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Original article

# Lymphocyte-predominant triple-negative breast carcinomas in premenopausal patients: Lower expression of basal immunohistochemical markers

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ABSTRACT

*Objectives:* Triple-negative breast carcinomas (TNBCs) correspond to a molecular heterogeneous disease defined by lack of estrogen and progesterone receptor expression, and the absence of overexpression and/or amplification of HER2. Recent data indicate that clinical outcome in TNBC is affected by tumor-infiltrating lymphocytes, suggesting that they can benefit from immunotherapies. We selected 116 consecutive premenopausal patients with TNBC to compare the immunohistochemical profile of the group rich in tumor-infiltrating lymphocytes with those without this characteristic.
*Materials and methods:* We reviewed all the original histological sections to assess pathological features, and to select a representative area for tissue microarrays and immunohistochemical study. Estrogen and progesterone receptors, HER2 and Ki-67 were evaluated in whole histological sections. The following markers were analyzed in tissue microarrays sections: androgen receptor, cytokeratin 5/6, cytokeratin 14, epidermal growth factor receptor (EGFR), vimentin, p16, claudin-3, -4, and -7, p63, and aldehyde dehydrogenase isoform 1 (ALDH1). Lymphocyte-predominant breast cancer (LPBC) was defined by the presence of more than 50% of lymphocytes in the intratumoral stroma.
*Results:* Twenty-six (22.4%) patients present tumors classified as LPBC and 90 (77.6%) as non-LPBC. The two groups were similar regarding age of patients, tumor grade and Ki-67 positive cells. LPBC cases presented lower frequency of expression of the basal cytokeratins, EGFR, and basal-like immunoprofile. There was a trend to higher expression of ALDH1 by stromal intratumoral cells. The expression of all other markers were similar in the two groups.
*Conclusions:* Lymphocyte-predominant TNBC in premenopausal patients are mostly of non-basal phenotype.



## Introduction

Triple-negative breast carcinomas (TNBCs) are defined by lack of estrogen and progesterone receptor expression, and the absence of overexpression and/or amplification of the erb-b2 receptor tyrosine kinase 2 (HER2). Although they are often associated with a basal-like genetic phenotype, 21.4% of them correspond to other molecular types, such as HER2-enriched (7.8%), normal (7.0%), luminal B (4.4%), and luminal A (2.2%) [1]. The basal-like phenotype of TNBCs was first defined by the immunohistochemical expression of basal cytokeratin 5/6 and/or epidermal growth factor receptor (EGFR) [2,3]. TNBCs are more prevalent in young patients and they usually have a clinically aggressive behavior [4,5].

Tumor-infiltrating lymphocytes (TILs) in breast cancer (BC) are

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the main actors of the immune response and have been recognized as an important prognostic and predictive factor, particularly among estrogen receptor-negative carcinomas [6–8]. A meta-analysis study with 2987 patients with TNBCs and early stage showed that tumors rich in TILs have a 30% reduction in recurrence, 22% in distant recurrence, and 34% in death [9]. Lymphocyte-predominant breast cancers (LPBC) correspond to tumors with at least 50–60% of stromal TILs [10]. The International TILs Working Group provided a standardized methodology for evaluating TILs in BC [10]. The criteria were applied in a cohort of 897 TNBCs from the European Institute of Oncology [11]. The authors reported that patients with LPBC had better disease-free survival, distant disease-free survival and overall survival. In addition, they observed that each 10% increase in TILs strongly predicted better survival independent of other prognostic variables.

The heterogeneous morphologic, gene-expression profiles and genomic changes of TNBCs have been a challenge in finding of targets for therapy. It is clear that potential targets will work only in subsets of TNBCs. Candidates of these subsets are BRCA-1 mutant, BRCA1-like tumors with underlying defects in homologous recombination-mediated DNA repair and androgen-receptor positive tumors [12]. Successful subgroups based on immunohistochemical profile are lacking. The luminal androgen receptor subtype is characterized by the expression of this receptor [12,13]. However, the prognostic value of androgen receptor is controversial [14,15]. Other attempts on the classification of TNBC based on the immunohistochemical profile included p16, e-cadherin, claudins, and Ki-67 [16,17]. Some immunohistochemical markers have been related to prognosis, for example, vimentin [18] and aldehyde dehydrogenase 1A1 (ALDH-1A1) [19].

In this study, our aim was to compare triple-negative LPBC with non-LPBC according to immunohistochemical profile.

## Patients and methods

This project was approved by the Scientific Committee of the Department of Pathology and by the Ethical Committee for Research Projects of the Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, Brazil (CAPPesq) (protocol 311/10). As the study was retrospective, informed patient consent was waived, and any form of patient identification was abolished.

