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Invasive Lobular Carcinoma Predicts Micrometastasis in Breast Cancer

Sarah M. Gainer, M.D.,

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

Ashutosh K. Lodhi, M.D.,

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

Anirban Bhattacharyya, M.D.,

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

Savitri Krishnamurthy, M.D.,

Department of Pathology, The University of Texas, MD Anderson Cancer Center, Houston, Texas

Henry M. Kuerer, M.D., Ph.D., and

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

Anthony Lucci, M.D.

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

Abstract

Background—Invasive lobular carcinomas (ILCs) are almost always estrogen receptor (ER) positive. Most delayed breast cancer recurrences occur in ER positive patients. Disseminated tumor cells (DTCs) and circulating tumor cells (CTCs) have been implicated in recurrence. The study purpose was to determine if DTCs and CTCs are associated with ILCs in stage I-III breast cancer.

Materials and Methods—Clinical stage I-III breast cancer patients consented to participate in an IRB-approved study involving collection of bone marrow and blood at surgery for their primary breast cancer. DTCs were assessed by anti-CK antibody cocktail following cytopsin. CTCs were detected using CellSearch™. CTCs were defined as nucleated cells lacking CD45 but expressing cytokeratin. One or more cells per 5 ml of bone marrow or 7.5 ml of blood was considered positive. Statistical analyses used chi-square and Fischer's exact tests.

Results—We prospectively enrolled 422 patients, 64 with ILC and 358 with invasive ductal carcinoma (IDC). ER positivity was higher in ILCs (92.2% vs. 66.2%). {P=0.0001} DTCs were identified 43.4% with ILC compared to 28.9% with IDC. {P = 0.03} CTC rates were similar.

Manuscript Correspondence Sarah M. Gainer, M.D. Department of Surgical Oncology The University of Texas, MD Anderson Cancer Center 1400 Pressler Street, Unit 1484 Houston, Texas 77030 Telephone: 713-792-1842 Fax: 713-745-1462 sgainer@mdanderson.org.

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Either DTCs or CTCs were identified in 75.6% with ILC compared to 51.7% with IDC. { $P = 0.002$ } No correlation was observed between presence of DTCs and CTCs in ILC patients and tumor size, grade, hormone receptor status, stage, lymph node status, or administration of NACT.

Conclusions—ILC independently predicted micrometastatic disease. Since most late recurrences are ER positive, this raises the question of whether DTCs and CTCs are indeed responsible for late breast cancer recurrence.

Introduction

Invasive lobular carcinoma (ILC) represents 5-15% of breast cancers [1-2]. Carcinomas of lobular histology are typically well-differentiated and the majority are estrogen receptor (ER) positive [3-4]. Recurrence in patients with ER positive disease tends to occur later than recurrence in those with ER negative disease, specifically more than 5 years following therapy [5-6].

Response to systemic treatment and risk of metastasis has been correlated with clinical and pathologic factors including primary tumor size, grade, hormone receptor status, HER2/neu amplification, and axillary nodal involvement. Despite a lack of axillary involvement, nearly a third of women will develop distant recurrence [7]. This suggests a method of spread independent of the lymphatic system.

Disseminated tumor cells (DTCs) and circulating tumor cells (CTCs) have been implicated in breast cancer recurrence [8-9]. Stathopoulos and colleagues showed that despite a lack of axillary disease, one-third of patients with operable breast cancer had micrometastatic disease at the time of surgery [10]. In a pooled analysis of 4,703 patients, Braun and colleagues assessed the prognostic significance of DTCs. They found that 30% of patients with stage I-III breast cancer harbored DTCs at diagnosis and those with DTCs had decreased disease-free survival (DFS) and overall survival (OS) [8]. In patients with metastatic disease, the presence of 5 or more CTCs prior to initiation of chemotherapy predicted worse progression-free survival (PFS) and OS [9]. Follow-up of this cohort of patients showed that presence of 5 or more CTCs detected at serial time points during therapy predicted worse PFS and OS [11]. Although the significance of CTCs in metastatic disease has been defined, that of CTCs in non-metastatic patients remains to be determined.

The purpose of this study was to determine if DTCs and CTCs are associated with ILCs in patients with non-metastatic breast cancer.

