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Original article

Detection of disseminated tumor cells in locally advanced breast cancer patients before primary systemic therapy

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ABSTRACT

Aim: To assess the prevalence and prognostic power of disseminated tumor cells (DTC) in patients with locally advanced breast cancer (LABC) before primary systemic therapy (PST).
Materials and methods: LABC patients attending our Breast Unit were studied between 2002 and 2012, all of them being considered for PST. To determine the presence of DTC, posterior iliac crest aspirates were obtained and marrow samples were processed by gradient separation with Ficoll (Lymphoprep®) and immunohistochemical staining using the antiCK A45-B/B3 (EPIMET) antibody. Clinicopathologic variables were recorded before and after PST to assess response. Disease-free survival (DFS) and overall survival (OS) were determined after follow-up. The presence of DTC as a predictor of response to PST and as a prognostic tool for OS and DSF was evaluated.
Results: DTC were observed in 26% of 47 patients included in the study. PST consisted of chemotherapy in 94% and hormone therapy in 6%. Breast-conserving therapy was attained in 33%. Mean follow-up was 68 months. Complete clinical response (CR) after PST was seen in 26%, disease recurrence in 38%, and cancer-related death in 8%; tumor size and negative estrogen receptors were significant predictors of CR and mastectomy was associated with DFS. Persistent axillary disease after PST and previous recurrence were predictive of OS. DTC were detected more often in patients who did not achieve CR and those who presented recurrence. DTC detection was a significant prognostic factor for a worse OS (OR = 7.62; CI95%: 1.46–39.61; $p = 0.009$) and a decreased survival time (62 versus 82 months, $p = 0.004$).
Conclusion: Presence of DTC before PST was found in a significant number of patients with LABC. DTC were found to be a significant prognostic factor for cancer-related death. DTC could be a surrogate predictor of response to PST and also of disease recurrence in LABC patients.

Introduction

The importance of the indication of primary systemic therapy (PST) for patients with locally advanced breast cancer (LABC) lies in its ability to promote conservative surgery through tumor downstaging.[1,2] PST regimens also permit assessment, both in vivo and using histology specimens of certain markers, and correlation of these markers with tumor response and long-term survival.[3] Several factors have been found to predict response to neoadjuvant chemotherapy, among them, tumor size, type, and grade, hormone-receptor expression, and erb-B2 over-expression.[4,5] Furthermore, clearing of primary and lymph node disease, that is, complete pathologic response, are well-established prognostic factors for overall survival (OS), and disease-free survival (DFS).[6,7]

Abbreviations: CR, complete clinical response; CTC, circulating tumor cells; DCIS, ductal carcinoma in situ; DFS, disease-free survival; DTC, disseminated tumor cells; ISHAGE, International Society of Hematotherapy and Graft Engineering; LABC, locally advanced breast cancer; OR, odds ratio; OS, overall survival; PST, primary systemic treatment; RR, relative risk.

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Early hematogenous dissemination has been proposed as a prognostic factor that might explain the errors in the prognostic assessment of breast cancer patients. Detection of disseminated tumor cells (DTC) at diagnosis could show potential for restaging purposes as well as for reconsideration of the therapeutic regimen used in any given patient. The importance of marrow DTC cannot be dismissed, although the prevalence reported varies widely from 13% to 43% in a pooled analysis of 4703 patients from 9 centers.[8]

The impact of DTC on prognosis has already been shown among patients with disease of similar stage, as defined by tumor size and grade, lymph node involvement, and hormone receptor status. Most studies[9–14] have been conducted at the time of diagnosis in patients considered for surgical treatment and, therefore, reflect initial status only. There is a paucity of information on the prevalence and prognostic power of DTC in more advanced stages, with locoregional spread. Hall et al.[15] detected DTC in 26% of patients after PST, a finding that proved to be of prognostic value for survival. Nevertheless, we are unaware of studies on DTC detection before PST in LABC patients, a time point when such information could be of significant prognostic interest.

The aim of our study was to determine DTC prevalence before PST in patients with LABC, as well as its value as a prognostic factor for disease recurrence and overall survival, compared to standard clinicopathologic parameters and response to chemotherapy.

Materials and methods

This observational study was carried out at the Germans Trias i Pujol University Hospital between 2002 and 2012 with a sample consisting of consecutive patients considered for PST, according to a decision agreed by the Breast Unit staff between 2002 and 2005. Most patients presented with LABC (stage II B or stage III breast cancer, where the term *locally advanced breast cancer* is used to describe a breast cancer that has progressed locally but has not yet spread outside the breast or local lymph nodes); however, some patients in stage II A were considered for PST, in order to promote conservative surgery.

