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Original Paper

Obvious Peritumoral Emboli: an Elusive Prognostic Factor Reappraised. Multivariate Analysis of 1320 Node-negative Breast Cancers

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This study was conducted to determine the prognostic influence of obvious peritumoral vascular emboli as prospectively determined by a simple routine slide examination in patients with operable node-negative breast cancer. Obvious peritumoral emboli (OPE) were defined by the presence of neoplastic emboli within unequivocal vascular lumina (including both lymphatic spaces and blood capillaries) in areas adjacent to but outside the margins of the carcinoma. OPE were assessed routinely on 5 μm thick haematoxylin and eosin-stained sections for each of 1320 primary operable node-negative breast cancers from 1975 to 1992 at our institution. OPE and other prognostic variables (tumour size, SBR grade, oestrogen and progesterone receptor status) were correlated to overall survival (OS) and metastasis-free interval (MFI) by means of univariate and multivariate analysis with a median follow-up of 103 months. OPE were found in 19.5% of tumours. In univariate analysis, OPE were related to tumour size ($P = 6.3 \times 10^{-5}$) and histologic grade ($P = 4.9 \times 10^{-7}$). Statistically significant correlations were found with OS ($P = 4.6 \times 10^{-5}$) and MFI ($P = 6.4 \times 10^{-9}$). Furthermore, in multivariate analysis, OPE was an independent prognostic variable, the most predictive factor for MFI ($P = 7.7 \times 10^{-7}$) before tumour size and grade, and was second after tumour grade for OS ($P = 0.002$). This study on a large unicentric series and with a long follow-up confirms the prognostic significance of vascular emboli in patients with operable node-negative breast carcinoma. Importantly, vascular emboli were found to be accurately detectable by a simple routine and non-time-consuming method. Therefore, such obvious vascular emboli should be considered as an important cost-effective, prognostic variable in patients with node-negative breast carcinoma. © **1998 Published by Elsevier Science Ltd.**

Key words: breast cancer, multivariate prognostic analysis, peritumoral emboli (lymphatic/blood invasion)
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INTRODUCTION

THE PROGNOSTIC significance of tumoral lymphatic or blood vessel invasion has been indicated in many studies for over 15 years [1–5]. However, this has not resulted in the integration of vascular invasion in the decision tree for breast carcinoma patients in clinical practice. The reason is that such studies on vascular invasion refer to definitions which are too different to be comparable or reproducible in routine practice. The various authors used different methods to assess vascular invasion (e.g. haematoxylin and eosin staining alone, or in association with immunohistochemical techniques), investigated different sites (intra and/or peritumoral region), and different types and numbers of invaded vessels (lymph and/or blood capillaries). These variations in the techniques explain the variable results obtained both in terms of frequency and prognostic significance of the various types of vascular invasions. Therefore, clinicians have considered this criterion as

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being non-reproducible, unreliable and unusable in routine practice. In the present study, we attempted to demonstrate that there is a simple and reproducible routine technique to detect vascular invasions, i.e. by taking into account only the obvious peritumoral embolus (OPE) irrespective of the type and number of invaded vessels. Our aims were to reduce interobserver discordance and to verify the possible prognostic value of such obvious peritumoral emboli. The analysis was done in a large series of primarily operable node-negative breast cancers with a long median follow-up.

PATIENTS AND METHODS

Between 1975 and 1992, 3975 consecutive patients with distant metastasis-free primarily operable breast cancers underwent surgery and were monitored at our institution. All were prospectively included in our clinical, histological and biological database. Among them, there were 2987 patients with invasive ductal carcinomas not otherwise specified (IDC-NOS) or infiltrating carcinoma of no special type (NST), which account for the largest category of mammary carcinoma and which are histologically universally accepted. Other histological types (invasive ductal carcinoma with proeminent intraductal component, lobular carcinomas and special histological types) were excluded (988 cases). Also excluded from analysis were 273 women with a previous (168 cases) or a concurrent contralateral (105 cases) breast cancer and 22 with too many essential missing data. The study was limited to 1320 node-negative patients among the 2692 (Table 1).

