ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN CANCER HORIZONS

ORIGINAL RESEARCH

Lack of prognostic impact of sentinel node micro-metastases in endocrine receptor-positive early breast cancer: results from a large multicenter cohort☆

G. Houvenaeghel[1*], A. de Nonneville[2], M. Cohen[1], N. Chopin[3], C. Coutant[4], F. Reyal[5], C. Mazouni[6], P. Gimbergues[7], A.-S. Azuar[8], M.-P. Chauvet[9], J.-M. Classe[10], E. Daraï[11], A. Martinez[12], R. Rouzier[13], C. T. de Lara[14], E. Lambaudie[1], J. Barrou[1] & A. Goncalves[2]

Departments of [1]Surgical Oncology; [2]Medical Oncology, CRCM, Institut Paoli-Calmettes, Aix-Marseille Univ, CNRS, INSERM, Marseille; [3]Department of Surgical Oncology, Centre Léon Bérard, Lyon; [4]Department of Surgical Oncology, Centre Georges François Leclerc, Dijon; [5]Department of Surgical Oncology, Institut Curie, Paris Cedex 05, Paris; [6]Department of Surgical Oncology, Institut Gustave Roussy, Villejuif; [7]Department of Surgical Oncology, Centre Jean Perrin, Clermont Ferrand; [8]Department of Surgical Oncology, Hôpital de Grasse, Grasse; [9]Department of Surgical Oncology, Centre Oscar Lambret, Lille; [10]Department of Surgical Oncology, Institut René Gauducheau, St Herblain; [11]Department of Surgical Oncology, Hôpital Tenon, Paris; [12]Department of Surgical Oncology, Centre Claudius Regaud, Toulouse; [13]Department of Surgical Oncology, Hôpital René Huguenin, Saint Cloud; [14]Department of Surgical Oncology, Institut Bergonié, Bordeaux, France

Available online xxx

Background: Prognostic impact of lymph node micro-metastases (pN1mi) has been discordantly reported in the literature. The need to clarify this point for decision-making regarding adjuvant therapy, particularly for patients with endocrine receptor (ER)-positive status and HER2-negative tumors, is further reinforced by the generalization of gene expression signatures using pN status in their recommendation algorithm.

Patients and methods: We retrospectively analyzed 13 773 patients treated for ER-positive breast cancer in 13 French cancer centers from 1999 to 2014. Five categories of axillary lymph node (LN) status were defined: negative LN (pN0i−), isolated tumor cells [pN0(i+)], pN1mi, and pN1 divided into single (pN1 = 1) and multiple (pN1 > 1) macro-metastases (>2 mm). The effect of LN micro-metastases on outcomes was investigated both in the entire cohort of patients and in clinically relevant subgroups according to tumor subtypes. Propensity-score-based matching was used to balance differences in known prognostic variables associated with pN status.

Results: As determined by sentinel LN biopsy, 9427 patients were pN0 (68.4%), 546 pN0(i+) (4.0%), 1446 pN1mi (10.5%) and 2354 pN1 with macro-metastases (17.1%). With a median follow-up of 61.25 months, pN1 status, but not pN1mi, significantly impacted overall survival (OS), disease-free survival (DFS), metastasis-free survival (MFS), and breast-cancer-specific survival. In the subgroup of patients with known tumor subtype, pN1 = 1, as pN1 > 1, but not pN1mi, had a significant prognostic impact on OS. DFS and MFS were only impacted by pN1 > 1. Similar results were observed in the subgroup of patients with luminal A-like tumors (n = 7101). In the matched population analysis, pN1macro, but not pN1mi, had a statistically significant negative impact on MFS and OS.

Conclusion: LN micro-metastases have no detectable prognostic impact and should not be considered as a determining factor in indicating adjuvant chemotherapy. The evaluation of the risk of recurrence using second-generation signatures should be calculated considering micro-metastases as pN0.

Key words: breast cancer, sentinel node, micro-metastases, survival

INTRODUCTION

The most commonly accepted prognostic factors for proposing adjuvant therapy in early breast cancer (BC) include patient age, tumor size, axillary lymph node (LN) status, and tumor pathology, including grade, endocrine receptors (ER) status, HER2 status, lympho-vascular invasion (LVI), and proliferation assays such as the Ki67 labeling index.[1-3]

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

*Correspondence to: Prof. Gilles Houvenaeghel, Department of Surgical Oncology, CRCM, Institut Paoli-Calmettes, 232 Boulevard de Sainte Marguerite, 13009 Marseille, France. Tel: +33-4-91-22-35-32; Fax: +33-4-91-22-36-13
E-mail: houvenaeghelg@ipc.unicancer.fr (G. Houvenaeghel).

☆Note: This work was approved by our institutional review board (IPC – Comité d'Orientation Stratégique).

overall survival (OS) and highlighted the importance of prognostic factors in prescribing individualized treatments with regard to the magnitude of expected clinical benefit.[1]

Sentinel LN biopsy (SLNB) practice, followed by serial sectioning of the sentinel node (SN) and immunohistochemical (IHC) analysis, resulted in increased detection of occult nodes metastases compared with axillary LN dissection (ALND) that is usually associated with a single hematoxylin- and eosin-stained (HES) section. The prognostic value of micro-metastases has been discordantly reported in the literature according to the periods of inclusions, the technique used for micro-metastases identification (SLNB or ALND), the cohort sizes, and different adjustments in multivariate analysis.[4-32] The detection rates of micro-metastases were up to 8%-10%[32-34] of patients with early BC and SLNB, representing 10%-25% of patients with positive SN.[33-37] IHC analysis increased the SN involvement rate from 9% to 47% when compared with HES only.[37,38] However, different rates of LN involvement according to molecular-like tumor subtypes were reported with lower rates in triple-negative BC and higher rates in HER2-positive BC.[33,39-41] The lack of consensus on the importance of micro-metastasis when deciding upon adjuvant chemotherapy (AC) was further emphasized by the recent and increasing utilization of gene expression signature assay results in making adjuvant decisions, particularly for patients with ER-positive and HER2-negative tumors.[42-48]

To investigate the impact of LN micro-metastases on patient outcomes, we retrospectively analyzed a large, national, multicenter cohort of 13 773 patients with SLNB for their independent prognostic impact. Analyses were carried out on the entire population of patients with ER-positive BC, in subgroups of clinical interest according to tumor subtypes, and in a propensity-score-matched population to balance numeric differences in known prognostic variables.

