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Peritumoural vascular invasion: A major determinant of triple-negative breast cancer outcome

Renaud Sabatier [a,b,*], *Jocelyne Jacquemier* [a,c], *François Bertucci* [a,b,d], *Benjamin Esterni* [e], *Pascal Finetti* [a], *Francine Azario* [c], *Daniel Birnbaum* [a], *Patrice Viens* [b,d], *Anthony Gonçalves* [b,d,f], *Jean-Marc Extra* [b]

[a] *Molecular Oncology Department, Centre de Recherche en Cancérologie de Marseille, UMR891 Inserm, Institut Paoli-Calmettes, Marseille, France*

[b] *Medical Oncology Department, Institut Paoli-Calmettes, Marseille, France*

[c] *Department of Biopathology, Institut Paoli-Calmettes, Marseille, France*

[d] *Université de la Méditerranée, Marseille, France*

[e] *Biostatistics Department, Institut Paoli-Calmettes, Marseille, France*

[f] *Department of Cell Polarity, Cell Signaling and Cancer, Centre de Recherche en Cancérologie de Marseille, UMR891 Inserm, Institut Paoli-Calmettes, Marseille, France*

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ABSTRACT

Purpose: Triple-negative breast cancers (TNBC) have the worst outcome of all breast cancer subtypes. Nevertheless TNBC are heterogeneous in terms of pathological, biological and prognostic behaviours. We explored clinical and pathological factors correlated with outcome in this phenotype.

Methods: We retrospectively studied clinical and pathological factors correlated with prognosis in a series of 344 early TNBC. Staining for blood (CD31) and lymphatic (Podoplanin) vascular endothelium markers was performed to best characterise peritumoural vascular invasion (PVI) in 108 cases available for pathological reviewing.

Results: Univariate and multivariate analyses performed on our whole cohort underlined PVI as an independent predictive factor of distant metastasis ($p = 0.00012$, HR = 2.72 [1.63–4.52]). Standardised pathological reviewing of 101 histologically confirmed TNBC showed that PVI, observed in 41% (28% by haematoxylin and eosin staining plus 13% by immunohistochemistry), was confirmed as the first prognostic factor in TNBC, particularly in node-negative tumours. Five-year metastasis-free survival in this subset was 87.5% and 50.8% without and with PVI, respectively ($p = 0.003$).

Conclusions: Vascular invasion diagnosis is improved by the combination of HES and IHC. Moreover it is a major prognostic feature and must take a greater part in therapeutic management of early TNBC with the possibility to adapt the adjuvant treatment according to the predicted relapse risk.

* *Corresponding author:* Address: Département d'Oncologie Médicale, Institut Paoli Calmettes, UMR891 Inserm, 232 Bd. Sainte-Marguerite, 13273 Marseille Cedex 09, France. Tel.: +33 4 91 22 35 37; fax: +33 4 91 22 36 70.
E-mail address: sabatierr@marseille.fnclcc.fr (R. Sabatier).

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1. Introduction

Breast cancer is the first female cancer worldwide and has an incidence close to 50,000 cases per year in France.[1] Standard therapy of early stages consists in surgery, adjuvant radiation therapy and if needed, depending on the prediction of metastatic risk, in systemic adjuvant treatment including chemotherapy, hormone therapy and human epidermal growth factor receptor 2 (ERBB2) inhibitors. These last two treatments provide a major advantage for patients with hormone receptors and/or ERBB2-expressing tumour.

Triple-negative breast cancers (TNBC), i.e. cancers with no expression of oestrogen receptor (ER), progesterone receptor (PR), and ERBB2 protein, represent 15–20% of all cases.[2–5] Women with TNBC cannot receive currently available tumour cells targeted therapies, hormone therapy or ERBB2 inhibitors. These tumours display some other unfavourable features when compared with hormone receptors or ERBB2-positive tumours. They are described to occur in younger patients, to have a higher frequency of histological high grade, high proliferation, central necrosis or acellular zones, lymphocytic infiltrate, BRCA1 pathway alterations and *TP53* mutations than hormone receptors or ERBB2 positive tumours.[6–8] Despite the fact that most of them (nearly 85%) receive chemotherapy and that they are highly chemosensitive[4], they present more numerous and earlier relapses.[9] Furthermore, metastasis sites are also different with more visceral metastasis (particularly lung and brain lesions)[3,10] that lead to the worst prognosis of all breast cancers (with equivalent age, tumour size, lymph node involvement) with a 5-year metastasis-free survival of 60%[11] and a 5-year overall survival of ~70%.[12,13] Thus a large part of TNBC seems to have a better outcome. But this prognostic heterogeneity is poorly understood with currently available tools, and there are few relevant prognostic factors identified within TNBC.

We present a retrospective study of nearly 350 patients treated in our institution for TNBC. Our aims were to identify clinical or histological factors influencing prognosis (metastasis-free survival) in this population in order to improve specific therapeutic management of TNBC patients that will lead to a more tailored medicine.

2. Patients and methods

2.1. Patients selection

We collected data from women treated in a tertiary referral centre (Institut Paoli-Calmettes) between 1995 and 2008. Our inclusion criteria were patients with first treatment for invasive early breast cancer, without metastasis at diagnosis, with no expression of oestrogen receptor, progesterone receptor or ERBB2 identified by immunohistochemistry. Exclusion criteria were T4 (TNM stage), bilateral disease, and any personal history of cancer (including *in situ* breast cancer). Follow-up was stopped on March 2009.