Methods and Methods

A prospectively collected database of women with stage I-III breast cancer treated at The University of Texas MD Anderson Cancer Center between February 2005 and October 2011 was reviewed. The enrollment of these patients in research protocol 04-0657 was approved by our institutional review board. To be enrolled, patients must have had biopsy proven invasive carcinoma with no clinical or radiographic evidence of metastatic disease. Both chemo-naïve patients and those who underwent neoadjuvant chemotherapy (NACT) were eligible. Consent was received for collection of bone marrow, blood, and tissue.

Prior to tumor resection, 2, 5 ml tubes of bone marrow and 2, 7.5 ml tubes of blood were collected under anesthesia. Bone marrow was collected from the bilateral anterior superior iliac crests using a Jamshidi needle. Samples were transferred to a tube containing ethylenediamine tetra-acetic acid (EDTA) and centrifuged. DTCs were assessed by anti-CK antibody cocktail following cytopspin. A positive result was defined as one or more cells per 5 ml of bone marrow. Peripheral blood was collected from the bilateral upper extremities.

CTCs were assessed using the CellSearch™ system. CTCs were defined as nucleated cells lacking CD45, but expressing cytokeratin (CK). A positive result was defined as the presence of one or more cells per 7.5 ml of blood since the threshold for positivity has not been established in non-metastatic breast cancer.

Statistical analyses were performed using chi-square and Fischer's exact tests.

Results

A total of 422 patients were prospectively enrolled. The clinical characteristics of the patients, the pathologic characteristics of the tumors, and the chemotherapy administration strategies are summarized in Tables I and II. The mean patient age was 52 years (range 25 to 91). Median follow-up was 32 months (range 3 to 94). Sixty four patients (15.2%) had ILC while 358 (84.8%) had invasive ductal carcinoma (IDC). Based on AJCC criteria, 180 patients had T1 tumors, 147 had T2 tumors, 43 had T3 tumors, and 52 had T4 tumors. One hundred and thirty six patients had stage I disease, 176 were stage II, and 110 were stage III. Fifty-six tumors were low grade, 186 were intermediate grade, and 179 were high grade. Tumor grade was unknown for 1 patient. Two hundred and thirty-nine patients (56.6%) had ER positive disease. Of the 64 patients with ILC, 59 (92.2%) had ER positive disease while only 66.2% of patients (237/358) with IDC were ER positive. { $P = 0.0001$ } NACT was administered to 136 patients (32.2%) and 286 (67.8%) were chemo-naïve. Only 21.9% percent of patients (14/64) with ILC underwent NACT compared to 34.1 % of patients (122/358) with IDC.

Rates of micrometastases in the study population are summarized in Table III. Analysis for DTCs has been completed in 298 patients to date. DTCs were identified in 109/298 patients (36.6%). Twenty-three of the 53 patients (43.4%) with ILC analyzed to date had DTCs. Only 81/298 patients (28.9%) with IDC had DTCs. { $P = 0.03$ } Analysis for CTCs has been completed in 365 patients to date. CTCs were identified in 90/365 patients (24.7%). Fifteen of the 55 patients (27.3%) with ILC analyzed to date had CTCs. Seventy-five of 310 patients (24.2%) with IDC had CTCs. { $P = 0.33$ } Of the 281 patients analyzed for both DTCs and CTCs, 170 (60.5%) harbored either DTCs or CTCs. Of the 45 patients with ILC analyzed for both DTCs and CTCs, 34 (75.6%) had either compared to 136/263 (51.7%) with IDC. { $P = 0.002$ }

Only 2/14 (14.3%) of patients with ILC treated with NACT achieved a pathologic complete response (pCR) compared to 34/122 patients (27.9%) with IDC. { $P = 0.09$ } No correlation was observed between the presence of DTCs and / or CTCs in ILC patients and tumor size, tumor grade, hormone receptor status, stage, lymph node status, or administration of NACT.

Discussion

Our study reports on 422 patients with stage I-III breast cancer and compares the rates of DTCs and CTCs by type of invasive carcinoma. In our series, almost one-third of patients harbored DTCs and one-third harbored CTCs. This is in agreement with previously published results [8, 12-14]. As anticipated, 15% of patients in our series had ILC. Patients with ILC were 1.5 times more likely to harbor DTCs compared to those with IDC. No difference was found between rates of CTCs between histologies. However, patients with ILC were more likely to harbor either DTCs or CTCs than those with IDC. { $P = 0.002$ } No correlation was found between the presence of DTCs and CTCs in patients with ILC and clinicopathologic characteristics. This represents one of the first series of patients showing different rates of micrometastatic disease reported by histology. Although it is too early in our series to report on differences in DFS and OS, many have studied the outcomes of those

