DNA Flow Cytometry Does Not Predict 5- or 10-Year Recurrence Rates for T1-2 Node-Negative Breast Cancer

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Background: A small proportion of T1 or T2 node-negative breast cancer tumors will recur in patients by 5 years, and more by 10 years. Results of recent studies have suggested improvement in overall survival with administration of adjuvant chemotherapy to all patients. More sensitive and specific methods are needed to identify patients at highest risk for recurrence who might benefit most from adjuvant therapy, saving others from unnecessary treatment. Some investigators have suggested DNA flow cytometry as a method to discriminate patients at greatest risk for recurrence.

Hypothesis: DNA flow cytometry has predictive value for breast cancer recurrence in node-negative patients.

Methods: The cancer registry of a medium-sized university-affiliated hospital was used to identify patients with T1-2 N0 M0 breast cancer treated with a uniform surgical approach and no adjuvant therapy who had completed at least 5 years of follow-up or had recurrence. Flow cytometric analysis was performed on paraffin-embedded specimens.

Results: Of 115 patients, 92 (80%) had disease-free survival without recurrence and 23 (20%) had recurrence. Comparison of diploid and nondiploid tumors for likelihood of recurrence revealed no association ($P = .79$). Furthermore, the DNA index and S-phase fraction were not significantly different between recurrent and nonrecurrent groups.

Conclusions: The likelihood of recurrence of small node-negative breast cancers after mastectomy cannot be accurately predicted on the basis of DNA flow cytometric analysis. Traditional methods for determining risks—such as nuclear and histological grade, lymph node status, and tumor size—seem to be more useful. Sentinel lymph node biopsy techniques may increase the detection of micrometastases.

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PATIENTS AND METHODS

PATIENT SELECTION

All patients treated for breast cancer at McLaren Regional Medical Center (MRMC), a medium-sized university-affiliated hospital in Flint, Mich, between January 1, 1982, and January 1, 1987, were eligible for inclusion in this study. Selection criteria were as follows: patients entered through the cancer registry had confirmed invasive breast cancers that were less than 5.0 cm (T1-2), lymph node negative (N0), and without metastases (M0). Surgical treatment was limited to modified radical mastectomies because breast-conserving operations were uncommon during that period. Patients with fewer than 5 axillary lymph nodes in the specimen were excluded. The study group could not, before recurrence, have received any form of adjuvant therapy (hormonal therapy, chemotherapy, or radiotherapy). Furthermore, all patients were required to have completed follow-up in the hospital's cancer registry to an end date: (1) 5-year disease-free survival, (2) survival at 3 years with recurrence, or (3) death from disease within 5 years. A further subset analysis was carried out on patients who completed 10 years of follow-up (or died between 3 and 10 years with known disease status at the time of death). One hundred fifteen patients met all criteria and completed 5- and 10-year follow-up, and 128 were excluded from final analysis. Reasons for exclusion from the final study group are as follows:

<table>
<thead>
<tr>
<th>Reason for Exclusion</th>
<th>Patients, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lost to follow-up &lt;5 y</td>
<td>17</td>
</tr>
<tr>
<td>Unknown tumor status at 5 y</td>
<td>1</td>
</tr>
<tr>
<td>No modified radical mastectomy</td>
<td>10</td>
</tr>
<tr>
<td>Received adjuvant therapy</td>
<td>6</td>
</tr>
<tr>
<td>Unknown tumor status at death</td>
<td>1</td>
</tr>
<tr>
<td>Unknown primary tumor size</td>
<td>11</td>
</tr>
<tr>
<td>Original paraffin blocks could not be found or were lost during retrieval or analysis</td>
<td>27</td>
</tr>
<tr>
<td>Had only ductal carcinoma in situ on independent pathological review</td>
<td>22</td>
</tr>
<tr>
<td>Had metastases at time of diagnosis</td>
<td>1</td>
</tr>
<tr>
<td>Had bilateral breast cancer during the study</td>
<td>4</td>
</tr>
<tr>
<td>Original biopsy was not done at MRMC</td>
<td>3</td>
</tr>
<tr>
<td>Unable to perform DNA analysis for technical reasons</td>
<td>24</td>
</tr>
<tr>
<td>Had male breast cancer</td>
<td>1</td>
</tr>
</tbody>
</table>

Only 24 patients were excluded for not having flow cytometry successfully performed, leaving 83% (115 of 139 patients) of the total eligible patient group to form the study group.