PATIENTS AND METHODS

Patient selection

Medical records of early BC patients treated from January 1999 to December 2014 were retrieved from clinical databases of 13 different comprehensive cancer centers in France for retrospective analysis (ClinicalTrials.gov NCT02869607). Patient and tumor characteristics, treatments, periods, and clinical outcomes were collected. Out of an initial cohort of 23 134 patients, 13 773 were included in the present study based on histologically proven invasive BC, stage cT0, cT1, or cT2 (TNM breast cancer 8th edition) pathological tumor size $\leq$5 cm, clinically negative axillary lymph node (LN), positive ER status (estrogen and/or progesterone staining >10% of cells by IHC in line with the French guidelines), and evaluation of LN status determined by SLNB with or without completion of ALND. Patients with HER2-negative tumors and HER2-unknown status were included. Exclusion criteria were the use of systemic neoadjuvant therapies, failure to identify SN, clinically positive axillary LN, and known HER2-positivity. Among 10 826 ER-positive patients with known HER2 status, 719 patients with HER2 positivity were excluded, corresponding with a HER2-positive tumor rate of 6.64% (719/10 826). Consequently, estimates of HER2-positive status among the 3666 patients with unknown HER2 status was 243.

All procedures carried out in this study involving human participants were done in accordance with the French ethical standards and with the 2008 Helsinki declaration.

All included patients provided written informed consent before surgery, including the use of their data for research.

Pathology

Involvement of SN was diagnosed by serial sections with standard HES. If all the serial sections were negative, an additional IHC analysis was carried out. Five categories of LN status were defined: negative lymph node (pN0i−), isolated tumor cells [$\leq$0.2 mm: pN0(i+)], micro-metastases (pN1mi, >0.2-2 mm), and macro-metastases (>2 mm), divided into single (pN1 = 1) and multiple (pN1 > 1) macro-metastases. There was no central review. The method used for the detection of SN was a combined technique or isotopic only detection during the last years. Two tumor subtypes were defined as surrogates for molecular subtypes based on tumor grade and ER and HER2 status (patients with unknown HER2 status were consequently excluded): luminal A-like (ER+/HER2-/grade 1 or 2 [SBR grading]) and luminal B-like (ER+/HER2-/grade 3 [SBR grading]).[49]

Statistics

The associations between categorical values were evaluated via χ^2 tests. Multivariate survival analyses [overall survival (OS), disease-free survival (DFS), metastasis-free survival (MFS), and breast-cancer-specific survival (BCSS)] were carried out using the Cox proportional hazard regression model adjusted for variables significantly associated with final pN status (pathologic results of SLNB and ALND when it was carried out), in the entire population and in the subgroup of patients with known tumor subtypes with distinction between pN0, pN0(i+), pN1mi, pN1 and then with distinction between pN0, pN0(i+), pN1mi, pN1 = 1, and pN1 > 1 (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2021.100151). We conducted the same analysis according to SN status regardless of ALND when it was carried out in the sub-population of patients without AC, in all patients with positive ER status, and in the subgroup of patients with known tumor subtype with distinction between pN0, pN0(i+), and pN1mi. To balance differences in known prognostic variables associated with pN status, we generated 1 : 1 : 1 matched cohorts of the three following groups: pN0(i−), pN1mi, and pN1macro. Coefficients of a logistic regression adjusted by age, SLNB/ALND, LVI, tumor size, type of breast surgery, endocrine therapy, AC, and tumor grade were used to compute a propensity score for each pN0 and pN1macro patient first (population 1), and for matched population 1 and pN1mi second. Optimal 1 : 1 matching using Mahalanobis distance was carried out with a caliper of 0.2 for population 1, and then 2 : 1 matching for matched population 1 and pN1mi

