Original Study

Seroma Cytology in Breast Cancer: An Underappreciated Issue

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Abstract

The presence of cancer cells in postoperative drain fluid has been ignored when achieving local disease control in breast cancer. We designed a prospective study to examine the drain cytology and demonstrated malignant cells in the drainage fluid from 4 of 68 cases, mostly independent of the axillary status. These findings highlight the danger regarding the overall objective of "disease-free local control" in breast cancer surgery.

Background: The presence of malignant cells in postoperative seroma has been ignored in current breast cancer treatment. We aimed to assess the presence of malignant seroma cytology and to evaluate its relationship with the known prognostic factors for breast cancer. **Patients and Methods:** The solution from irrigation of the operation field and postoperative drainage fluid from 68 patients were prospectively collected and examined for malignant cytology. The results were evaluated according to the tumor characteristics and patient demographics. **Results:** Malignant cytology was found in none of the intraoperative samples but was found in the postoperative samples from 4 patients. Of these 4 patients, 3 were free of axillary metastasis. None of the common risk factors for breast cancer was associated with the finding of malignant cytology. **Conclusion:** Malignant cells can be seen in the drainage fluids from breast cancer patients independent of any contamination occurring during surgery, even in those without axillary metastasis.

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Introduction

Local disease control plays a pivotal role in achieving successful outcomes in breast cancer. Adequate local control after breast cancer surgery can be described as clear surgical margins and either dissected locoregional lymph nodes or lymph nodes confirmed to be free of metastasis. Malignant cells dormant in the operation field can have an effect on the systemic outcome of the disease. Some expensive molecular methods such as reverse transcriptase polymerase chain reaction (RT-PCR) have been studied for the early detection of circulating tumor cells that have entered into the circulation from the local area in nonmetastatic breast cancer patients.[1,2] However, the presence of cancer cells persisting in the operation field could perhaps be determined using simple cytology of the drainage fluid.

Although the importance of the cytologic diagnosis of malignancy in fluids such as pleural effusions and ascites has previously been demonstrated for other cancer types,[3,4] this concept has been underappreciated for breast cancer. Vujičić et al[5] drew attention to the importance of seroma cytology in 1986. However, further studies are still lacking.

Our objective was to study the presence of malignant cells in postoperative seroma and to evaluate its relationship with the known prognostic factors for breast cancer. The demonstration of the presence of malignant cells in drainage fluids could lead the way for staging and forward radiotherapy planning of postmastectomy patients, especially those with axillary negativity.

Materials and Methods

The present prospective study included 68 women with a diagnosis of, and undergoing surgery for, nonmetastatic breast cancer from December 2013 to August 2014. The clinical research and

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ethics board of Baskent University approved the present study (approval no. KA13/189, December 2, 2013). Sample size estimation was performed according to the free cancer cell ratio reported previously.[5] Patients who had undergone surgery for breast cancer, were known to be free of nonaxillary metastasis, and had not received any type of neoadjuvant therapy were enrolled in the present study. Those with distant metastasis, those who had undergone previous systemic therapy or radiotherapy, and those undergoing surgery for recurrent disease were excluded. The eligible patients agreeing to participate in the study signed an informed consent form. Surgeons experienced in breast surgery performed all the surgical procedures. Sufficient experience was considered the performance of 30 operations for breast cancer annually for $\geq$ 5 years. The type of surgery was left to the discretion of the surgeon. However, vacuum drains were mandatory after any type of breast cancer surgery. All surgical fields (ie, mastectomy, lumpectomy, and sentinel or axillary dissection) of each case were irrigated with 1 L of saline for 5 minutes, and this irrigation fluid was collected in a container labeled "intraoperative sample" for cytologic examination to assess for the presence of cancer cells from operative contamination. Vacuum drains of 12 Ch (Bicakcilar, Istanbul, Turkey) were placed in the operative field before skin closure. The drainage fluid, mostly bloody, within the first 24 hours was discarded, and the serous fluid content that formed within the subsequent 48 hours was collected in another container labeled "postoperative sample" for cytologic examination to assess for any subsequently exfoliated cancer cells. A late cytologic drainage fluid examination was also planned if any postoperative drainage lasted $>$ 15 days.[6] The patient demographics and tumor characteristics were recorded.