Table 1. *Characteristics of the* 1320 *patients*

Characteristics	*N* (%)
Mean age	57.13 ± 11.64
< 50 years	363 (27.5)
≥ 50 years	957 (72.5)
Tumour size* (mm)	17.9 ± 8.4
≤ 10	235 (17.8)
11–20	700 (53.0)
> 20	329 (24.9)
Not measured	56 (4.2)
SBR grade†	
1	364 (27.6)
2	549 (41.6)
3	407 (30.8)
Hormonal receptor status‡	
EP−PR−	277 (21.0)
ER−PR+	32 (2.4)
ER+PR−	233 (17.6)
ER+PR+	489 (37.0)
Not specified	289 (21.9)

*Histological tumour size. †SBR grade, Scarff, Bloom and Richardson grade. ‡ER, Oestrogen receptor status; PR, progesterone receptor status.

Tumour size was measured on fresh surgical specimens except in 56 cases in which it was not possible because the tumour was divided into fragments. Tissue samples were fixed in Bouin-Holland and were paraffin-embedded. 5 μm thick sections were stained with haematoxylin and eosin (H&E) for histological examination from each of three blocks per tumour and from one block or more per node according to our serial macroscopic sectioning routine technique [6]. The mean number of lymph nodes examined per case was 15 (range 3–36); the Scarff–Bloom and Richardson (SBR) method was used for grading. There were 935 node-negative patients with a tumour diameter of 20 mm or less, including 235 with tumours of 10 mm or less. There were 364 node-negative patients with a SBR grade I tumour. Oestrogen receptor (ER) and progesterone receptor (PR) status had only been determined since 1980 (in 1035 and 1031 cases, respectively) by the dextran-coated charcoal method using a single saturation dose assay with a cut-off level of 10 and 15 fmol/mg of protein, respectively [7]. Therefore, ER and PR status were not available in 289 cases treated before 1980.

Patients were treated with surgery by either modified radical mastectomy (637 cases) or local tumour resection (683 cases), with axillary node dissection followed by postoperative breast irradiation (695 cases). Adjuvant therapy with chemotherapy and/or hormone therapy was decided according to nodal status and hormone receptor results [7, 8] (Table 2). Treatment protocols varied over time. From 1975 to 1985, node-negative patients had no chemotherapy. After 1985, node-negative patients under 50 years of age, with ER and PR negative and SBR grade 3 tumours, had chemotherapy. All patients were followed-up every 3 months for 2 years, twice a year for the next year and then once a year. The median follow-up was 103 months (range 13.33–252.34). The total number of recurrences observed was 281 (21%); 239 patients presented distant metastases with or without locoregional recurrences and 42 patients presented locoregional recurrences without distant metastases. 66 patients developed a contralateral breast cancer (of which 15 were *in situ*). At the time of analysis, 129/1320 (10%) patients had

Table 2. *Characteristics of treatment, recurrences and deaths*

	Local tumour resection No of observations: 683	Mastectomy No of observations: 637
Treatment		
Surgery alone (no radiotherapy, no systemic adjuvant treatment)	15	480
Surgery + postoperative radiotherapy (no systemic adjuvant treatment)	607	88
Surgery + systemic adjuvant treatment (± radiotherapy)		
Chemotherapy*	56	68
Tamoxifen	4	1
Chemotherapy + tamoxifen	1	
Recurrences and deaths		
Distant metastases	74	165
Locoregional recurrences	38	4
Deaths	63	185

*Cyclophosphamide, methotrexate, 5-fluorouracil; or epirubicin, vincristine, methotrexate or mitomycin-C, thiotepa, vindesine.

died. Among the 235 node-negative patients with small tumours 10 mm or less, there were 11 deaths, 9 distant metastases and 4 locoregional recurrences. Among the 364 node-negative patients with an SBR grade I tumour, there were 28 deaths, 23 distant metastases and 12 locoregional recurrences.

Definition of obvious peritumoral emboli (OPE)

All the breast carcinomas were examined in our pathology department by one of three senior pathologists. OPE was one of the 140 pathological criteria systematically and prospectively entered into a computer database for each tumour. No special review of these pathological data was done for this study, so the analysis was made on raw initial data.