2.2. TNBC definition

Data were retrospectively extracted from individual clinical files. Breast cancers were initially considered as TN if the first pathological examination showed less than 10% of cancer cells expressing ER and PR and if the ERBB2 expression score was 0 or 1. This was done because not all the patients treated at our institution were diagnosed by us and histological samples could not be available for all patients. Antibodies used during this period were heterogeneous. ER expression was assessed with three different clones: 1D5, Dako (before 1998), 6F11, Abcam (from 1998 to 2007) or SP1 clone, Dako (from 2007 until December 2008). PR expression was evaluated with 1A6 clone, Abcam (before 1998), and PgR636 clone, Dako (1998–2008). ERBB2 expression was evaluated with different clones: TAB250 before 2000, NeuAB3 (France Biochem), DA485 (Dako) or DA485 and CB11 (Ventana) combination from 2000 to 2003, and Dako Herceptest™ from 2003 to 2008.

The second step of our study was focused on histologically confirmed TNBC. All available formalin-fixed paraffin-embedded (FFPE) samples were reviewed by a single pathologist (JJ) with homogeneous antibodies currently used in routine practice and specific to ER (Dako, SP1 clone), PR (Dako, PgR636 clone), and ERBB2 protein (Dako, Herceptest™). As defined by the 9th St Gallen consensus Conference, the cut-off of 1% of marked cells was used in this second part. This subset of patients was named 'histologically confirmed' TNBC subset.

2.3. Peritumoural vascular invasion

First vascular invasion evaluation was done by the pathologist who examined the tumour soon after surgery by haematoxylin and eosin staining (HES). We considered only peritumoural vascular invasion, and intratumoural emboli were not taken into account.

For the 'histologically confirmed' TNBC cohort, FFPE samples were examined by a single pathologist (JJ) to confirm vascular invasion by HES according to the European guidelines.[14] In order to determine if lymphatic or blood vessels were involved, samples were also stained with Podoplanin antibody (Dako, D2-40 clone, 1:20 dilution), widely used as a specific marker for lymphatic endothelial cells and lymphangiogenesis[15], and with CD31 (or PECAM1) antibody (Dako, JC70A clone, 1:50 dilution), expressed on blood endothelial cells but not on lymphatic endothelium. Samples were diagnosed as positive as soon as endothelial staining was observed, whatever the percentage of marked cells was.

2.4. Statistical analysis

Clinical and histological parameters included in this study were patients' age, pathological tumour size, pathological axillary lymph node involvement, histological grade according to the Scarff Bloom and Richardson (SBR) grading system, lymphocytic infiltrate and peritumoural vascular invasion.

Follow-up was measured from the date of diagnosis to the date of last news for living patients. Metastasis-free survival (MFS) was defined as the time from diagnosis to first distant metastasis or death.

Correlations between sample groups and histoclinical variables were calculated with the Fisher's exact test or $\chi 2$ test when appropriate. Comparisons between different populations were done using the Wald test or Kaplan–Meier method. Statistical significance of observed differences was evaluated

using the log-rank test. Correlations between usual histo-clinical parameters and outcome were performed using Cox regression analyses. Only factors associated with survival with a p-value < 0.15 in univariate analyses were included in multivariate Cox regression analyses. All tests were two-sided at the 5% significance level. Analyses were done using SPSS software (version 16.0).

This prognostic study was performed according to the REMARK criteria.[16]

3. Results

3.1. Population description

Of the 3408 patients treated for non metastatic invasive breast cancer with hormone receptors and ERBB2 status available, 411 (12.1%) were diagnosed with TNBC. Patients with exclusion criteria or without treatment data available were excluded. Thus, a total of 344 patients were included in our first analysis (Fig. 1). From this series, only 108 cases underwent surgery in our institution with paraffin blocks available for histological reviewing.

All histoclinical and treatment characteristics are resumed in Table 1 and were close to previously published data. Regarding adjuvant chemotherapy (delivered to 289 patients), it consisted in 6 to 8 cycles of anthracycline-based regimen (213 cases, 61.9%), anthracycline and taxane-based regimen (58 cases, 16.9%), and anthracycline and taxane-free regimen (18 cases, 5.6%). The 5-year OS and MFS for the entire population were 78% (Fig. 2A) and 75%, (Fig. 2B) respectively. It is of note that very few events (4 out of 67 in the whole cohort, and none of the 26 events in the pathologically reviewed subset) occurred after 5 years of follow-up, close to the median follow-up in our set.

3.2. Prognostic impact of the usual histo-clinical parameters in the whole cohort

We looked for clinical and histological data associated with MFS. First, univariate analysis was done on the entire cohort (344 patients) with usual histoclinical parameters. MFS was influenced by age as a continuous variable (younger the patients were, earlier they relapsed ($p = 0.019$)), tumour size ($p <$ E-04, HR = 3.3, 95%CI [1.9–5.9], Fig. 3A), lymph node involvement ($p =$ 2E-04, HR = 2.5, 95%CI [1.5–4.1], Fig. 3B) and presence of PVI ($p <$ E-04, HR = 3.2, 95%CI [2–5.3], Fig. 3C). The 5-year MFS was 84% for PVI-negative samples versus 54% for positive cases.

In multivariate analysis (data not shown), three parameters were still significant: age ($p =$ 3.4 E-03), tumour size ($p =$ 1.8 E-04) and presence of PVI ($p =$ 1.0 E-04).

3.3. Hormone receptors and ERBB2 expression reviewing

One hundred and eight FFPE samples were available. Out of these samples, one hundred and one were confirmed to be TNBC with a standardised immunohistochemistry reviewing. The characteristics of this 'histologically confirmed' TNBC cohort were close to those of the initial 344 TNBC population (Table 1).

All prognostic analyses described above were applied to this 'histologically confirmed' TNBC subset. Pathological tumour size and PVI were significant in univariate analysis, but PVI was the only significant prognostic marker in multivariate analysis (Table 2).