Table 1. Characteristics of patients according to pN status

	pN0		pN0(i+)		pN1mi		pN1macro		Chi^2
	Nb	%	Nb	%	Nb	%	Nb	%	*P* value
	9427	68.4	546	4.0	1446	10.5	2354	17.1	<0.0001
HER2 status									
Negative	6967	73.9	329	60.3	970	67.1	1841	78.2	
Unknown	2460	26.1	217	39.7	476	32.9	513	21.8	
Age, years									
≤40	354	3.8	39	7.1	92	6.4	163	6.9	<0.0001
40.1-50	1797	19.1	121	22.2	351	24.3	629	26.7	
50.1-74.9	6470	68.7	355	65.0	912	63.1	1403	59.6	
≥75	801	8.5	31	5.7	91	6.3	159	6.8	
Histology									
Ductal	7098	75.3	399	73.1	1186	82.0	1827	77.6	<0.0001
Lobular	1362	14.4	103	18.9	143	9.9	378	16.1	
Mixed	153	1.6	20	3.7	22	1.5	76	3.2	
Others	814	8.6	24	4.4	95	6.6	73	3.1	
SLNB/ALND									
SLNB	8463	89.8	132	24.2	244	16.9	107	4.5	<0.0001
SLNB+ALND	964	10.2	414	75.8	1202	83.1	2247	95.5	
LVI									
No	7382	78.3	354	64.8	960	66.4	1238	52.6	<0.0001
Yes	827	8.8	176	32.2	372	25.7	803	34.1	
Unknown	1218	12.9	16	2.9	114	7.9	313	13.3	
Periods									
<2005	3843	40.8	267	48.9	583	40.3	955	40.6	0.002
≥2005	5584	59.2	279	51.1	863	59.7	1398	59.4	
Localization T									
Outer	4530	48.1	305	55.9	759	52.5	1269	53.9	<0.0001
Inner	2230	23.7	160	29.3	308	21.3	471	20.0	
Unknown	2667	28.3	81	14.8	379	26.2	614	26.1	
RNI									
No	6785	86.9	277	57.1	616	48.2	322	15.6	<0.0001
Yes	1027	13.1	208	42.9	661	51.8	1738	84.4	
T size, mm									
≤5	871	9.4	21	3.9	48	3.3	52	2.2	<0.0001
5.1-10	3395	36.5	123	22.8	309	21.5	304	13.0	
10.1-19.9	3590	38.6	222	41.1	663	46.2	894	38.4	
20-50	1362	14.7	159	29.4	370	25.8	944	40.5	
>50	76	0.8	15	2.8	45	3.1	136	5.8	
Surgery breast									
BCS	8227	87.3	444	81.3	1216	84.1	1704	72.4	<0.0001
Mastectomy	913	9.7	91	16.7	201	13.9	599	25.4	
Unknown	287	3.0	11	2.0	29	2.0	51	2.2	
Subtypes									
Luminal A-like	6423	91.8	291	83.6	905	87.6	1409	83.5	<0.0001
Lum B Her2- like	576	8.2	57	16.4	128	12.4	279	16.5	
Final pN									
pN0	919	92.9	0	0	0	0	0	0	<0.0001
pN0(i+)	0	0	414	90.6	0	0	0	0	
pN1mi	0	0	0	0	1203	90.8	0	0	
pN1macro	70	7.1	43	9.4	122	9.2	1965	0	
Endocrine therapy									
No	1228	13.0	31	5.7	55	3.8	57	2.4	<0.0001
Yes	8199	87.0	515	94.3	1391	96.2	2297	97.6	
Chemotherapy									
No	7947	84.3	352	64.5	704	48.7	382	16.2	<0.0001
Yes	1459	15.5	191	35.0	721	49.9	1893	80.4	
Neo-adjuvant	18	0.2	2	0.4	19	1.3	75	3.2	
Unknown	3	0.0	1	0.2	2	0.1	4	0.2	
Grade									
1	4276	45.4	146	26.7	571	39.5	676	28.7	<0.0001
2	4253	45.1	316	57.9	689	47.6	1282	54.5	
3	769	8.2	79	14.5	172	11.9	384	16.3	
Unknown	129	1.4	5	0.9	14	1.0	12	0.6	
Radiotherapy and mastectomy									
No	526	56.4	33	34.0	48	21.5	34	6.2	<0.0001
Yes	407	43.6	64	66.0	175	78.5	510	93.8	

ALND, axillary lymph node dissection; BCS, breast conservative surgery; LVI, Lympho-vascular invasion; Nb, number; RNI, regional nodal irradiation; T, tumor; SLNB, sentinel lymph node biopsy.

Table 2. Characteristics of patients according to pN status in the propensity-score-matched cohort

	pN0(i−)		pN1mi		pN1macro		Chi²
	Nb	%	Nb	%	Nb	%	*P* value
	409	32	449	35	436	34	
Age, years							
≤40	18	4.4	24	5.3	20	4.6	0.982
40.1-50	86	21.0	96	21.4	93	21.3	
50.1-74.9	260	63.6	284	63.3	282	64.7	
≥75	45	11.0	45	10.0	41	9.4	
SLNB/ALND							
SLNB	69	16.9	77	17.1	73	16.7	0.987
SLNB + ALND	340	83.1	372	82.9	363	83.3	
LVI							
No	327	80.0	353	78.6	348	79.8	0.866
Yes	82	20.0	96	21.4	88	20.2	
Tumor size, mm							
≤5	12	2.9	14	3.1	17	3.9	0.357
5.1-10	69	16.9	69	15.4	67	15.4	
10.1-19.9	157	38.4	165	36.7	134	30.7	
20-50	146	35.7	177	39.4	188	43.1	
>50	25	6.1	24	5.3	30	6.9	
Surgery breast							
BCS	311	76.0	339	75.5	323	74.1	0.791
Mastectomy	98	24.0	110	24.5	113	25.9	
Endocrine therapy							
No	17	4.2	22	4.9	15	3.4	0.555
Yes	392	95.8	427	95.1	421	96.6	
Chemotherapy							
No	197	48.2	219	48.8	197	45.2	0.523
Yes	212	51.8	230	51.2	239	54.8	
Grade							
1	133	32.5	148	33.0	141	32.3	0.975
2	216	52.8	232	51.7	234	53.7	
3	60	14.7	69	15.4	61	14.0	

ALND, axillary lymph node dissection; BCS, breast conservative surgery; LVI, Lympho-vascular invasion; Nb, number; SLNB, sentinel lymph node biopsy.

patients.[50-52] Final matched pN0(i−), pN1mi, and pN1macro cohorts resulted in 409, 449, and 436 patients, respectively. The impact of pN status on DFS, MFS, OS, and BCSS was assessed on this matched population by log-rank tests stratified on the pairs.[53] Statistical significance was set as $P \leq 0.05$. Analyses were carried out with SPSS version 16.0 (SPSS Inc., Chicago, Illinois) and R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient population and association of LN status with other clinico-pathological features

Among 13 773 patients with pN status determined by SLNB alone or SLNB with completion ALND (cALND), 9427 (68.4%) were pN0, 546 (4.0%) pN0(i+), 1446 (10.5%) pN1mi, and 2354 (17.1%) pN1 with macro-metastases (Table 1). For 4736 patients with cALND and pathologic results of ALND known, we observed macro-metastases at cALND in 70 (7.1%) patients of 989 pN0sn, 43 (9.4%) patients of 457 pN0(i+)sn, and in 122 (9.2%) patients of 1325 pN1mi-sn ($P < 0.0001$).

In univariate analysis, axillary LN status was significantly associated with all clinical and pathological characteristics analyzed when considering the entire cohort (Table 1). No association between clinico-pathological features and axillary LN status remains statistically significant after matching (Table 2). Clinico-pathological features distributions before and after matching are represented in Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2021.100151.