OPE were defined by the presence of neoplastic emboli within unequivocal vascular lymphatic or capillary lumina in areas adjacent to but outside the margins of the carcinoma. Moreover, we followed the recommendations of certain authors:

- Unequivocal lymphatic or capillary lumina are lined by recognisable endothelial cells. 'A coexistent blood vessel and/or multiple lymphatics' [9], 'and/or nerves' [10] are 'confirmatory evidence' (Rosen) [11].
- Evaluation was limited to tissue peripheral to the carcinoma [10–19]. 'This requires that the focus in question be outside the margin of the invasive carcinoma. This distance is usually one high-power microscopic field or more across, although on occasion very convincing evidence of lymphatic invasion is found closer to the lesion' (Rosen) [11]. Therefore, OPE were considered only if observed in vessels located in normal mammary fibrous fatty tissue surrounding the tumour margins or in the normal contiguous mammary tissue between non-neoplastic lobules and ducts.
- The type of vascular space involved is not specified. For Rosen [10–12, 18–20] there are two reasons: first 'the certainty of differentiating lymphatic vessels from small veins is poor' [21] and such 'a distinction may be of minor relevance to the overall purpose of a prognostic study' [10].

Histologically, emboli were defined by the presence of cohesive aggregates of neoplastic cells of variable sizes but smaller than the lumen. They did not usually conform exactly to the space where they were observed. The number of emboli was not recorded.

Only samples with unequivocal emboli were considered as OPE+, doubtful cases being considered as negative (OPE−). This was to reduce and even abolish interobserver discordance [9, 11, 17, 18, 22–24].

Statistical analysis

Correlations between OPE and other qualitative parameters were assessed by the chi-square-test; Student's *t*-test was used to study correlation with age. Survival curves were determined by the Kaplan–Meier method. Overall survival (OS) was calculated from date of surgery to death, or the date they were last known alive. All causes of death were considered as events. For metastasis-free interval (MFI), time-to-failure was computed from the date of surgery until metastasis or the date they were last known to be disease free. Univariate analyses for survival were performed using the log-rank test and BMDP software, program 1L. Multivariate analysis was performed stepwise using the Cox regression model [25] using BMDP. To validate Cox's model, the patient group was randomly divided into two subgroups: one, the test group, for the elaboration of the model (1000 patients) and the other for its validation (320 patients).

RESULTS

OPE were observed in 258 of 1320 patients studied (19.5%). The OPE+/OPE− ratio was found to be relatively uniform over the 17-year study period. Moreover, the OPE+/OPE− ratio per year was similar for the three senior pathologists. As shown in Table 3, OPE were more often detected in patients under 50 years of age (24%) than in patients aged 50 years or more (18%), but without statistical significance. The frequency of OPE increased with tumour size, from 10% for tumours with a diameter ≤ 10 mm to 24% in tumours with a diameter >20 mm ($P = 0.000063$). Similarly, OPE occurred in only 10% of SBR grade 1 tumours compared with 22% and 25% in SBR 2 and SBR 3, respectively ($P = 0.00000049$).

The prognostic significance of classical parameters in univariate analysis is shown in Table 4. Tumour size (≤ 20 mm), SBR grade 1 or 2 and PR (≥ 15 fmol/mg of protein) were correlated with a better outcome with respect to survival and MFI. Patients with OPE-positive tumours had a significantly worse prognosis compared with OPE-negative tumours with poorer 10-year OS (70.3% versus 80.7%)

Table 3. Correlation between obvious peritumoral emboli (OPE) and classical prognostic parameters

	OPE−		OPE+				
Classical parameters	No. of observations	(%)	No. of observations	(%)	Total	(%)	*P* value
Age							
< 50 years	276	(76)	87	(24)	363	(27.5)	0.067
≥ 50 years	786	(82)	171	(18)	957	(72.5)	
Tumour size*							
≤ 10 mm	212	(90)	23	(10)	235	(18)	
11–20 mm	552	(79)	148	(21)	700	(53)	0.000063
> 20 mm	250	(76)	79	(24)	329	(25)	
SBR grade							
1	327	(90)	37	(10)	364	(28)	
2	428	(78)	121	(22)	549	(42)	0.00000049
3	307	(75)	100	(25)	407	(31)	

*Not measurable in 56 cases.

Table 4. *Univariate analysis in* 1320 *patients: significant prognostic factors in overall survival (OS) and metastasis-free interval (MFI)*