3.4. Exploration of peritumoural vascular invasion

As PVI was the major prognostic factor in our population, we decided to best characterise it. The point we focused on was to identify whether lymphatic or blood vessels were involved in this invasion. We stained the 101 available FFPE samples confirmed as TNBC with two antibodies: CD31, specific of blood vessels, and D2-40 (or Podoplanin), specific of lymphatic vessels.

Out of the 28 samples with vascular invasion diagnosed by HES (Fig. 4A), some were positive for D2-40 (10 out of 25 with both assessment, e.g. 40%; Fig. 4B and C), or for CD31 staining (41%, Fig. 4D). Among them, two expressed both proteins.

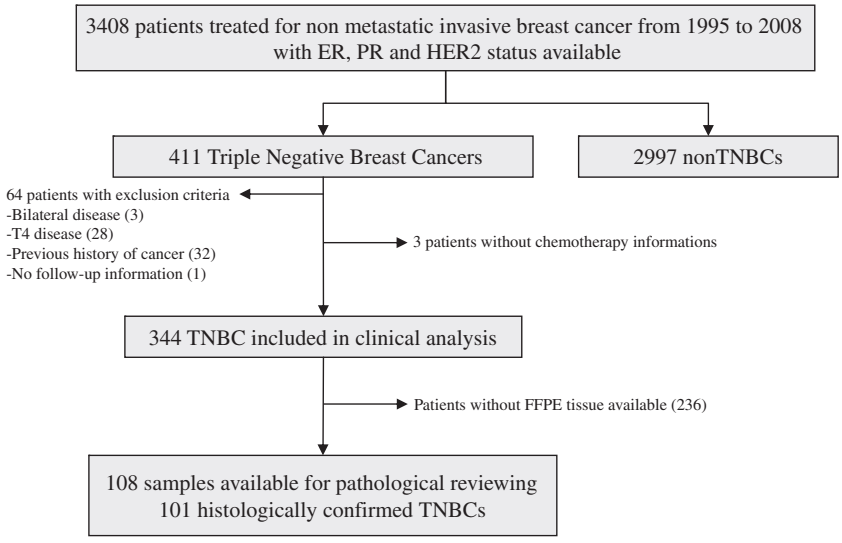

Fig. 1 – Patients selection flowchart.

Table 1 – Histo-clinical features.

		Whole population (n = 344)	Histologically confirmed TNBC subset (n = 101)
Age	Median	53 [27–86]	52 [29–84]
pT	⩽20 mm	157 (49%)	33 (32%)
	>20 mm	164 (51%)	68 (68%)
	NI	23	0
Histological subtype	Ductal	256 (74%)	75 (74%)
	Lobular	23 (7%)	6 (6%)
	Medullary	28 (8%)	12 (12%)
	Metaplastic	13 (4%)	4 (4%)
	Other	24 (7%)	4 (4%)
SBR grade	1	27 (8%)	3 (3%)
	2	86 (25%)	19 (19%)
	3	225 (67%)	78 (78%)
	NI	6	1
pN	Negative	214 (63%)	65 (65%)
	Positive	126 (37%)	35 (35%)
	NI	4	1
Peritumoural vascular invasion	Negative	235 (71%)	73 (72%)
	Positive	94 (29%)	28 (28%)
	NI	15	0
Lymphocytic infiltrate	Absent to low	NI	75 (74.2%)
	Moderate to high	NI	26 (25.8%)
Surgery	Mastectomy	89 (26%)	28 (28%)
	BCT	253 (74%)	73 (72%)
	NI	2	0
Radiation therapy	No	20 (6%)	5 (5%)
	yes	320 (94%)	95 (95%)
	NI	4	1
Chemotherapy	No	54 (16%)	14 (14%)
	yes	289 (84%)	86 (85%)
	NI	1	1 (1%)
Follow-up (years)	Median	4.03	5.5
Events (metastasis or death)		67 (19.5%)	26 (26%)
5-year MFS		75.20%	70.9%
5-year OS		78%	72.8%

BC, triple negative breast cancer; pT, pathological tumour size; NI, not available; SBR, Scarff-Bloom and Richardson; pN, pathological lymph node involvement; BCT, breast conserving therapy; MFS, metastasis-free survival; OS, overall survival.

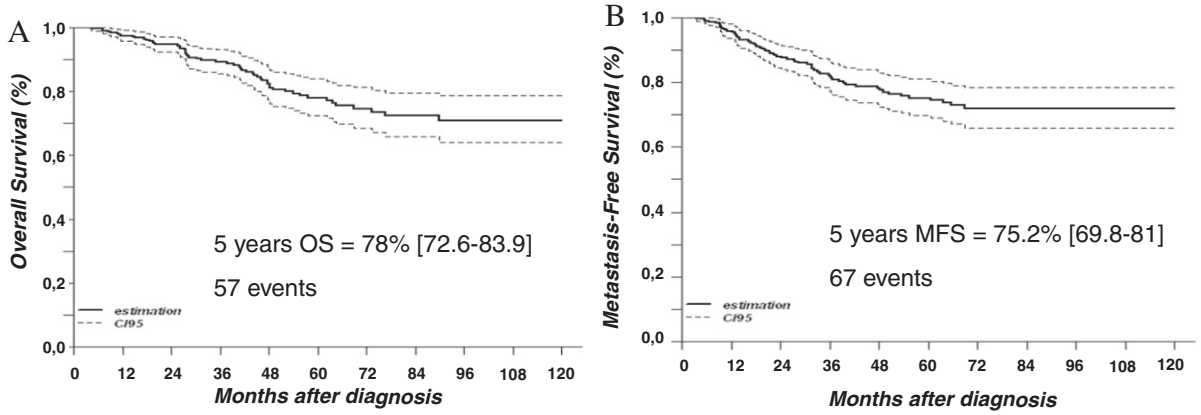

Fig. 2 – Survival curves of our whole TNBC cohort (n = 344). OS: overall survival; MFS: metastasis-free survival.