Prognostic impact of axillary LN status on DFS, MFS, and OS in the entire population (13 773 patients): univariate analysis

Median follow-up was 61.25 months [95% confidence interval (CI) 62.1-63.3], 58.9 months (95% CI 58.5-59.8) for pN0, 66.0 months (95% CI 68.1-74.0) for pN0(i+), 66.8 months (95% CI 68.6-72.4) for pN1mi, and 67.8 months (95% CI 70.2-73.4) for pN1. OS and DFS were significantly different according to axillary LN status, age, LVI, tumor size, grade, HER2 status, type of breast surgery, tumor subtypes, endocrine therapy, chemotherapy, and regional nodal irradiation (RNI). All these factors had significant prognostic impact, as well as tumor histology and periods (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2021.100151). Five-year OS were 97.7%, 97.9%, 97.6%, and 95.5% for pN0, pN0(i+), pN1mi, and pN1, respectively (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2021.100151). Five-year DFS are reported in Supplementary Table S2 and Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2021.100151. The maximal potential difference that these

Table 3. Survival results (overall survival, disease-free survival, metastasis-free survival and breast cancer-specific survival): Cox model adjusted on significant univariate criteria for all patients and for patients with tumor subtypes known, according to pN status [pN0, pN0(i+), pN1mi, pN1macro]

All patients	All patients		
	HR	*P* value	95% CI
Overall survival			
Positive ER			
pN0	1		
pN0(i+)	0.872	0.595	0.527-1.444
pN1mi	0.930	0.665	0.670-1.292
pN1a	**1.828**	**<0.0001**	1.368-2.442
Subtypes			
pN0	1		
pN0(i+)	1.089	0.788	0.585-2.026
pN1mi	0.999	0.994	0.674-1.479
pN1a	**1.973**	**<0.0001**	1.391-2.798
Disease-free survival			
Positive ER			
pN0	1		
pN0(i+)	1.102	0.524	0.817-1.487
pN1mi	0.936	0.551	0.751-1.165
pN1a	**1.408**	**0.001**	1.148-1.726
Subtypes			
pN0	1		
pN0(i+)	1.328	0.133	0.918-1.921
pN1mi	0.960	0.759	0.739-1.246
pN1a	**1.505**	**0.001**	1.178-1.921
Metastasis-free survival			
Positive ER			
pN0	1		
pN0(i+)	0.728	0.250	0.423-1.251
pN1mi	0.851	0.371	0.598-1.212
pN1a	**1.443**	**0.016**	1.071-1.943
Subtypes			
pN0	1		
pN0(i+)	0.847	0.635	0.426-1.685
pN1mi	0.905	0.633	0.601-1.363
pN1a	**1.509**	**0.021**	1.064-2.140
Breast-cancer-specific survival			
Positive ER			
pN0	1		
pN0(i+)	0.848	0.663	0.404-1.779
pN1mi	1.122	0.372	0.787-1.897
pN1a	**1.960**	**0.001**	1.328-2.893
Subtypes			
pN0	1		
pN0(i+)	0.893	0.829	0.321-2.489
pN1mi	1.355	0.255	0.803-2.288
pN1a	**2.216**	**0.001**	1.382-3.554

Significant values are indicated in bold.

analyses have the power to identify between pN0 and pN1mi status was 1.7% and hazard ratio (HR) of 2.0 with a power ≥85%.

Prognostic impact of axillary LN status on DFS, MFS, OS, and BCSS: multivariate analysis

In the entire population as well as in the subgroup of patients with known tumor subtype, with distinction between pN0, pN0(i+), pN1mi, pN1, only pN1, but not pN0(i+) or pN1mi, were independent prognostic factors for OS, DFS, MFS, and BCSS (Table 3). The other independent prognostic factors in the entire population are reported in Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2021.100151.

In the entire population, with distinction between pN0, pN0(i+), pN1mi, pN1 = 1 (1008 patients), and pN1 > 1 (1173 patients), both pN1 = 1 and pN1 > 1 status were independent prognostic factors for OS and DFS. Only pN1 > 1 status significantly impacted MFS (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2021.100151). In the subgroup of patients with known tumor subtype, an independent adverse prognostic effect was observed in OS in patients with pN1 = 1 and pN1 > 1. Only pN1 > 1 status significantly impacted DFS and MFS (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2021.100151, Figure 1).

In the population with luminal A-like tumors (grade 1-2, *n* = 8547), significant results were observed for OS for patients with pN1 = 1 and pN1 > 1 status, for DFS in patients with pN1 > 1 status, and also in patients with pN0(i+) status and for MFS and BCSS in patients with pN1 > 1 status (Supplementary Table S5 and Figure S4, available at https://doi.org/10.1016/j.esmoop.2021.100151). Other independent prognostic factors in the population with luminal A-like tumors are reported in Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2021.100151. For 1040 luminal B-like tumors, including 128 pN1mi, significant results were observed for OS, DFS, and MFS for patients with pN1 status, but not for pN1mi status (Supplementary Table S7, available at https://doi.org/10.1016/j.esmoop.2021.100151).

Results of analysis according to SN status whatever ALND pathologic results were similar without significant survival impact of pN1mi(sn) (data not shown).

Results in the population without AC with pN0, pN0(i+), and pN1mi adjusted on ER status (*n* = 7305) and adjusted on tumor subtypes (*n* = 5154) did not show any significant survival impact (OS, DFS, MFS) for pN1mi status in comparison with pN0 (Supplementary Table S8, available at https://doi.org/10.1016/j.esmoop.2021.100151). A significant BCSS difference was observed for pN1mi, only for the model applied on all patients according to ER status, without difference in the model adjusted on tumor subtypes.

Prognostic impact of axillary LN status on OS, DFS, MFS, and BCSS in the matched population

In the final pN0(i−) (*n* = 409), pN1mi (*n* = 449), and pN1macro (*n* = 436) matched cohorts, log-rank tests stratified on the pairs revealed the statistically significant impact of pN1macro compared with pN1mi on OS, MFS, and BCSS, but not DFS (Figure 2 and Supplementary Table S9, available at https://doi.org/10.1016/j.esmoop.2021.100151). When considering pN0(i−) as the reference category, the trends in the HRs of pN1mi versus pN1macro were strictly opposite: 0.62 (95% CI 0.32-1.18) versus 1.48 (95% CI 0.87-2.52), 0.97 (95% CI 0.61-1.53) versus 1.45 (95% CI 0.94-2.23), 0.64 (95% CI 0.38-1.08) versus 1.65 (95% CI