Formalin-fixed, paraffin-embedded tissue specimens from 116 consecutive patients aged 45 years or younger with triple-negative primary breast carcinomas diagnosed between July 2009 and March 2011 were selected for this study. All specimens corresponded to surgical or incisional biopsies prior to any local or systemic treatment.

The same pathologist (FMC) reviewed all the slides and evaluated their histological types based on World Health Organization criteria [20]. Tumor grading was assigned according to the Nottingham criteria [21]. TILs were evaluated in whole histological sections within the borders of the invasive tumor as percentage of area of intratumoral stromal compartment occupied by mononuclear cells (Fig. 1). TILS as a continuous variable was defined by level of percentage each 10% increments: 1 (0–10%); 2 (11–20%); 3 (21–30%); 4(31–40%); 5(41–50%), and 6 (>50%). Cases with more than 50% of mononuclear cells were defined as LPBC, according criteria recommended by Salgado et al. [10]. A representative area of each tumor was selected for the construction of tissue microarray (TMA) blocks and for the immunohistochemical study; Ki-67 was evaluated in whole histological sections.

## TMA construction

Tissue sections were stained with hematoxylin/eosin and the corresponding sections in each paraffin donor block were marked. Then one cylinder of the material (2.0 mm in diameter) was punched from each of these regions and these were mounted into recipient paraffin blocks at 2-mm intervals using a precision microarray instrument (Beecher Instruments, Silver Spring, MD, USA). A grid system was established such that each core had an x- and y-coordinate reference for sample identification. The blocks were sealed at 60 °C for 10 min. Sections (3 μm) from each TMA block were prepared using standard techniques and were mounted on Starfrost® slides. The first histological sections cut were stained with hematoxylin/eosin to ensure that the appropriate sections of the tumor had been obtained.

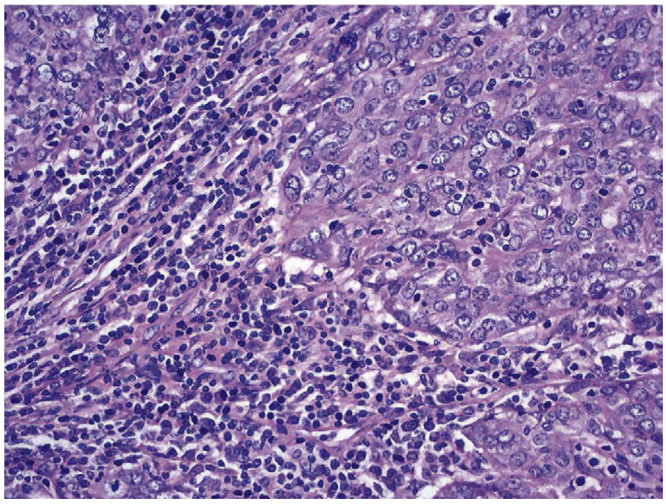

**Fig. 1.** Invasive carcinoma with intratumoral stroma rich in lymphocytes (Hematoxylin-eosin – original magnification 200×).

## Immunohistochemistry and scoring

The following markers were selected for this study: Estrogen receptor (ER), progesterone receptor (PR), HER2, Ki-67, androgen receptor (AR), cytokeratin 5/6 (CK 5/6), cytokeratin 14 (CK 14), EGFR, vimentin, p16, claudin-3, -4, and -7, p63, and aldehyde dehydrogenase isoform 1 (ALDH1). The immunohistochemical detection of ER, PR, HER2 and Ki67 were performed in whole tumor sections. The other markers were evaluated on the TMA sections. Immunohistochemical studies were performed using the Envision Flex (Dako, Carpinteria, USA) as the detection system. A pressure cooker was used in all cases for epitope retrieval, except for HER2. For HER2, we used a microwave oven. Technical specifications of immunohistochemistry staining are given in Table 1. Peroxidase activity was visualized with diaminobenzidine staining (DAKO, USA).

Nuclear staining was considered positive for ER, PR, AR, Ki-67, and p63; cytoplasmatic staining for cytokeratins, vimentin, and ALDH1; cytoplasmatic and/or nuclear for p16; and membrane for HER2, EGFR, and claudins.

Negative ER and PR were defined by less than 1% of nuclear positive cells. The criteria of HER2 negative were those described by ASCO/CAP recommendations [22]. Ki-67 was expressed by percentage of positive cells calculated by scoring 500 tumor cell nuclei. For AR, CK 5/6, CK 14, vimentin, p16, p63, and ALDH1, any moderate to strong intensity in at least 1% of cells was considered positive. Positive EGFR expression was defined based on the presence of complete moderate/strong membrane staining in ≥10% of the cells. The same criteria were considered for claudins. A basal-like