multi-institutional

cohort. In addition, as mentioned previously, the analyses were carried out by individual pathologists and could allow for the misclassification of LVI if said analyses were not consistent between centers. However, our multicenter study does provide the advantages of limiting biases inherent in single-center studies while also reflecting real-world practice. Additional strengths of our study include the large sample size of >17 000 patients in multivariate and propensity score-matched analyses of the associations between LVI, numerous clinical characteristics, and OS, DFS, and MFS.

In conclusion, our study had the notable advantage of analyzing LVI in a large cohort of BC patients from 13 cancer institutions, which consequently allowed for more powerful statistical analyses and provided more applicable findings. Our results demonstrated a significant association of the presence of LVI with patient age; tumor size, histology, location, and grade; pN status; breast or axillary surgery; and adjuvant therapy; all regardless of ER status; as well as multiple subtypes of BC. Furthermore, the strong negative association between LVI and the length of OS, DFS, and MFS in all patients regardless of AC and in both ER-positive and ER-negative patients, with the notable exceptions of patients with luminal A-like tumors treated with AC, grants additional support to the argument that LVI is a more important prognostic factor than previously thought concerning the decision for adjuvant therapy. Indeed, the fact that only patients with luminal A-like tumors with AC had no association with a negative impact of LVI on OS, DFS, and MFS indicates that LVI could be especially important regarding adjuvant treatment decisions for a subset of patients with a supposed reduced risk of recurrence that may still benefit from adjuvant therapy. Accordingly, the respective role of genomic assays versus the prognostic significance of LVI should be discussed when making adjuvant decisions, while the integration of LVI into the calculation of risk provided by such assays (similarly to tumor size and node status) for luminal A-like cancers (G1-2) could improve prognostication in this subset of patients.

Another important application of our results may be to contribute to better define clinically high-risk tumors. Since genomic signature in the clinical routine may be predominantly indicated in these latter forms, the presence of LVI might identify tumors in which these tests have a high clinical utility. In addition, once a genomic signature is carried out, LVI might also help to orient adjuvant therapeutic decision, especially in case of intermediate-risk classes. We proposed that patients with luminal A-like tumors associated with LVI should be considered as clinically high risk, while there is room for considering that patients with intermediate genomic score (Oncotype DX or Prosigna) might also be considered as high risk. Nevertheless, more data are needed to elucidate the prognostic value of LVI in the context of current genomic signatures.

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