Patient characteristics	No of patients (%)	(%) OS			(%) MFI		
		5 years	10 years	*P* value	5 years	10 years	*P* value
Tumour size							
≤ 20 mm	935 (71)	93.2	81.4		90.9	82.2	
>20	329 (25)	85.4	71.7	0.0001	76.6	66.8	6.4×10^{-9}
not measured	56 (4)						
SBR grade							
1	364 (28)	96.5	86.6		94.6	87.3	
2	549 (42)	93.3	80.5	0.000001	89.2	78.6	2×10^{-8}
3	407 (31)	84.3	69.7		78.2	70.4	
Obvious peritumoral emboli							
OPE−	1062 (80)	92.4	80.7	0.000046	90.1	81.3	6.4×10^{-9}
OPE+	258 (20)	86.8	70.3		75.9	65.9	
PR							
<15	510 (39)	87.9	73.9		84.8	76.7	
≥ 15	521 (39)	94.1	84.6	0.0012	89.9	81.8	0.0342
not done	289 (22)						
ER							
<10	312 (24)	88.3	82		86.1	82.8	
≥ 10	723 (55)	92.3	78.3	0.95	87.9	77.9	0.63
not done	285 (22)						
Age							
<50 years	363 (28)	90.8	81.5	0.1916	83.5	77.6	0.4981
≥ 50 years	957 (73)	91.5	77.3		88.7	78.5	

PR, progesterone receptor; ER, oestrogen receptor.

($P = 4.6 \times 10^{-5}$) and MFI (65.9% versus 81.3%) ($P = 6.4 \times 10^{-9}$).

In patients with SBR grade 1 tumours, the 5- and 10-year MFI were, respectively, 82.7% and 70.2% when OPE were present, versus 96% and 89.4% when OPE were not ($P = 4 \times 10^{-4}$). These 5- and 10-year MFI differences were also found in patients with tumours ≤ 10 mm according to whether OPE was present or not, but were not statistically significant (only 9 distant metastases).

In Cox multivariate analysis on the 1000-patient test group, six factors were tested (Table 5): age (<50 versus ≥ 50 years), tumour size (≤ 20 mm versus >20 mm, SBR grade (grade 2 versus grade 1 and versus grade 3), OPE (OPE− versus OPE+), PR (≥ 15 fmol/mg versus <15 fmol/mg and 'not done'), ER (≥ 10 fmol/mg versus <10 fmol/mg and 'not done'). OPE positivity was the most predictive factor before tumour size and SBR grade for MFI ($P = 7.7 \times 10^{-7}$) and ranked second after tumour size ($P = 1 \times 10^{-4}$) for OS ($P = 2 \times 10^{-3}$). Moreover, for MFI there was a difference, on the one hand, between patients with none of these three adverse factors and patients with only one adverse factor, and on the other, between patients with one adverse factor and patients with two or three adverse factors (Figure 1). This difference was also found for MFI in the validation group (Figure 2).

Table 5. *Multivariate analysis for overall survival (OS) and metastasis-free interval (MFI)*

1320 patients	RR	[Confidence interval]	*P* value
OS			
SBR grade 3	1.78	[1.33–2.39]	0.0001
OPE+	1.65	[1.19–2.28]	0.002
Tumour size >20	1.57	[1.16–2.13]	0.003
MFI			
OPE+	2.18	[1.6–2.9]	0.00000077
Tumour size >20	1.82	[1.3–2.4]	0.0001
SBR grade 3	1.79	[1.3–2.4]	0.00011

*RR, Relative risk.

DISCUSSION

Variations in the techniques previously used explain the differences in the frequency of vascular invasions reported in the literature. Therefore, pathological assessment of emboli in the literature is variable according to the type and/or topography and/or the criteria used in assessing vascular involvement. Some authors evaluated lymphatic invasion and blood invasion separately [12–14, 22, 26–30] or only lymphatic invasion [3, 4, 9–11, 15, 16, 31, 32, 33–38], whereas others studied vascular invasion encompassing both lymphatic and blood vessel invasion [12, 18, 39–43]. Lymphatic and/or blood invasion were evaluated either inside and outside the tumour [9, 15, 23, 42, 44] or exclusively outside, in the immediate periphery adjacent to the main tumour mass [10–12, 14–19, 22, 36, 38]. Some authors specified a distance at which tumour emboli are to be considered, for example, at 'more than one not otherwise specified high power microscopic field' [11, 13] or at 'not less than one microscopic field at $\times 250$ magnification' (0.7 mm) [10]. Moreover, assessment criteria for vascular involvement have been extremely variable, according to whether studies involved H&E routine staining [9, 11, 23, 44] or immunohistochemical techniques. So in view of the above, some authors' skill and experience may eventually influence the ability to detect vascular invasion, while for others immunohistochemical techniques may facilitate identification of lymphatic and blood vessel invasion by identifying additional peritumoral emboli and by eliminating pseudo-emboli resulting from a shrinkage artefact [41, 45–50]. Consequently, for almost all authors, the histological criteria to be used for recognising lymphatic and/or

blood invasion are debatable. This heterogeneity of diagnostic criteria, in addition to differences in patient population characteristics (tumour size, histological type, nodal status, size of study population, heterogeneity of patients), may explain the wide range of lymphatic and/or blood invasion found in different studies. For example, the incidence of the lymphatic invasion detected on H&E-stained slides in node-negative patients varies from 9% (15/171) [3] to 49% (157/318) [13] and peritumoral emboli encompassing both lymphatic and blood vessel invasion vary from 14.9% (33/221) [12] to 23.5% (62/263) [42].