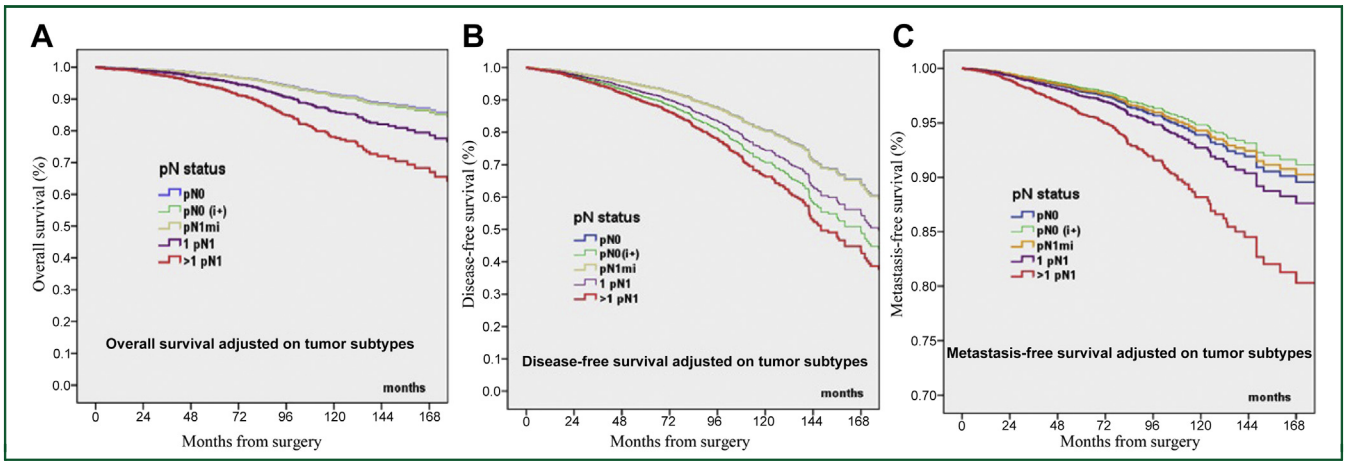

Figure 1. Survival results adjusted on tumor subtypes with distinction between pN0, pN0(i+), pN1mi, 1pN1, and more than 1pN1.
(A) Overall survival. (B) Disease-free survival. (C) Metastasis-free survival.

1.07-2.54), and 0.59 (95% CI 0.24-1.44) versus 1.93 (95% CI 0.95-3.91) for OS, DFS, MFS, and BCSS, respectively.

DISCUSSION

In this large retrospective cohort of patients with SLNB and ER-positive tumors, only macro-metastatic LN involvement, including pN1 = 1 and pN1 > 1, but not pN1mi, had a significant and independent pejorative prognostic impact on survival outcomes. However, the maximal potential difference that these analyses have the power to identify between pN0 and pN1mi status was 1.7% and HR 2.0.

These results differ from previous studies that have reported different survival rates between micro-metastases and pN0.[5-23,31,32] In contrast, our results are consistent with recent studies with no[20,24,26-28,54] or only little[22,29,30] significant survival impact of pN1mi. In the recent study reported by Andersson et al.,[30] including 123 patients with SN micro-metastases with distinction of tumor subtypes, a negative BCSS impact of pN1mi was shown. In agreement with Andersson et al.,[30] we share the point of view that a follow-up >7 years or 10 years is contributive since ER-positive BC recurrences can be observed many years after treatment.[55] In our study, the number of pN1mi patients still at risk after 7 years and 10 years of follow-up were 404 and 121, respectively, in comparison with 111 and 93 patients at risk at 6 years and 10 years, respectively in the Andersson et al. study.[30] We identified six main reasons that could potentially explain the discordant results with Andersson et al.: (i) more AC in our study, 51.25% (740/1444) versus 24.4% (30/123), (ii) more endocrine therapy in our study, 96.2% (1391/1446) versus 81.3% (100/123), (iii) more radiotherapy in our study, 91.9% (11 825/12 864) versus 71.5% (88/123), (iv) lower rate of mastectomies in our study, 10.0% (913/9140) versus 24.4% (30/123), (v) a multivariate model adjusted on more criteria which was possible due to a large number of patients in our study, and (vi) serial sectionings of SN were not carried out in the Andersson et al. study, and only 3.7% of the patients had micro-metastases (with a probably higher rate of large micro-metastases) versus 10.5% in our study. Interestingly, we did not observe any significant survival impact of pN1mi

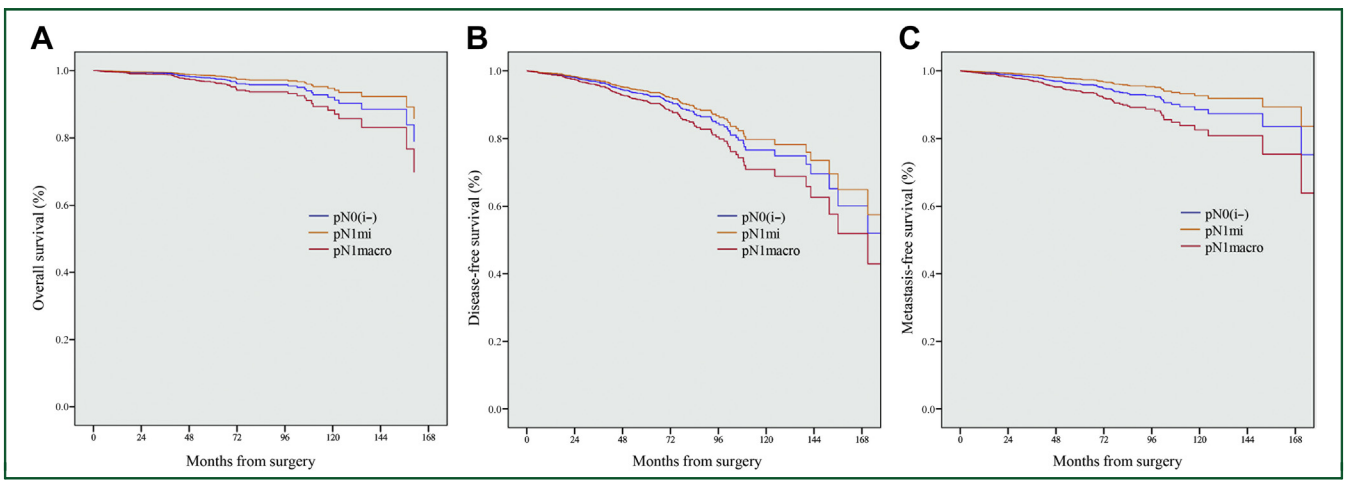

Figure 2. Kaplan Meier estimates for propensity-score-matched population with distinction between pN0(i−), pN1mi, and pN1macro.
(A) Overall survival. (B) Disease-free survival. (C) Metastasis-free survival.

in comparison with pN0 status for patients without AC. The numerically worse outcome of pN0(i+) may be in relation to the short follow-up.