GENERAL METHODS

Patients were identified from the cancer registry, and their medical records were obtained. Operative notes and pathology reports were checked for indication of the presence of invasive cancer, size of tumor, and number of lymph nodes. Hormone receptor status was rarely ascertained during the early years of the study; thus, this information was not compiled.

Paraffin-embedded tumor specimens from the patients were reviewed by a pathologist at MRMC for selection of an appropriate sample. Only 27 patients were excluded for lack of paraffin specimens. The specimens, identified only by specimen number, were shipped in batches of 8 to 12 to United Hospital in St Paul, Minn, for further analysis.

The pathologist (S.M.) at United Hospital in charge of DNA flow cytometry was blinded to patient identity. Identifying specimens thereafter by number, he individually reviewed every specimen for the presence of invasive breast cancer, measured and recorded standard morphometric variables, and then conducted DNA flow cytometry. Each batch analysis took approximately 8 weeks to complete. Approximately 170 specimens underwent complete analysis, including those later dropped from the study for reasons listed in the “Patient Selection” subsection.

LABORATORY METHODS

Paraffin-embedded tumor specimens from each patient were prepared for flow analysis by modification of the method of Hedley et al. Three 50-µm sections were dewaxed in xylene, rehydrated in an ethanol gradient, and digested in 1 mL of 0.5% (wt/vol) pepsin solution (Sigma-Aldrich, St Louis, Mo) for 60 minutes at 37°C. The suspension was filtered, mixed with pepsin-neutralizing solution (Gibco, Grand Island, NY), and centrifuged at 300g for 10 minutes. The final suspension was washed in water and centrifuged and the pellet was processed for DNA (propidium iodide) staining with the DNA-Prep system (Beckman Coulter Inc, Hialeah, Fla), which provides a permeabilizer, propidium iodide, and RNase in a proprietary mixture. DNA content was measured on a flow cytometer (Epics Elite; Beckman Coulter Inc). At least 104 nuclei were collected for each histogram. Cell cycle histograms were constructed using a software program (Modfit; Verity Software House, Topsham, Me). DNA ploidy and S-phase fraction were assigned by conversion.

STATISTICAL ANALYSIS

To determine whether the variables of S-phase fraction, ploidy pattern, DNA index, pathological classification, Scarff-Bloom and Richardson (SBR) classification, modified SBR classification, and tumor size could be used to predict recurrence or overall survival, 3 separate logistic regressions were performed, with 5-year recurrence, 10-year recurrence, and 3-year overall survival as outcome variables. A receiver operating characteristic curve was created to compare sensitivity and specificity for 5-year survival from logistic regression analysis (Figure). This can compare multiple variables simultaneously. In addition, descriptive statistical methods were used, including t tests and chi² analysis.

RESULTS

Of 115 patients who met the study criteria, 92 (80%) experienced no recurrence and 23 (20%) had recurrence of their breast cancer during the first 5 years after surgery. This number of recurrences almost doubled (n=45 or 39%) by 10 years (Table 1). Pathology reports indicated that tumor size was similar in the 2 groups of patients (t test, P=.72). The mean ± SD size of tumors in patients whose disease did not recur during this time was 1.82 ± 0.97 cm, and patients with disease recurrence had tumors measuring 1.89 ± 0.78 cm.

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Tables 1, 2, and 3 summarize the differences between recurrence and nonrecurrence groups for each of the independent variables. Seventy-two (63%) of the total number of tumors were diploid and 43 (37%) were nondiploid. No statistical differences were noted in the distribution of diploid and nondiploid tumor cells between patients with and without recurrent disease (Table 1).

S-phase fraction was also determined in the tumors. No significant differences were found in these values between the groups whose disease recurred during the study period and those whose did not. Likewise, the DNA index was not significantly different between groups (Table 2). Finally, when the tumors were graded according to the SBR classification, no differences could be detected between the 2 groups. Tumors from all patients were evenly divided among the 3 SBR classes (Table 3).

To determine whether the variables in combination can predict outcomes, receiver operating characteristic curves were computed for outcome variables (Table 4 and the Figure). The curves demonstrated that the predictive power of the variables was extremely low.

The search for prognostic indicators in patients with node-negative breast cancer was more academic than practical until 1990, when the National Institutes of Health published the conclusions of a Consensus Development Conference. Based on 5 prospective, randomized trials (4 of chemotherapy and 1 of tamoxifen), there was a clear acknowledgment that “the majority of patients with node-negative breast cancer are cured by breast conservation treatment or total mastectomy and axillary dissection.” However, it was also stated that “the rate of local and distant recurrence is decreased by both adjuvant combination cytotoxic chemotherapy and adjuvant Tamoxifen.” With this statement, adjuvant systemic therapy for patients with node-negative breast cancer soon was seen as the standard of care by most medical oncologists.