However, when methodologies are comparable, the results may also be compared. With a comparable methodology, we found the same percentage of OPE in the node-negative group of patients as did Pinder and colleagues (18.6% of 'definite' emboli) [18] and Clemente and colleagues (19.6% in his second review) [10]. These values are higher but of the same order as those found by Lee (14.9%) in 221 patients. Lauria and colleagues [14] studied lymphatic vessel invasion (LVI) and blood vessel invasion (BVI) in the peritumoral areas of 543 node-negative patients, and found vascular invasion in 16% of cases (14.2% LVI and 1.8% BVI).

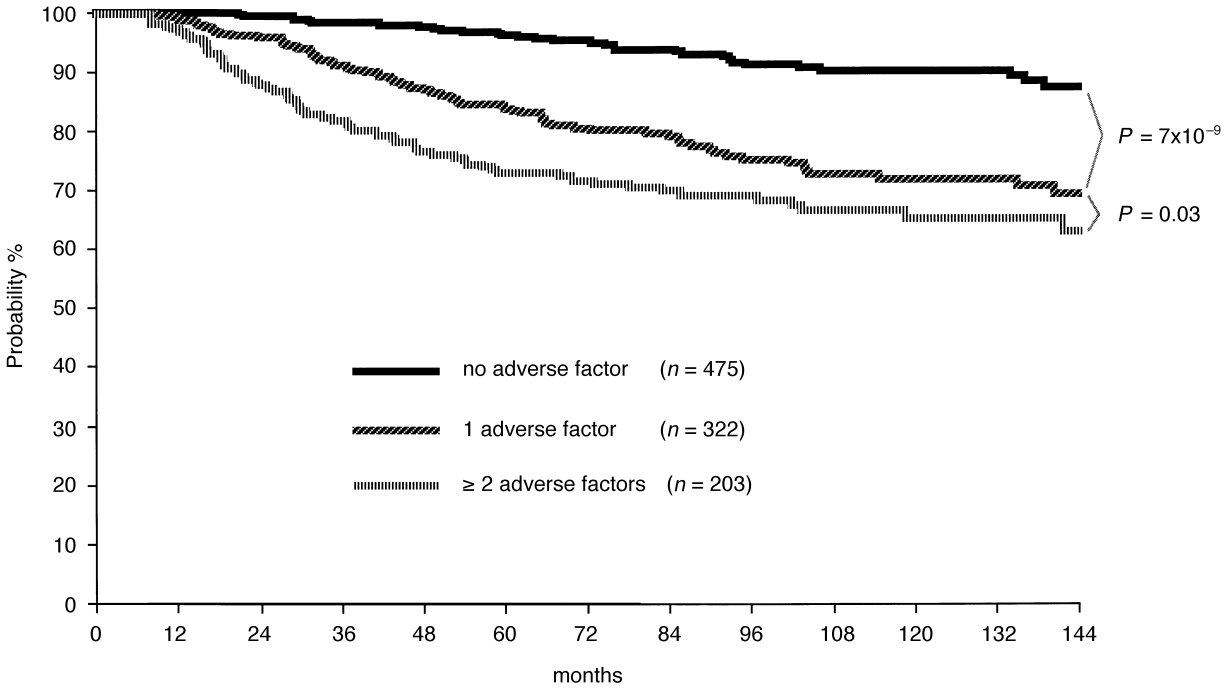

Figure 1. MFI in the 1000 patient test group with the presence of no, 1 or $\geq$ 2 adverse factors (size > 20 mm, SBR grade 3 and OPE+).