Interestingly, this method allowed us to discover small size emboli that were not described by HES. Finally, 41 samples (41%), instead of 28 (28%), presented RPVI – revised vascular invasion – i.e. PVI diagnosed by HES combined with CD31 and/or D2-40 staining. The correlation of RPVI with MFS was analysed: there was a 3-fold metastatic risk increase for

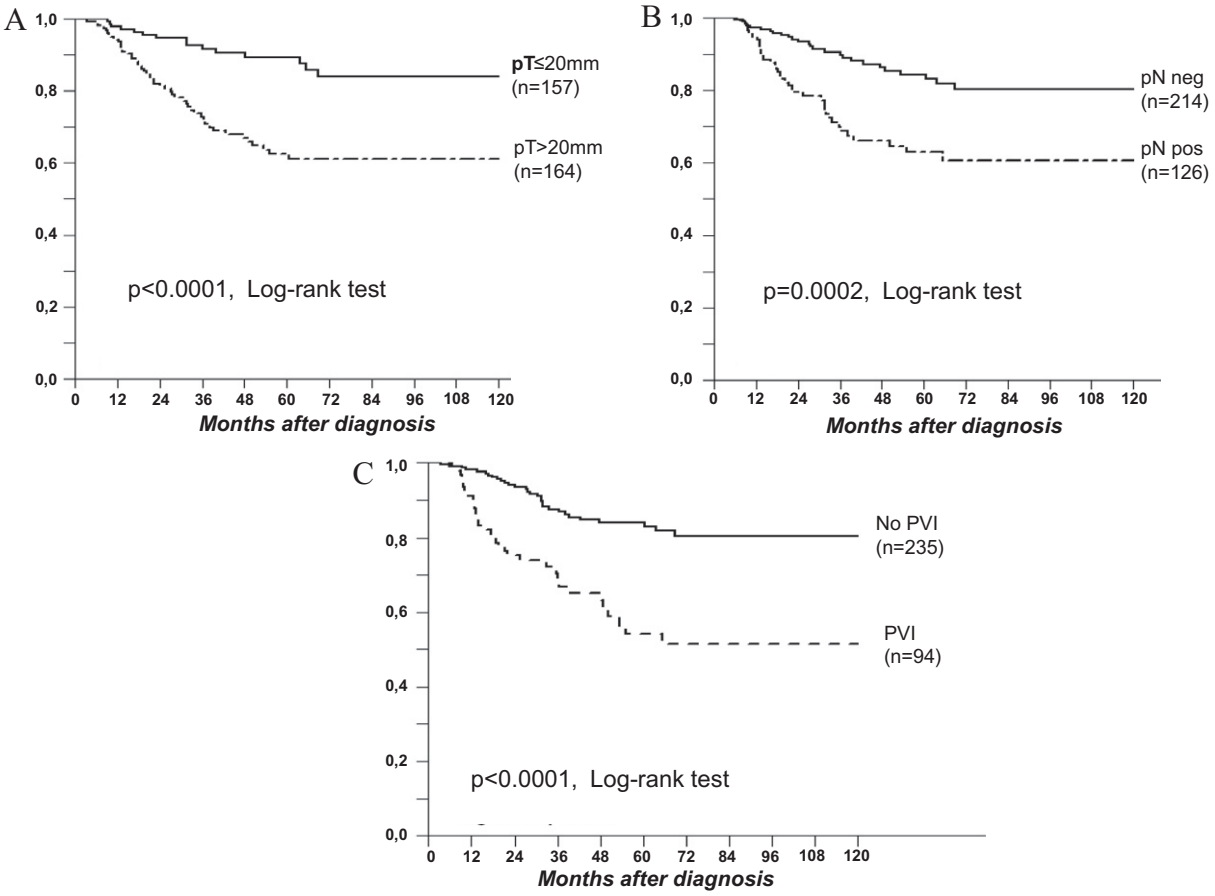

Fig. 3 – Metastasis-free survival curves according to the main parameters influencing metastasis-free survival in the whole TNBC cohort (*n* = 344). (A) pathological tumour size; (B) lymph node involvement; (C) peritumoural vascular invasion. pT: pathological tumour size; pN: pathological lymph node involvement; PVI: peritumoural vascular invasion.

Table 2 – MFS, Cox regression analysis, histologically confirmed TNBC subset (*n* = 101).

	Univariate analysis			Multivariate analysis		
	N	HR [95%CI]	*p*-Value	*N*	HR [95%CI]	*p*-Value
Age	101	1.01 [0.98–1.04]	0.45			
pT (⩽20 mm versus >20 mm)	101	2.9 [1–8.42]	0.05	101	2.39 [0.81–7.06]	0.12
SBR grade (1 versus 2–3)	100	0.047 [0–819]	0.54			
pN (pos versus neg)	101	1.71 [0.79–3.69]	0.17			
Lymphocytic infiltrate	101	0.52 [0.19–1.52]	0.23			
Vascular invasion (pos versus neg)	101	2.79 [1.30–6.03]	0.009	101	2.41 [1.10–5.26]	0.028

MFS, metastasis-free survival; TNBC, triple negative breast cancer; pT, pathological tumour size; SBR, Scarff–Bloom–Richardson; pN, pathological lymph node involvement; *N*, number of cases of available data; HR, hazard ratio; CI, confidence interval

RPVI-positive samples ($p = 0.007$, HR = 3.05, 95% CI [1.36–6.84], Wald test); RPVI was the only factor significantly associated with MFS in multivariate analysis (data not shown).