Contrary to the present work, no analysis of SN micro-metastases impact according to tumor subtypes was carried out in other series. In a previous research article, we reported the negative survival impact of occult axillary LN metastases for triple-negative tumors.[56] Nevertheless, axillary micro-metastases do not impact the indication of adjuvant treatment with chemotherapy +/− trastuzumab in HER2-positive and triple-negative tumors with pathologic size >2 cm, and even >5 mm or 10 mm.[57,58] For patients with HER2-positive tumors, the benefit of AC with trastuzumab has been reported, even for patients with pT1b node-negative tumors. Consequently, SN micro-metastases have no impact on AC indication in this situation.[58] In contrast, the impact of pN1mi on adjuvant chemotherapy indication may be of particular interest for ER+ HER2- tumors with grade 1-2 and/or with high proliferation index (Ki67 > 20%). We did not capture proliferation index such as Ki67 in our study, which may be of interest for the distinction of ER-positive, HER2-negative, grade-2 tumors between luminal A-like and luminal B-like HER2-negative tumor subtypes.

Our study is also the first one generating a 1 : 1 : 1 matched pN0(i−), pN1mi, and pN1macro cohort. This statistical technique attempts to control for confounding factors by matching individual patients from a pN status group with a patient with the same clinico-pathological features in the other two groups. Thus, patients in the 1 : 1 : 1 matching cohort no longer differed for clinical and pathological characteristics (as described in Table 2 and illustrated by Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2021.100151), except for pN status, allowing an estimate of its independent impact. Finding corresponding matches in each cohort for all features included in the model is not always possible and results in the exclusion of many unmatched patients from the analysis, underlining the need to start with a large number of patients. In our case, the matching process resulted in a cohort of 1294 patients with consequent loss in statistical power compared with the analyses carried out in the entire population. Nevertheless, pN1macro, but not pN1mi, was significantly associated with worse MFS. This point is critical since most of the benefit expected from AC is the prevention of ultimately lethal metastases.

St. Gallen consensus confirmed the important role of adjuvant therapy in reducing recurrence and improving survival. Subsequently, there is a need for precise prognostic factors to further improve the specificity of treatment with regard to the magnitude of clinical benefit.[1] In the present study, patients were treated at 13 centers and adjuvant treatments may have differed. However, this multicenter cohort reflects clinical reality out of clinical trials. The decision to offer AC is most often made for patients with node-positive BC, while prognostic factors such as tumor size, tumor grade, proliferation factors (Ki-67 in particular), ER and HER2 status are used to identify a subset of node-negative patients who are at lower risk of recurrence without additional therapy.[4] For ER-positive HER2-negative tumors, detection of occult metastases could lead to a discussion about AC indication. SN isolated tumor cells (SN-ITC) are usually not considered of negative prognostic impact and treated as pN0(i−). Our result support that micro-metastases do not negatively impact patient outcomes. Therefore, the relevance of pN1mi on AC indication should be the same as pN0(i−) or pN0(i+). Interestingly, we did not observe a significant prognostic difference between pN0(i−) and pN0(i+) in contrast with Anderson et al. study.[31] This observation was also suggested in a recent review from Tsuda[12] and results of de Boer et al. study for patients with ITC and micro-metastases in the absence of AC.[9,10]

Moreover, the results of our national, multicenter study demonstrated that the presence of micro-metastases was not an independent significant prognostic factor on OS, DFS, and MFS in the entire cohort, in patients according to tumor subtypes, in luminal A-like tumors, and in a 1 : 1 : 1 matched population compared with N0(i−) on OS, DFS, and BCSS. The apparent and counterintuitive positive effect of pN1mi on MFS compared with pN0 patients, while supporting the difference in these patients compared with those with pN1macro involvement, should be interpreted with caution, and may be, as mentioned, in relation to a short follow-up.

In the predictive tool Oncotype DX, a first-generation genomic test, pN1mi patients were classified at the same level of pN0. However, second generation of gene expression signatures, such as EndoPredict or Prosigna, includes tumor size and nodal status in the final recurrence risk score for ER-positive HER2-negative tumors.[47,48] These signatures consider micro-metastases and macro-metastases as equivalent factors and may consequently overestimate the risk of recurrence for patients with micro-metastases. Considering our results, which show no significant independent impact of SN micro-metastases for ER-positive tumors and ER-positive HER2-negative tumors, including luminal A-like tumors, we strongly believe that the recurrence risk assessment for pN1mi tumors should no longer be calculated as equivalent to pN1, but as pN0.

Finally, neo-adjuvant treatment was excluded and residual LN tumor as ypN1mi for patients initially cN1 probably have a negative prognosis impact.

Conclusions

The results of our study, based on a large retrospective multicenter cohort, demonstrated that the presence of LN micro-metastases has no detectable prognostic impact on ER-positive early BC. Consequently, LN micro-metastases should not be considered a determining factor in indicating AC, and the recurrence risk assessment using second-generation signatures should be calculated considering micro-metastases as pN0. However, this study adds to the knowledge in this field but there will always some debate and further prospective studies should continue to accrue

data with longer follow-up and with analysis of cohorts within genomic testing.

FUNDING

None declared.

DISCLOSURE

The authors have declared no conflicts of interest.

DATA SHARING

The data sets generated and/or analyzed during the current study are not publicly available, as the study has used clinical databases of 13 different comprehensive cancer centers in France (ClinicalTrials.gov NCT02869607).