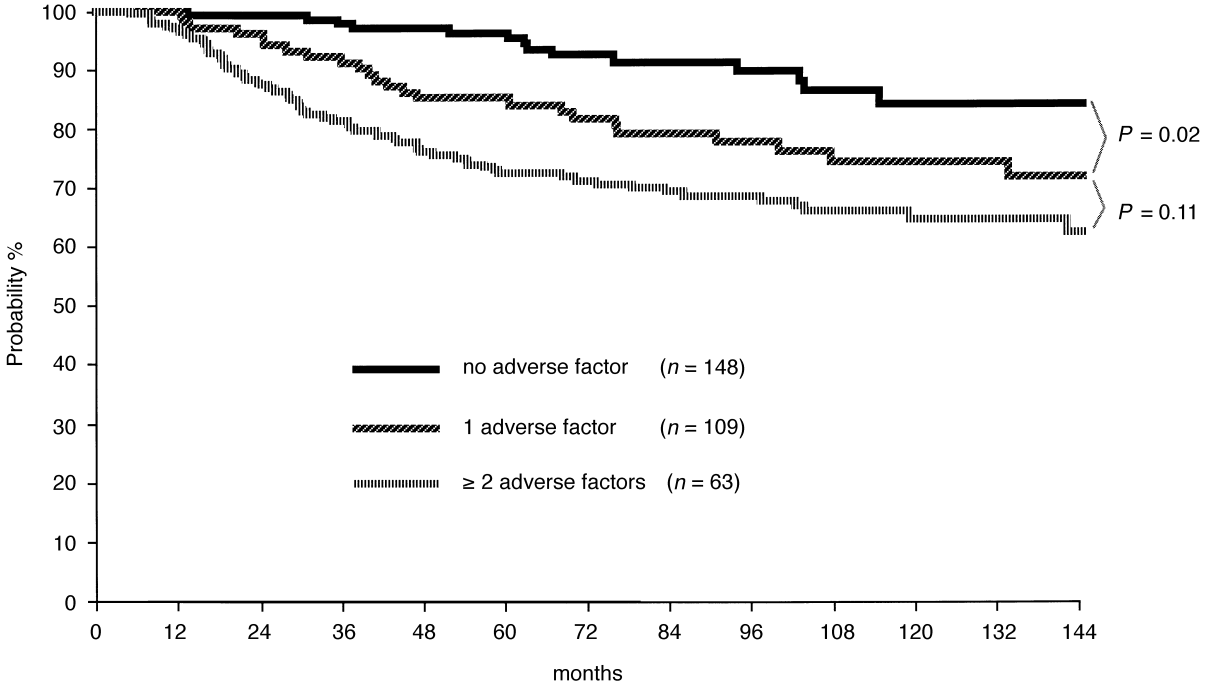

Figure 2. MFI in the 320 patient validation group with the presence of no, 1 or $\geq$ 2 adverse factors.

Therefore, strict morphologic criteria of vascular invasion minimise intra- [10, 37] and interobserver variation. Thus, the reproducibility of OPE determination 'is not a significant problem... the pathologists agreed in preparation prior to the review based on concurrent evaluation with a multihead microscope' [22], and 'when artefactual tissue spaces without an endothelial lining are ignored, agreement in over 90% of cases of vascular invasion may be expected between pathologists' [51]. Several studies have reported a high concurrence between pathologists: Orbo and associates [9] demonstrated an 82% reproducibility in the diagnosis of lymphatic invasion, and Pinder and associates [18] an 85.8% overall agreement in 400 cases examined separately by two pathologists when two classes of vascular invasion were used ('definite' or 'none' in peritumoral areas). Even the negative conclusions of Gilchrist and associates [23] after the review of 35 cases by three pathologists (five criteria for lymphatic invasion; agreement in 12 cases) do not plead in favour of interobserver variations for two reasons: on the one hand, 'the disagreement they described between the three pathologists were minor including distinguishing no lymphatic invasion from no observed lymphatic vessels' [18], and on the other hand, when considering only the presence or absence of lymphatic invasion peripheral to an invasive breast carcinoma, the authors 'reached at least a 77% (27/35) level of agreement' [11].