3.5. *Correlation between PVI and lymph node involvement*

As vascular invasion has been described as differentially associated with prognosis in node-positive and in node-negative tumours, we analysed the correlation between lymph node (LN) involvement and PVI in our pathologically reviewed subset. Vascular invasion (defined by HES) was highly correlated with LN involvement (74% accuracy): 86% of node-negative samples were PVI-free; and 53% of node-positive samples presented PVI ($p = 3$ E-05, Fisher's exact test). RPVI, defined by HES and Podoplanin-CD31 staining, still correlated with LN involvement (65% accuracy, $p = 7$ E-03, Fisher's exact test), but with a weaker ratio. This suggested that some node-negative tumours had small size peritumoural vascular emboli not observed by HES. Indeed, out of the 13 tumours positive for Podoplanin and/or CD31 and without PVI observed by HES, 11 (85%) did not present lymph node involvement.

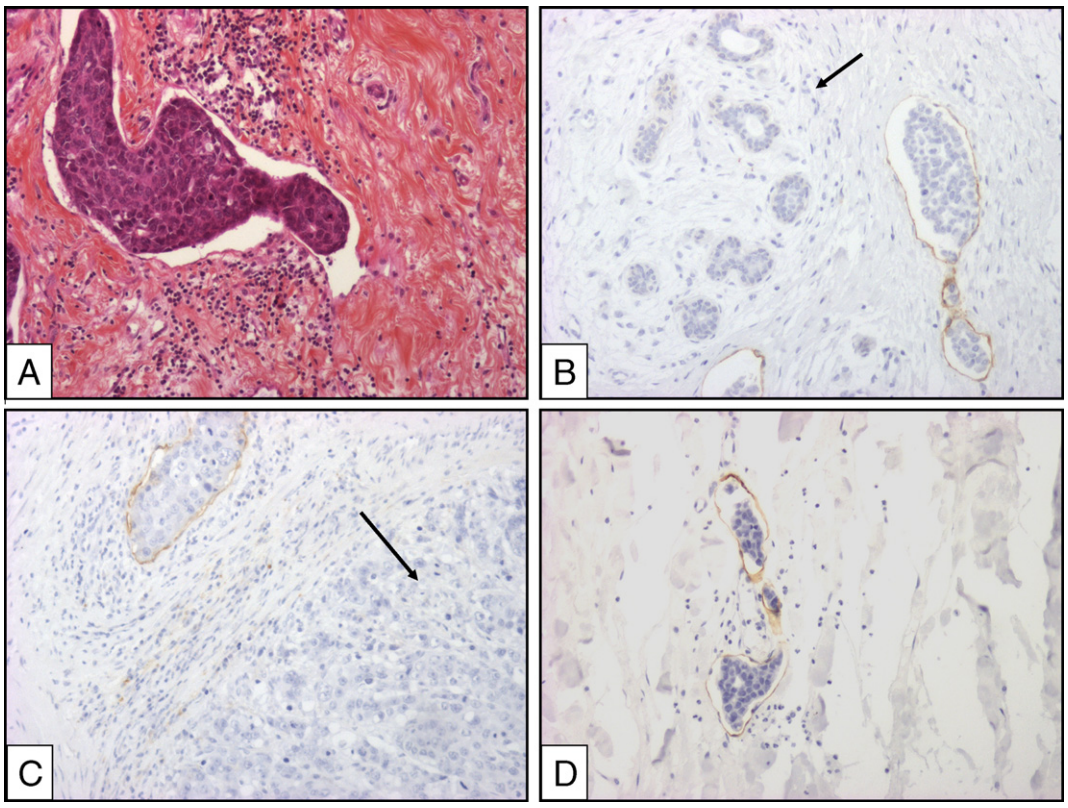

Fig. 4 – Different observations of vascular invasion in triple negative breast cancer. (A) Haematoxylin and eosin staining; (B) Peritumoural blood vessel invasion diagnosed by CD31 staining. Note the normal breast tissue (arrow); (C) Lymphatic invasion diagnosed in the tumour periphery (arrow) by D2-40 staining; (D) Lymphatic vessel invasion diagnosed far from the tumour border by D2-40 staining.

3.6. *Does IHC improve prognostic value of peritumoural vascular invasion?*

In the 'histologically confirmed' TNBC subset, MFS was correlated with both PVI – defined with HES – (HR = 2.60, 95%CI [1.19–5.70]) and RPVI – defined with combination of HES and IHC – (HR = 3.05, 95%CI [1.36–6.84], Wald test).

In node-negative tumours, MFS was also correlated with these two parameters: the hazard ratios were 3.71 (95%CI [1.24–11.09]) and 4.61 (95%CI [1.54–13.76]) for PVI and RPVI, respectively. Multivariate analysis including both PVI and RPVI showed that this later was more strongly associated with MFS in this subset ($p = 0.58$ for PVI versus $p = 0.046$ for RPVI). According to RPVI status, 5-year MFS were 88% and