REFERENCES

1. Burstein HJ, Curigliano G, Loibl S, et al. Estimating the benefits of therapy for early-stage breast cancer: the St. Gallen International Consensus Guidelines for the primary therapy of early breast cancer 2019. *Ann Oncol*. 2019;30(10):1541-1557.
2. Schwartz AM, Henson DE, Chen D, Rajamarthandan S. Histologic grade remains a prognostic factor for breast cancer regardless of the number of positive lymph nodes and tumor size: a study of 161 708 cases of breast cancer from the SEER program. *Arch Pathol Lab Med*. 2014;138(8):1048-1052.
3. Mohammed RAA, Martin SG, Gill MS, Green AR, Paish EC, Ellis IO. Improved methods of detection of lymphovascular invasion demonstrate that it is the predominant method of vascular invasion in breast cancer and has important clinical consequences. *Am J Surg Pathol*. 2007;31(12):1825-1833.
4. Cianfrocca M. Prognostic and predictive factors in early-stage breast cancer. *Oncologist*. 2004;9(6):606-616.
5. Jafferbhoy S, McWilliams B. Clinical significance and management of sentinel node micrometastasis in invasive breast cancer. *Clin Breast Cancer*. 2012;12(5):308-312.
6. Salhab M, Patani N, Mokbel K. Sentinel lymph node micrometastasis in human breast cancer: an update. *Surg Oncol*. 2011;20(4):e195-e206.
7. Chen SL, Hoehne FM, Giuliano AE. The prognostic significance of micrometastases in breast cancer: a SEER population-based analysis. *Ann Surg Oncol*. 2007;14(12):3378-3384.
8. Colleoni M, Rotmensz N, Peruzzotti G, et al. Size of breast cancer metastases in axillary lymph nodes: clinical relevance of minimal lymph node involvement. *J Clin Oncol*. 2005;23(7):1379-1389.
9. de Boer M, van Dijck JA, Bult P, Borm GF, Tjan-Heijnen VC. Breast cancer prognosis and occult lymph node metastases, isolated tumor cells, and micrometastases. *J Natl Cancer Inst*. 2010;102(6):410-425.
10. de Boer M, van Deurzen CH, van Dijck JA, et al. Micrometastases or isolated tumor cells and the outcome of breast cancer. *N Engl J Med*. 2009;361(7):653-663.
11. Wada N, Imoto S. Clinical evidence of breast cancer micrometastasis in the era of sentinel node biopsy. *Int J Clin Oncol*. 2008;13(1):24-32.
12. Tsuda H. Histological examination of sentinel lymph nodes: significance of macrometastasis, micrometastasis, and isolated tumor cells. *Breast Cancer*. 2015;22(3):221-229.
13. Andersson Y, Frisell J, Sylvan M, de Boniface J, Bergkvist L. Breast cancer survival in relation to the metastatic tumor burden in axillary lymph nodes. *J Clin Oncol*. 2010;28(17):2868-2873.
14. Cox CE, Kiluk JV, Riker AI, et al. Significance of sentinel lymph node micrometastases in human breast cancer. *J Am Coll Surg*. 2008;206(2):261-268.
15. Grabau D, Jensen MB, Rank F, Blichert-Toft M. Axillary lymph node micrometastases in invasive breast cancer: national figures on incidence and overall survival. *APMIS*. 2007;115(7):828-837.
16. Kuijt GP, Voogd AC, van de Poll-Franse LV, Scheijmans LJ, van Beek MW, Roumen RM. The prognostic significance of axillary lymph-node micrometastases in breast cancer patients. *Eur J Surg Oncol*. 2005;31(5):500-505.
17. Tan LK, Giri D, Hummer AJ, et al. Occult axillary node metastases in breast cancer are prognostically significant: results in 368 node-negative patients with 20-year follow-up. *J Clin Oncol*. 2008;26(11):1803-1809.
18. Truong PT, Vinh-Hung V, Cserni G, Woodward WA, Tai P, Vlastos G. The number of positive nodes and the ratio of positive to excised nodes are significant predictors of survival in women with micrometastatic node-positive breast cancer. *Eur J Cancer*. 2008;44(12):1670-1677.
19. Iqbal J, Ginsburg O, Giannakeas V, Rochon PA, Semple JL, Narod SA. The impact of nodal micrometastasis on mortality among women with early-stage breast cancer. *Breast Cancer Res Treat*. 2017;161(1):103-115.
20. Maaskant-Braat AJ, van de Poll-Franse LV, Voogd AC, et al. Sentinel node micrometastases in breast cancer do not affect prognosis: a population-based study. *Breast Cancer Res Treat*. 2011;127(1):195e203.
21. Hansen NM, Grube B, Ye X, et al. Impact of micrometastases in the sentinel node of patients with invasive breast cancer. *J Clin Oncol*. 2009;27(28):4679e84.
22. Weaver DL, Ashikaga T, Krag DN, et al. Effect of occult metastases on survival in node-negative breast cancer. *N Engl J Med*. 2011;364(5):412-421.
23. Yang ZJ, Yu Y, Hou XW, et al. The prognostic value of node status in different breast cancer sub-types. *OncoTarget*. 2017;8(3):4563-4571.
24. Gobardhan PD, Elias SG, Madsen EV, et al. Prognostic value of lymph node micrometastases in breast cancer: a multicenter cohort study. *Ann Surg Oncol*. 2011;18(6):1657-1664.
25. Giuliano AE, Hawes D, Ballman KV, et al. Association of occult metastases in sentinel lymph nodes and bone marrow with survival among women with early-stage invasive breast cancer. *JAMA*. 2011;306(4):385-393.
26. Meattini I, Desideri I, Saieva C, et al. Impact of sentinel node tumor burden on outcome of invasive breast cancer patients. *Eur J Surg Oncol*. 2014;40(10):1195-1202.
27. Rovera F, Fachinetti A, Rausei S, et al. Prognostic role of micrometastases in sentinel lymph node in patients with invasive breast cancer. *Int J Surg*. 2013;11(1):60022-60029.
28. Maibenco DC, Dombi GW, Kau TY, Severson RK. Significance of micrometastases on the survival of women with T1 breast cancer. *Cancer*. 2006;107(6):1234-1239.
29. van Roozendaal LM, Schipper RJ, Van de Vijver KK, et al. The impact of the pathological lymph node status on adjuvant systemic treatment recommendations in clinically node negative breast cancer patients. *Breast Cancer Res Treat*. 2014;143(3):469-476.
30. Andersson Y, Bergkvist L, Frisell J, de Boniface J. Long-term breast cancer survival in relation to the metastatic tumor burden in axillary lymph nodes. *Breast Cancer Res Treat*. 2018;171(2):359-369.
31. Houvenaeghel G, Classe JM, Garbay JR, et al. Prognostic value of isolated tumor cells and micrometastases of lymph nodes in early-stage breast cancer: a French sentinel node multicenter cohort study. *Breast*. 2014;23(5):561-566.
32. Madsen EV, Elias SG, van Dalen T, et al. Predictive factors of isolated tumor cells and micrometastases in axillary lymph nodes in breast cancer. *Breast*. 2013;22(5):748-752.
33. Houvenaeghel G, Lambaudie E, Classe J-M, et al. Lymph node positivity in different early breast carcinoma phenotypes: a predictive model. *BMC Cancer*. 2019;19(1):45.
34. Yi M, Giordano SH, Meric-Bernstam F, et al. Trends in and outcomes from sentinel lymph node biopsy (SLNB) alone vs. SLNB with axillary lymph node dissection for node-positive breast cancer patients: experience from the SEER database. *Ann Surg Oncol*. 2010;17(suppl 3):343-351.
35. Bilimoria KY, Bentrem DJ, Hansen NM, et al. Comparison of sentinel lymph node biopsy alone and completion axillary lymph node dissection for node-positive breast cancer. *J Clin Oncol*. 2009;27(18):2946-2953.

