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

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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.

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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