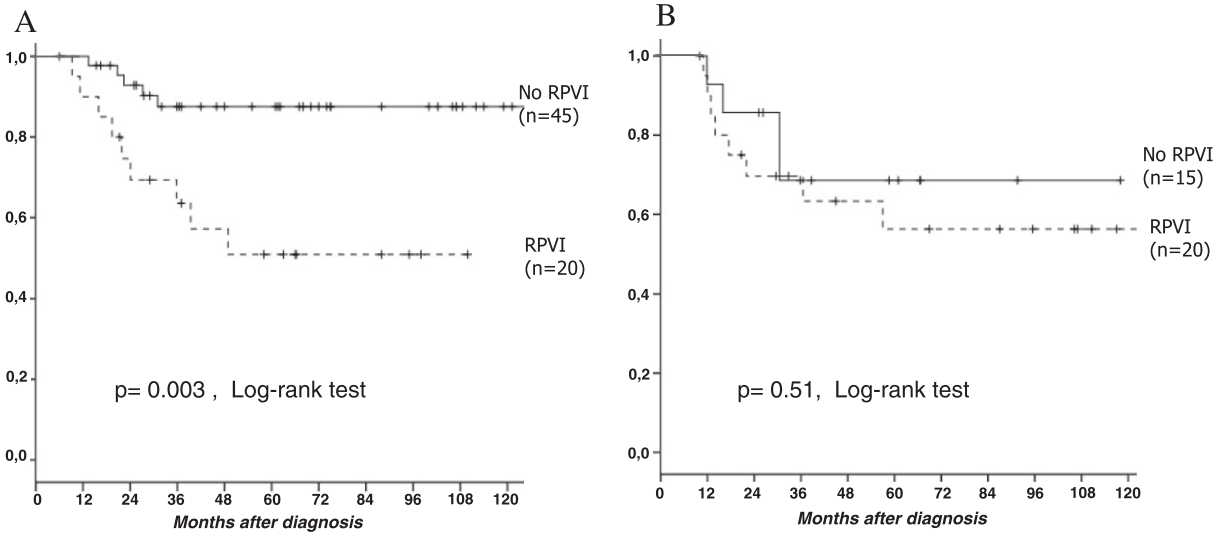

Fig. 5 – Metastasis-free survival curves according to vascular invasion status after HES an IHC reviewing (revised peritumoural vascular invasion), ($n = 101$). (A) pathological lymph node-negative tumours; (B) pathological lymph node-positive tumours.

51% without and with vascular invasion, respectively ($p = 0.003$) (Fig. 5). As well as PVI, RPVI could not predict outcome in node-positive tumours: 5-year MFS was 71% for negative samples *versus* 56% for positive ones ($p = 0.41$).

4. Discussion

This study is to our knowledge the first to report that PVI is an independent predictor of metastasis within TNBCs. PVI could be observed by HES and Podoplanin-CD31 staining in 41% of subset of 101 histologically confirmed TNBCs. This is more than the usually described PVI incidence in breast cancers, which is close to 20–25% after HES examination.[17–19] Among all usual histo-clinical parameters, using a Cox regression analysis, PVI was an independent prognostic factor for MFS. Its impact was predominant for node-negative tumours with a 5-year MFS difference of 37% in this population. These results are not only consistent with recently published data suggesting that triple-negative phenotype may be correlated with PVI[19], but they also show that PVI is a major factor involved in TNBC outcome decrease, notably in node-negative tumours.

4.1. *The difficulties to predict TNBC prognosis*

Fifteen to twenty percent of breast cancers do not express ER, PR, and ERBB2 protein and are so-called triple-negative breast cancers. They display the worst prognosis of all breast tumours.[9,20] Nevertheless, even in this aggressive phenotype, outcome is heterogeneous because of pathological and biological heterogeneities poorly understood with currently used prognostic markers.[5]

The only markers currently admitted are molecular and pathological. Pathological heterogeneity may affect prognosis. The majority of TNBC are high grade ductal carcinomas displaying poor prognosis; but TNBC also contain medullary[21], and adenoid cystic carcinomas[22], known to have a good prognosis despite their TN phenotype. Recent molecular studies based on gene expression profiling have shown that TNBC outcome may be decreased in samples (~85% of TNBCs) expressing basal markers such as CK5/6, c-kit and EGFR.[13,23–25] Immune response genes under-expression may also be correlated with poor prognosis in ER-negative tumours, as well as androgen receptor and steroid responsive genes.[26–28] All these recent data derive from molecular analyses and are still limited to the research area without direct clinical application. Therefore only few studies have explored TNBC outcome with a single histoclinical approach using currently available modern tools. The present work has been performed in order to explore, in a large cohort of TNBCs, correlation between histoclinical parameters and patients outcome.

4.2. *Vascular invasion is an independent prognostic factor in TNBC*

Vascular invasion has been described to be a predictor of breast cancer outcome for decades. Bettelheim et al[29] discovered that it distinguished two groups of non-metastatic (stage I to III) breast cancer with a significant difference in DFS even when adjusted for tumour size and axillary status. Neville et al[30] showed in the Ludwig trial V that the presence of vascular emboli was a significant prognostic factor but could not predict response to chemotherapy in 1275 node-negative breast cancer patients. Lee et al[31] assessed lymphovascular invasion in 2760 node-negative breast cancers. They showed that vascular invasion was observed in 19% of tumours and was predictive of overall survival on multivariate analysis. Mohammed et al[32] recently reported in a series of 1005 lymph node-negative breast cancers that 22% presented VI, with 97% and 3% of lymphovascular and blood vascular invasion, respectively. VI was independently associated with both decreased disease-free interval and overall survival. All these studies introduced the influence of PVI in breast cancer of all phenotypes. However, to our knowledge, no study focused on vascular peritumoural invasion and TNBCs outcome has been published to date. We report here that four histo-clinical factors can predict TNBCs MFS in univariate analysis in our entire cohort (344 patients): age, pathological tumour size, lymph node involvement and PVI. PVI is an independent prognostic factor in multivariate analysis with a nearly 3-fold metastasis risk enhancement.