36. Meretoja TJ, Leidenius MH, Heikkilä PS, et al. International multicenter tool to predict the risk of non-sentinel node metastases in breast cancer. *J Natl Cancer Inst*. 2012;104(24):1888-1896.
37. Dowlatshahi K, Fan M, Anderson JM, Bloom KJ. Occult metastases in sentinel nodes of 200 patients with operable breast cancer. *Ann Surg Oncol*. 2001;8(8):675-681.
38. Cserni G. Metastases in axillary sentinel lymph nodes in breast cancer as detected by intensive histopathological work up. *J Clin Pathol*. 1999;52(12):922-924.
39. Cheang MC, Voduc D, Bajdik C, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res*. 2008;14(5):1368-1376.
40. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406(6797):747-752.
41. Reyal F, Rouzier R, Depont-Hazelzet B, et al. The molecular subtype classification is a determinant of sentinel node positivity in early breast carcinoma. *PLoS One*. 2011;6(5):e20297.
42. Nielsen TO, Jensen M-B, Burugu S, et al. High-risk premenopausal luminal a breast cancer patients derive no benefit from adjuvant cyclophosphamide-based chemotherapy: results from the DBCG77B clinical trial. *Clin Cancer Res*. 2017;23(4):946-953.
43. Sparano JA, Gray RJ, Makower DF, et al. Prospective validation of a 21-gene expression assay in breast cancer. *N Engl J Med*. 2015;373(21):2005-2014.
44. Sparano JA, Gray RJ, Makower DF, et al. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. *N Engl J Med*. 2018;379(2):111-121.
45. Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-gene signature as an aid to treatment decisions in early-stage breast cancer. *N Engl J Med*. 2016;375:717-729.
46. Sparano JA, Gray RJ, Ravdin PM, et al. Clinical and genomic risk to guide the use of adjuvant therapy for breast cancer. *N Engl J Med*. 2019;380(25):2395-2405.
47. Gnant M, Filipits M, Dubsky P, et al. Predicting risk for late metastasis: the PAM50 risk of recurrence (ROR) score after 5 years of endocrine therapy in postmenopausal women with HR+ early breast cancer: a study on 1478 patients from the ABCSG-8 trial. *Ann Oncol*. 2013;24(suppl 3):iii29.
48. Dubsky P, Filipits M, Jakesz R, et al. EndoPredict improves the prognostic classification derived from common clinical guidelines in ER-positive, HER2-negative early breast cancer. *Ann Oncol*. 2013;24(3):640-647.
49. von Minckwitz G, Untch M, Blohmer JU, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J Clin Oncol*. 2012;30(15):1796-1804.
50. Rosenbaum PR, Rubin DB. Constructing a control group using multivariate matched sampling methods that incorporate the propensity score. *Am Stat*. 1985;39(1):33-38.
51. Little RJ, Rubin DB. Causal effects in clinical and epidemiological studies via potential outcomes: concepts and analytical approaches. *Annu Rev Public Health*. 2000;21:121-145.
52. Rosenbaum PR. Modern algorithms for matching in observational studies. *Annu Rev Stat Appl*. 2020;7(1):143-176.
53. Klein J, Moeschberger M, Hawkins K. Survival analysis: techniques for censored and truncated data. *Pharm Stat*. 2004;3:303-304.
54. Bae HW, Yoon KH, Kim JH, et al. Impact of micrometastatic axillary nodes on survival of breast cancer patients with tumors $\leq$2 cm. *World J Surg*. 2018;42(12):3969-3978.
55. Pan H, Gray R, Braybrooke J, et al. 20-year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years. *N Engl J Med*. 2017;377(19):1836-1846.
56. Houvenaeghel G, Sabatier R, Reyal F, et al. Axillary lymph node micrometastases decrease triple-negative early breast cancer survival. *Br J Cancer*. 2016;115(9):1024-1031.
57. de Nonneville A, Gonçalves A, Zemmour C, et al. Benefit of adjuvant chemotherapy with or without trastuzumab in pT1ab node-negative human epidermal growth factor receptor 2-positive breast carcinomas: results of a national multi-institutional study. *Breast Cancer Res Treat*. 2017;162(2):307-316.
58. de Nonneville A, Gonçalves A, Zemmour C, et al. Adjuvant chemotherapy in pT1ab node-negative triple-negative breast carcinomas: results of a national multi-institutional retrospective study. *Eur J Cancer*. 2017;84:34-43.