The collected intraoperative irrigation fluid samples and drainage fluid samples were centrifuged at 1600 rpm for 5 minutes. Homogeneity was achieved by pipetting the sediment. The sediment was spread on 2 slides each and cytocentrifuged at 1000 rpm for 5 minutes and then fixed in alcohol for 5 minutes. The slides were stained with either Papanicolaou or hematoxylin Papanicolaou and examined under a light microscope. Cell blocks were prepared in suspected cases and were examined in 4-μm-thick sections stained with hematoxylin and evaluated under a light microscope. The cytodiagnosis was determined from the ratio of nucleus/cytoplasm, nucleus shape, amount and distribution of nuclear chromatin, and the status of nucleoli. The results were classified as either positive or negative. If positive, the number of cancer cells per patient was also counted. Borderline positive results, in which only a small number (range 1-3) of cells with highly severe atypia or possible malignancy were detected, were also classified as positive. Cells were excluded from the evaluation if they were too degenerate for a diagnosis.

All cytologic examinations were performed by a single pathologist (F.A.B.). The cytologic results were documented and compared with patient demographics and tumor characteristics. These included patient age, tumor diameter, tumor location, multicentricity, preoperative clinical and radiologic status of the axilla, pathologic features in the sentinel and axillary lymph nodes, lymphovascular invasion, number of mitotic figures counted in 10 high power fields, histologic type, surgery type, and major molecular subtypes. The major molecular subtypes included luminal A, luminal B, triple negative, and human epidermal growth factor 2 (HER2) according to the estrogen receptor, progesterone receptor, and HER2/neu status of the tumor as described previously.[7] The patients were followed up for the preliminary results of local recurrence and mortality.

Descriptive statistics for patient demographics and tumor characteristics were computed. Comparative statistical analysis to determine correlations between the various clinicopathologic parameters and cytologic cell count results in lymphatic fluid could not be calculated because the number of patients with positive cytology results was insufficient. All statistical analyses were performed with the Statistical Package for Social Sciences, version 20 (SPSS, Chicago, IL).

Results

The mean age of the patients was 51.5 $\pm$ 11.4 years (range, 29-80 years). The mean tumor diameter was 20.5 $\pm$ 13.5 mm (range, 7-89 mm). The tumors were located most frequently in the upper outer quadrants (44.1%) and less commonly in the lower inner quadrants (5.9%). Axilla were positive clinically in 32.4% and radiologically in 44.1% of the patients preoperatively. Infiltrating ductal carcinoma was the leading histologic subtype (88.2%). Lymphovascular invasion was positive in 89.8%, and the mean number of mitosis was 17.8 $\pm$ 11.6 (range, 4-65). Luminal A was the major molecular subtype (55.9%), with luminal B in 22.1%, triple negative in 5.9%, and HER2 in 16.2%.

Mastectomy (60.3%), skin-sparing mastectomy (11.8%), and lumpectomy (27.9%) were the procedures of choice, with sentinel lymph node biopsy (SLNB) and/or axillary lymph node dissection. In 17.6% of the patients, immediate breast reconstruction was performed with a transversus rectus abdominis muscle flap, latissimus dorsi flap, or breast implants. SLNB was performed in 40 of the 68 cases, and sentinel node was positive in 47.5%. However, 65% of the axillary dissection procedures for positive sentinel nodes resulted in negative nonsentinel axillary nodal status. Immediate axillary dissection was performed in 28 of the 68 patients, omitting the SLNB step. Axillary lymph nodes were positive in 89.2% of this group. All the surgical margins were clear ($\geq$ 1 mm).

Malignant cells were not detected in the intraoperative irrigation fluid of any of the patients. However, cancer cells were found in the cytologic analysis of the postoperative samples from 4 patients. The postoperative cytologic results of these 4 patients and their tumor characteristics are listed in Table 1 compared with the average values of the patients with negative cytology. The tumor characteristics of these 4 patients were identical with those of the entire group. Modified radical mastectomy without SLNB was performed in the first patient because of the presence of cancer invading the areola and palpable nodes in the ipsilateral axilla. Although the sentinel node was negative, axillary dissection was performed in the second and third patients because of the presence of palpable voluminous axillary nodes. None of these 3 patients had axillary nodal involvement. SLNB of the fourth patient demonstrated a metastatic sentinel nodal focus; however, further axillary lymph node dissection resulted in a metastasis-free axillary dissection specimen. Drainage did not exceed 15 days in any patient, and no cytologic examination of late drainage fluid was performed. Five patients with negative drain cytology were lost to follow-up. Local recurrence in 1 patient and brain metastasis in another patient, both with negative cytologic findings, were observed at the end of a mean follow-up period of 18 $\pm$ 2.6 months. Those with