4.3. *IHC improves PVI diagnosis and prognostic value, notably in node-negative tumours*

It is of note that most of the published studies have focused on vascular invasion prognostic value in node-negative breast cancers.[20,33] Indeed PVI may be a putative early predictor of lymph node involvement or distant metastasis. Recent observations[34] showed, in a cohort of node-negative breast cancers including 20% of hormone receptors-negative tumours, that Podoplanin could identify some HES-negative vascular emboli and improved vascular invasion prognostic significance. We also show in this study, with Podoplanin and CD31 stainings, that PVI do not predominantly occur in a specific vessel type. Podoplanin and CD31 stainings improve vascular invasion diagnosis with a 13% enhancement (41% versus 28%), and improve prognostic capacity of vascular invasion in node-negative tumours. Both HES and HES-IHC combined methods show significant prognostic values for PVI (HR = 2.60 and 3.05, respectively). Moreover, in multivariate analysis including both parameters, the combination of HES and IHC, and not HES alone, remains significant. The combined method thus improves prognostic value of vascular invasion.

PVI is correlated with lymph node status in our histologically confirmed TNBC subset ($p = 3$ E-05, Fisher exact test). Nevertheless, lymph node involvement was an independent prognostic factor neither in our entire cohort, nor in our pathologically reviewed subset. Thus PVI assessment may give us new information on disease evolution. In node-negative tumours, RPVI-positive samples had a worst outcome compared to RPVI-negative ones (5-year MFS: 51% versus 88%). Prognosis of lymph node-negative/RPVI-positive cancers (51% 5-year MFS) was even poorer than those of lymph node-positive tumours (63% 5-year MFS). Vascular emboli thus do not seem to be a predictor or a reflection of lymph node involvement but had a real independent prognosis impact. It is consistent with the fact that TNBC need an angiogenesis enhancement to support their rapid growth and early metastatic behaviour and have been found to express high levels of VEGF.[35]

An important bias of such a study may be the lack of central reviewing, which could be associated with mistakes due to antibodies, platform and inter-observer variability. We tried to avoid this issue in the 'histologically confirmed TNBC subset' by realising our stainings using the same platform and the same antibodies. Samples were also examined by the same pathologist to avoid inter-observer variability. However, the absence of double pathological reviewing may have led to uncorrected mistakes, even if a standardised method was used. It is also of note that there is caution for the whole series because our results have been validated only in the 101 samples from the reviewed subset. However, prognostic results in the whole cohort were similar to those of this 'histologically confirmed' TNBC subset.

In conclusion, this is the first study which reports vascular invasion as an histo-clinical parameter independently correlated with outcome inside this phenotype. Peritumoural vascular invasion (PVI) is not included in current prognostic tools daily used by clinicians such as 'Adjuvant on line' and the Saint–Gallen consensus criteria. Thus its impact on therapeutic decision is weaker than other parameters such as patients' age, tumour size, lymph node involvement, hormone receptors and ERBB2 expression. We have shown in this study that it better predicts distant relapses than lymph node involvement, usually considered as one of the more accurate prognostic factors in breast cancer.[36,37] Better appraisal of PVI improved by combination of HES with CD31 and D2-40 stainings, may enhance prognostic prediction and therapeutic management of TNBC patients. On one hand, treatment and/or follow-up increase may be warranted for patients with the worst prognosis[38], as well as development of new therapeutics such as Poly (ADP)-ribose Polymerase inhibitors or antiangiogenic agents.[39–41] On the other hand, adjuvant treatment decrease deserves to be assessed for the patients with the best prognosis.

Conflict of interest statement

None declared

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REFERENCES

1. Institut National de Veille Sanitaire. http://www.invs.sante.fr/applications/cancers/projections2009/donnees_localisation/sein.pdf.
2. Rakha EA, El-Sayed ME, Green AR, et al. Prognostic markers in triple-negative breast cancer. *Cancer* 2007;**109**:25–32.
3. Haffty BG, Yang Q, Reiss M, et al. Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. *J Clin Oncol* 2006;**24**:5652–7.
4. Liedtke C, Mazouni C, Hess KR, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. *J Clin Oncol* 2008;**26**:1275–81.
5. Gluz O, Liedtke C, Gottschalk N, et al. Triple-negative breast cancer–current status and future directions. *Ann Oncol* 2009;**20**:1913–27.
6. Dawson SJ, Provenzano E, Caldas C. Triple negative breast cancers: clinical and prognostic implications. *Eur J Cancer* 2009;**45**(Suppl 1):27–40.
7. Viale G, Bottiglieri L. Pathological definition of triple negative breast cancer. *Eur J Cancer* 2009;**45**(Suppl 1):5–10.
8. Silver DP, Richardson AL, Eklund AC, et al. Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. *J Clin Oncol* 2010;**28**:1145–53.
9. Parikh RR, Housman D, Yang Q, et al. Prognostic value of triple-negative phenotype at the time of locally recurrent conservatively treated breast cancer. *Int J Radiat Oncol Biol Phys* 2008;**72**:1056–63.
10. Kennecke H, Yerushalmi R, Woods R, et al. Metastatic behavior of breast cancer subtypes. *J Clin Oncol* 2010;**28**:3271–7.
11. Hugh J, Hanson J, Cheang MC, et al. Breast cancer subtypes and response to docetaxel in node-positive breast cancer: use of an immunohistochemical definition in the BCIRG 001 trial. *J Clin Oncol* 2009;**27**:1168–76.
12. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of oestrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. *Cancer* 2007;**109**:1721–8.
13. Tischkowitz M, Brunet JS, Bégin LR, et al. Use of immunohistochemical markers can refine prognosis in triple negative breast cancer. *BMC Cancer* 2007;**7**:134.
14. Sloane JP, Amendoeira I, Apostolikas N, et al. Consistency achieved by 23 European pathologists from 12 countries in diagnosing breast disease and reporting prognostic features of carcinomas. *Virchows Arch* 1999;**434**:3–10.
15. Breiteneder-Geleff S, Soleiman A, Kowalski H, et al. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. *Am J Pathol* 1999;**154**:385–94.
16. McShane LM, Altman DG, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies (REMARK). *Breast Cancer Res Treat* 2006;**100**:229–35.
17. Viale G, Zurrida S, Maiorano E, et al. Predicting the status of axillary sentinel lymph nodes in 4351 patients with invasive breast carcinoma treated in a single institution. *Cancer* 2005;**103**:492–500.
18. Viale G, Giobbie-Hurder A, Gusterson BA, et al. Adverse prognostic value of peritumoural vascular invasion: is it abrogated by adequate endocrine adjuvant therapy? Results from two International Breast Cancer Study Group randomized trials of chemoendocrine adjuvant therapy for early breast cancer. *Ann Oncol* 2010;**21**:245–54.
19. Colleoni M, Rotmensz N, Maisonneuve P, et al. Prognostic role of the extent of peritumoural vascular invasion in operable breast cancer. *Ann Oncol* 2007;**18**:1632–40.
20. Rakha EA, Ellis IO. Triple-negative/basal-like breast cancer: review. *Pathology* 2009;**41**:40–7.
21. Jacquemier J, Padovani L, Rabayrol L, et al. Typical medullary breast carcinomas have a basal/myoepithelial phenotype. *J Pathol* 2005;**207**:260–8.
22. Azoulay S, Laé M, Fréneaux P, et al. KIT is highly expressed in adenoid cystic carcinoma of the breast, a basal-like carcinoma associated with a favorable outcome. *Mod Pathol* 2005;**18**:1623–31.