The final sample included 55 patients. The study was approved by the hospital's ethics committee and all subjects gave written informed consent to participate.

All patients received systemic therapy before surgery, according to our hospital's guidelines at the time of inclusion. Chemotherapy regimens were based on anthracycline or anthracycline plus taxane, depending on the specific protocol in use. Patients with erb-B2 over-expression received chemotherapy and trastuzumab. Surgical treatment followed chemotherapy, and patients were subsequently re-evaluated to decide on postoperative chemotherapy. Hormone therapy was administered after chemotherapy in patients positive for estrogen or progesterone receptors. In patients who had undergone breast-conserving surgery, radiotherapy was tailored to residual breast, chest wall, and lymphatic basins, as necessary, according to our guidelines.

The following clinicopathologic variables were recorded before and after PST, as well as during follow-up: clinical stage at onset, including tumor size (T) and lymph node status (N); initial and post-PST histologic type; residual tumor size and grade, post-PST lymph node status; hormone receptor (HR) status and erb-B2 over-expression.

Clinical response was assessed according to clinical examination and mammography following RECIST criteria.[16] Patients with no evidence of breast or residual nodal disease were considered to have had a complete response (CR).

Values for DFS and OS were established at the end of the follow-up period. DFS was defined as the period between patient inclusion and disease progression, whereas OS was defined as the period between patient inclusion and death from any cause.

Detection of DTC

DTC were detected in marrow samples, using the International Society of Hematotherapy and Graft Engineering (ISHAGE)[17] procedural guidelines, modified for our setting.

Marrow aspirates consisting of 10 mL from both posterior iliac crests were obtained using local anesthetics. The marrow samples were then immediately processed using a gradient-separation technique based on Ficoll (Lymphoprep®). Centrifugation leads to a density product redistribution, such that mononuclear marrow elements together with any possible cancer cells are forced into an interphase from which they can be easily separated. After recovery, counting, and smearing, immunohistochemistry stains were prepared. The resulting samples were analyzed for epithelial cells with cytokeratin expression. The alkaline phosphatase/anti-alkaline phosphatase (APAAP) technique was used with the antiCK A45-B/B3 monoclonal antibody in a total of 2×10^6 cells. This is an antibody cocktail against cytokeratin 8, 18, and 19 (EPIMET Epithelial Cell Detection Kit®, Baxter Europe, Micromet) (Fig. 1).

In the case of positive stained cells, a morphologic assessment was needed to rule out false staining due to sample contamination from squamous cells, which were easily identified. A DTC+ result was defined as the detection of at least one positive cell. As a negative control, 10 marrow samples from patients with various hematologic conditions and very low pretest probability were analyzed. Cultured breast cancer cells (MCF-7) were used as a positive control. The outlined method had been used and validated in a previous study with early breast cancer patients.[18]

Data analysis

A descriptive analysis of all variables was performed. Qualitative variables were described using frequency tables for different categories, and quantitative variables were described as the mean and range. Associations between the variables of interest were analyzed. To compare qualitative variables, the chi-square or Fisher's exact test were used. For comparisons between

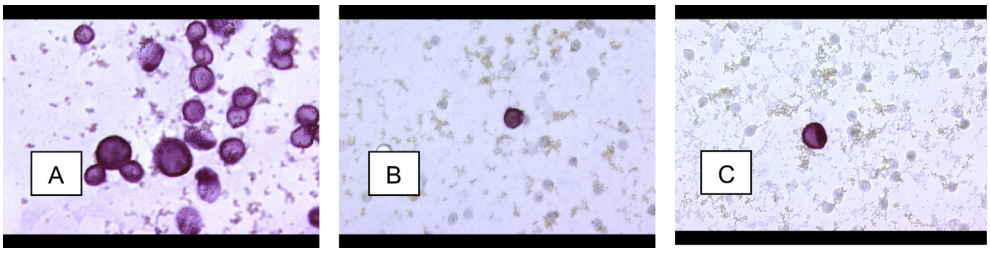

Fig. 1. Immunohistohemical staining: A. positive control of MCF-7 line; B. positive control of MCF-7 line. Recovery of 1×10^6 cells; C. positive staining in a patient.