with micrometastatic disease. In a pooled analysis of 4703 patients, Braun and colleagues found the presence of DTCs in patients with operable breast cancer to be an independent risk factor for significantly decreased DFS and OS. However, no differences in the rates of micrometastatic disease were seen by histologic type [8]. Hall et al found no correlation between pCR and presence of DTCs. However, in their series patients with DTCs following NACT had worse OS [14]. While the presence of CTCs has been shown to predict decreased PFS and OS in patients with metastatic breast cancer, their significance in operable breast cancer remains to be delineated [9]. In smaller series of patients with stage I-III breast cancer, CTCs have been shown to predict both DFS and OS [15-16]. These studies support the hypothesis that presence of DTCs and CTCs may be an independent predictor of prognosis in patients with operable breast cancer.

While the presence of micrometastases is not currently utilized for staging purposes, the 7th edition of the American Joint Commission of Cancer (AJCC) Staging Manual includes the classification of cM0(i+) disease which identifies patients with micrometastatic disease in either bone marrow or blood. However, these patients are still considered negative for metastatic disease [17]. Continuing studies assessing the presence of and characterizing DTCs and CTCs in patients with breast cancer may result in incorporation into staging models and provide valuable prognostic information.

Fewer patients with ILC received NACT compared to those with IDC in our series. This is not surprising as the role of NACT for patients with lobular histology has been debated due to lower rates of pCR [18-20]. Those with IDC were twice as likely to achieve a pCR with NACT compared to patients with ILC. Multiple studies have shown that the ability to achieve a pCR results in significantly improved DFS and OS [21-23]. Currently, our follow-up is too brief to determine if those with ILC who harbor micrometastatic disease have differences in DFS and OS in comparison to those with IDC. However, we are continuing to follow these patients to determine if tumor histology may result in survival differences in patients with micrometastatic disease.

Patients with ER positive disease tend to have delayed breast cancer recurrence, often later than 5 years following treatment [5-6]. In our study, patients with ILC were more likely to have ER positive disease (92.2% vs 66.2%, $P = 0.0001$), to harbor DTCs (43.4% vs. 28.9%, $P=0.03$), and to harbor either DTCs or CTCs (75.6% vs. 51.7%, $P = 0.002$). Since most late recurrences occur in those with ER positive disease, this raises the question of whether DTCs and CTCs are indeed responsible for late breast cancer recurrence. Studies which characterized DTCs have shown discordance with the primary tumor in up to 94% of patients [24-25]. Currently, we are assessing DTCs and CTCs for ER status to determine if there are discordance rates between these cells and the primary tumor which may explain delayed recurrence patterns following endocrine therapy in patients with ER positive disease.

In summary, patients with ILC were more likely to have ER positive disease, more likely to harbor micrometastatic disease, and less likely to attain a pCR following NACT. Further study with longer follow-up is warranted to determine if DTCs and CTCs play a role in late recurrences in patients with ER positive disease.

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Table I

Patient and Disease Characteristics

	n	Percentage
Total number	422	
Median age (years)	52	
TNM Stage		
I	136	32.2
II	176	41.7
III	110	26.1
T Stage		
T1	180	42.7
T2	147	34.8
T3	43	10.2
T4	52	12.3
Tumor Grade		
Low	56	13.3
Intermediate	186	44.1
High	179	42.4
Unknown	1	0.2

Table II

ER Status and Chemotherapy Status

	ILC	IDC	Total
ER Status			
Positive	59/64 (92.2%)	237/358 (66.2%)	239/422 (56.6%)
Negative	5/64 (7.8%)	121/358 (33.8%)	183/422 (43.4%)
Chemotherapy Status			
Neoadjuvant chemotherapy	14/64 (21.9%)	122/358 (34.1%)	136/422 (32.2%)
Chemonaive	50/64 (78.1%)	236/358 (65.9%)	286/422 (67.8%)

Table III

Rates of Micrometastasis

	ILC	IDC	Total	P value
DTCs	23/53 (43.4%)	81/298 (28.9%)	109/298 (36.6%)	0.03
CTCs	15/55 (27.3%)	75/310 (24.2%)	90/365 (24.7%)	0.33
DTCs or CTCs	34/45 (75.6%)	136/263 (51.7%)	170/281 (60.5%)	0.002