Prognostic Value of Circulating Tumor Cells Identified Before Surgical Resection in Nonmetastatic Breast Cancer Patients

Carolyn S Hall, PhD, Mandar G Karhade, MBBS, MPH, Jessica B Bowman Bauldry, BA, Lily M Valad, BS, Henry M Kuerer, MD, PhD, FACS, Sarah M DeSnyder, MD, Anthony Lucci, MD, FACS

BACKGROUND: Circulating tumor cells (CTCs) can be identified in approximately 25% of nonmetastatic breast cancer patients, and data are emerging regarding their prognostic significance. We hypothesized that CTCs identified before resection of the primary tumor would predict worse outcomes in nonmetastatic breast cancer patients.

STUDY DESIGN: We performed CTC enumerations on 509 patients with nonmetastatic breast cancer as part of an IRB-approved study. The CTCs (per 7.5 mL blood) were identified using the CellSearch System (Janssen). The presence of $\geq$1 CTC meeting morphologic criteria for malignancy was considered a positive result. Log-rank test and Cox regression analysis were applied to establish the association of CTCs with relapse-free and overall survival.

RESULTS: Median follow-up was 48 months and mean age was 53 years. Fifty-nine percent of patients (299 of 509) had tumors larger than 2 cm, and 46% (234 of 509) had positive lymph nodes. One hundred sixty-six patients received neoadjuvant chemotherapy (NACT) before CTC assessment, and 343 patients were chemonaïve. One or more CTC was identified in 43 of 166 (26%) NACT treated patients, and in 81 of 343 (24%) chemonaïve patients. Circulating tumor cells were not associated with tumor size, grade, or lymph node status ($p = NS$). Detection of 1 or more CTCs predicted decreased relapse-free (log-rank $p < 0.001$, hazard ratio [HR] 2.72, 95% CI 1.57 to 4.72; $p < 0.001$) and overall survival (log-rank $p = 0.02$, HR 2.29, 95% CI 1.12 to 4.67; $p = 0.03$) at 48 months of follow-up.

CONCLUSIONS: One or more CTCs identified before resection of the primary breast tumor predicted worse relapse-free and overall survival, irrespective of primary tumor size, grade, or lymph node positivity. (J Am Coll Surg 2016;223:20−29. © 2016 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

Distant metastasis is the primary cause of death for breast cancer patients. Metastasis is a complex, multistep process orchestrated by a subpopulation of cells within a heterogeneous tumor, which acquire the ability to disseminate from the primary tumor and enter the bloodstream and/or lymph nodes. Currently, lymph node metastasis is considered to be the most powerful prognostic predictor for breast cancer, and it forms the basis of the current pN category of the American Joint Committee on Cancer Tumor Node Metastasis (TNM) staging system.[1] However, even lower grade, lymph node negative patients have relapse rates of 20% over 10 years, and the relapse rate increases to 30% for node negative patients with high grade tumors.[2] Conversely, many patients with lymph node metastases will not have a relapse after treatment.[2-4] These data suggest that occult dissemination of cancer cells

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From The University of Texas MD Anderson Cancer Center, Department of Surgical Oncology, Houston, TX.

Correspondence address: Anthony Lucci, MD, FACS, Department of Surgical Oncology, Unit 444, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030. email: alucci@mdanderson.org

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Abbreviations and Acronyms

CTC = circulating tumor cells
ER = estrogen receptor
HER2 = human epidermal growth factor receptor 2
HR = hazard ratio
NACT = neoadjuvant chemotherapy
OS = overall survival
PR = progesterone
RFS = relapse-free survival
TNM = Tumor Node Metastasis

mediates disease progression in a significant number of operable breast cancer patients, irrespective of lymph node involvement, and current staging procedures are not sensitive enough to reliably detect and predict disease progression in all patients.

Circulating tumor cells (CTCs) are rare cells ($\geq$1 CTC/10^6 hematopoietic cells)[5] within the peripheral blood that usually remain undetected by high-resolution imaging technologies.[6] For more than a decade, clinical researchers have demonstrated the prognostic significance of CTCs in metastatic breast cancer patients using the FDA-approved CellSearch System (Janssen). Circulating tumor cell counts of $\geq$5 CTCs/7.5 mL blood before administration of systemic treatment independently predict shortened progression-free (2.7 months vs 7.0 months in patients with less than 5 CTCs/7.5 mL blood) and overall survival (10.1 months vs >18 months) in metastatic patients.[7] In addition, CTC monitoring throughout therapy predicted treatment response better than standard radiologic imaging in metastatic patients.[8,9] Because metastatic patients account for only 5% to 8% of newly diagnosed breast cancer cases,[10] many clinical research groups have more recently focused on the prognostic significance of CTCs in nonmetastatic patients. In 2010, our group published one of the first studies demonstrating that CTCs can be identified in early stage breast cancer patients. Thirty percent of the T1/T2 patients in our study had $\geq$1 CTC/7.5 mL blood, indicative of the early dissemination of these cells,[11] and these data have been validated by several European studies.[12-16] However, despite the recent studies documenting that CTCs can be detected in a significant number (19% to 31%) of nonmetastatic patients,[11,13-19] data regarding their prognostic significance in these patients have been lacking.

We hypothesized that CTC identification before removal of the primary tumor would predict worse progression-free and overall survival in nonmetastatic breast cancer patients, irrespective of primary tumor characteristics, axillary lymph node status, or whether they had received neoadjuvant chemotherapy or not. If CTC presence were to contribute to the currently available prognostic information, it would be beneficial in identifying nonmetastatic patients at high risk for relapse, who could benefit from additional adjuvant therapies or inclusion in clinical trials of novel agents.

METHODS

Patients

This study included 509 stage I to III breast cancer patients undergoing surgery for their primary tumor between February 2005 and February 2014. All eligible patients with nonmetastatic breast cancer were offered enrollment by the participating surgeons (from 2005 to 2010: Dr Lucci, and from 2010 to 2014: Drs Lucci, Kuerer, and DeSnyder) at The University of Texas MD Anderson Cancer Center. The institutional review board at The University at Texas MD Anderson Cancer Center approved this prospective study (04-0698; principal investigator: AL), which included CTC assessment on samples taken before initial surgery for the primary breast cancer. We obtained informed written consent from all patients before collecting blood. Enrollment was strictly voluntary, and patient results were blinded from investigators by use of a random number system as the unique patient identifier. Patients with bilateral breast cancer, or any other malignancy within 5 years of diagnosis of the current cancer, were ineligible.

Staging and classification

The primary TNM staging and tumor grade were designated according to the criteria set by the American Joint Committee on Cancer[1] and Black's nuclear grading system,[20] respectively. Clinical stage was defined as TNM stage determined at the time of first diagnostic procedure confirming the invasive component of the tumor. Tumor sections were immunostained for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) using previously published procedures.[11] Immunostaining results for HER2 were scored as positive when >10% of the tumor cells had membranous staining, or when fluorescence in situ hybridization for HER2 gene amplification using the Abbott PathVysion HER2 DNA probe kit (Abbott Laboratories) HER2/CEP17 ratio was >2.2. Triple negative breast cancer was defined by absence of primary tumor ER, PR expression and HER-2 immunostaining and/or gene amplification.

Isolation, staining, and enumeration of circulating tumor cells

Peripheral blood (7.5 mL) was collected at the time of primary tumor surgery (but before any surgical manipulation of the primary tumor). Status of the CTC was determined