23. Bertucci F, Finetti P, Cervera N, et al. How basal are triple-negative breast cancers? *Int J Cancer* 2008;**123**:236–40.
24. Rakha EA, Elsheikh SE, Aleskandarany MA, et al. Triple-negative breast cancer: distinguishing between basal and nonbasal subtypes. *Clin Cancer Research* 2009;**15**:2302–10.
25. Seal MD, Chia SK. What is the difference between triple-negative and basal breast cancers? *Cancer J* 2010;**16**:12–6.
26. Sabatier R, Finetti P, Cervera N, et al. A gene expression signature identifies two prognostic subgroups of basal breast cancer. *Breast Cancer Res Treat* 2010 [Epub ahead of print].
27. Teschendorff AE, Miremadi A, Pinder SE, Ellis IO, Caldas C. An immune response gene expression module identifies a good prognosis subtype in estrogen receptor negative breast cancer. *Genome Biol* 2007;**8**:R157.
28. Speers C, Tsimelzon A, Sexton K, et al. Identification of novel kinase targets for the treatment of oestrogen receptor-negative breast cancer. *Clin Cancer Res* 2009;**15**:6327–40.
29. Bettelheim R, Penman HG, Thornton-Jones H, Neville AM. Prognostic significance of peritumoural vascular invasion in breast cancer. *Br J Cancer* 1984;**50**:771–7.
30. Neville AM, Bettelheim R, Gelber RD, et al. Factors predicting treatment responsiveness and prognosis in node-negative breast cancer. The International (Ludwig) Breast Cancer Study Group. *J Clin Oncol* 1992;**10**:696–705.
31. Lee AHS, Spinder SE, Macmillan RD, et al. Prognostic value of lymphovascular invasion in women with lymph node negative invasive breast carcinoma. *Eur J Cancer* 2006;**42**:357–62.
32. Mohammed RA, Martin SG, Mahmmod AM, et al. Objective assessment of lymphatic and blood vascular invasion in lymph node-negative breast carcinoma: findings from a large case series with long-term follow-up. *J Pathol* 2011;**223**:358–65.
33. Colleoni M, Rotmensz N, Maisonneuve P, et al. Prognostic role of the extent of peritumoural vascular invasion in operable breast cancer. *Ann Oncol* 2007;**18**:1632–40.
34. De Mascarel I, MacGrogan G, Debled M, et al. D2–40 in breast cancer: should we detect more vascular emboli? *Mod Pathol* 2009;**22**:216–22.
35. Greenberg S, Rugo HS. Triple-negative breast cancer: role of antiangiogenic agents. *Cancer J* 2010;**16**:33–8.
36. Jatoi I, Hilsenbeck SG, Clark GM, Osborne CK. Significance of axillary lymph node metastasis in primary breast cancer. *J Clin Oncol* 1999;**17**:2334–40.
37. Dent DM. Axillary lymphadenectomy for breast cancer. Paradigm shifts and pragmatic surgeons. *Arch Surg* 1996;**131**:1125–7.
38. Perez EA, Moreno-Aspitia A, Aubrey Thompson E, Andorfer CA. Adjuvant therapy of triple negative breast cancer. *Breast Cancer Res Treat* 2010;**120**:285–91.
39. Lord CJ, Ashworth A. Targeted therapy for cancer using PARP inhibitors. *Curr Opin Pharmacol* 2008;**8**:363–9.
40. Voduc D, Nielsen TO. Basal and triple-negative breast cancers: impact on clinical decision-making and novel therapeutic options. *Clin Breast Cancer* 2008;**8**(Suppl 4):S171–178.
41. Fong PC, Yap TA, Boss DS, et al. Poly (ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. *J Clin Oncol* 2010;**28**:2512–9.