At our institution, for 20 years we have chosen the simplest approach by deciding to take into account only obvious and unequivocal emboli, i.e. those which were indubitable and for which neither experience nor immunohistochemical techniques were required. Our goal is not to assess all of them but to take into account only those which are obvious on H&E staining without counting them and without identifying the type of vascular space involved. Under these conditions, we agree with those [16, 18, 24, 41, 45–47, 49] who conclude that H&E staining is the most effective and reliable method for identifying vascular invasion in breast cancers without resorting to special techniques. Immunohistochemical stains may be of assistance in occasional cases, but with the reagents currently available, this methodology is subject to false-positive and false-negative results. The ease and reproducibility of our method of detecting OPE is suggested by the homogeneity of the OPE+/OPE− ratio found in our analysis, where OPE was routinely evaluated by three senior pathologists during a 17-year period and by the similarity of the OPE+/OPE− ratio per year between the three senior pathologists. This is also suggested by the similar OPE+/OPE− ratio found by the four junior pathologists between 1995 and 1996 in a comparable primary operable node-negative IDC-NOS group operated at our institution. However, this could be confirmed by a study involving less experienced pathologists [52].

Whatever method of assessing vascular invasions, most studies [14, 17, 18, 24] emphasise their adverse prognostic significance. As in our study, emboli are correlated with classical adverse parameters: large tumour size and high SBR grade, and have the same P value of ≤ 0.0001 in all reported studies. It is the same by univariate analysis; for Rosen, emboli were correlated with DFI and OS in patients with TlN0M0 [5, 29] but not in patients with T2N0M0 [29], and Clemente in his reviewed material found a disease-free interval but not an OS prognostic significance of emboli. In 275 N-M0, Sears and colleagues [27] did not find lymphatic emboli to be a prognostic indicator, but this discordant result is difficult to interpret because there were only 9 systemic recurrences. For these studies there was a wide range of group sizes (from 122 [22] to 776 patients [18]).

By multivariate analysis, most authors have found emboli to be an independent prognostic factor. Toïkkanen [15] reported that lymphatic invasion was the most important independent prognostic factor for survival before tumour size and grade (median follow-up 30 years). Lauria and colleagues [14] in a node-negative subgroup of 543 patients, found lymphatic vessel invasion (LVI) to be a very strong independent prognostic factor, with a 2-fold increased risk of death when LVI is present, and its prognostic effect being more relevant than histological grade. Clayton [37], in 378 node-negative M0 patients found that the best combination of features predicting tumour-related death was high mitotic count followed by lymphatic invasion, large tumour size and skin or subjacent muscle or chest wall invasion. For Pinder and colleagues [18] in 776 node-negative patients and Gasparini and colleagues [16] in 254 node-negative patients (195 DCI-NOS, 41 infiltrating lobular carcinoma, 18 of other types), emboli were independently significant for disease-free interval (46 relapses among which only 7 were locoregional in Gasparini and colleagues' study). With respect to disease-free interval in, respectively, 122 and 221 node-negative subsets, emboli were 'the most' [22], or 'the only' [12] statistically significant predictive factor for recurrence. Fourquet and colleagues [35], in 518 T1T2N0M0 patients treated by tumorectomy and radiotherapy, found emboli to be an independent prognostic indicator for local recurrence, with age and incomplete surgical excision. In 218 node-negative patients with tumours of 10 mm or less, Leitner and colleagues [38] found lymphatic invasion to be correlated with MFI.

Immunohistochemical prognostic factors, described for primary operable node-negative cancers, rank in the Cox model after the classical morphological factors (grade and size) [53, 54], are more costly, and require more time in routine practice. Markers of cell proliferation [55–59] are interesting. Sigurdsson and colleagues [55], in 367 node-negative patients (median follow-up 4 years), found that S-phase fraction was the best prognostic factor. Van Diest and Baak [56] found that a morphometric prognostic index (a multivariate combination of the mitotic count, lymph node status and tumour size) was the best combined prognostic factor in 121 node-negative patients.

Our study indicates two important points: on the one hand, it emphasises the strong prognostic significance of emboli and on the other it widens the group of high-risk lymph node-negative patients, since the presence of just one of the three adverse factors (OPE+, tumour size > 20 mm, SBR grade 3) has a strong prognostic effect. It is likely that OPE, like grade and size, are the rough morphological reflection of complex cellular mechanisms indicated by the new markers.

In conclusion, including only OPE (i.e. those which are indubitable and for which neither experience nor immunohistochemical techniques are required), explains the homogeneity of the OPE+/OPE− ratio over the 17-year study period, and the concordance in the assessment between the three senior pathologists and between the four junior pathologists. Therefore, such OPE can be accurately detected by a simple routine and non-time-consuming method. The strong prognostic significance of such emboli in a large unicentric

series with a large follow-up makes them an important prognostic variable to be considered in therapeutic decision-making in node-negative patients.

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