Although it had been known that patients with node-negative breast cancer had a 10-year 30% recurrence rate, most surgeons before 1990 did not refer those patients to medical oncologists. Currently, adjuvant therapy is recommended for all patients with tumors measuring 1.0 cm or larger, even with negative nodes. To administer systemic therapy to 100% of node-negative patients to effect a small reduction in recurrence and mortality is a serious matter. Putting societal costs aside, there remain tremendous issues for patients and their families regarding toxic effects of such therapies and their concomitant effects on quality of life, including hair loss, lethargy, nausea, vomiting, pain, leukopenia with concomitant infections sometimes requiring hospitalization, sexual dysfunction, infertility, depression, anxiety, loss of work, separation from family and other daily activities, secondary cancers, and sometimes even death.
Framed in this manner, the search for prognostic indicators for women with node-negative breast cancer is a compelling one. Two questions need to be asked: (1) Does a given prognostic indicator predict recurrence? and (2) Does a given prognostic indicator predict response to systemic therapy? Although these might seem to be different versions of the same question, little is known about the biologic features of breast cancer and its treatment that these questions should be addressed separately. This article seeks to answer only the first.

Many groups have studied various objective tumor characteristics and correlated those with disease-free survival or overall survival in an effort to identify patients with lymph node–negative breast cancer at greatest risk for recurrence. The theoretical value of this information is that these high-risk patients could be offered neoadjuvant therapies to improve their prognosis, sparing those least likely to benefit from the need for additional treatment.

The wide range of patient and tumor characteristics examined include those definitely shown to be associated with prognosis (such as tumor size and lymph node status)\(^\text{31,14}\) and those definitely shown not to be associated with prognosis (such as tumor location and menopausal status).\(^\text{15,16}\) A host of other characteristics may be related to outcome, such as histological grade, nuclear grade, SBR class and its modifications, mitotic activity index, HER-2/neu onconeue, cathepsin-D, angiogenesis, and mutant p53.\(^\text{17,20}\)

DNA flow cytometry recently has been studied also as a prognosticator for node-negative breast cancer. DNA flow cytometric measurements of ploidy and S-phase fractions were initially received as the best discriminators of node-negative breast cancer into good or poor prognostic categories.\(^\text{21-24}\) By 1989 and 1990, a few studies began surfacing that suggested that diploid tumors with low S-phase fractions had a better prognosis than those with high S-phase fractions (90% compared with 70% disease-free survival at 5 years).\(^\text{22,25-29}\) As in so many other areas of medicine, industry rose to the occasion and promoted the technology.

However, between 1991 and 1993, several studies questioned the value of these measurements.\(^\text{30,31}\) Bosari et al,\(^\text{31}\) in patients who underwent mastectomy, found that ploidy status was not a useful prognostic factor and that S-phase fraction, although related to recurrence of disease, did not remain a prognostic determinant after multivariate analysis of various histopathologic variables. Stanton and coworkers\(^\text{32}\) found neither factor to correlate with survival in a multivariate analysis. Batsakis et al\(^\text{33}\) concluded the same, although it is unclear from their study whether patients were controlled for initial surgical treatment or application of neoadjuvant therapy.

Stal and colleagues\(^\text{34}\) evaluated patients with stage I breast cancer with T1 tumors and concluded that S-phase fraction was associated with distant recurrence and survival but that DNA ploidy was not. That study controlled for adjuvant therapy like the present study, but patients were not limited to mastectomy only.\(^\text{34}\) Meyer and Province\(^\text{35}\) also showed that lymph node status and tumor size predict long-term survival and that nuclear size was the strongest independent predictor in node-negative patients, but they found no evidence for the independent prognostic value of DNA ploidy or S-phase fraction. Their patient population differed from ours in that mastectomy and breast-conserving surgeries were included, and some node-negative patients received adjuvant therapy.\(^\text{35}\)

In 1993, Witzig et al\(^\text{36}\) found no association between ploidy and overall or disease-free survival, but using a cutoff value of 12.3 for S-phase fraction suggested a separation for overall and disease-free survival. When S-phase fraction was adjusted for clinical factors in the multivariate analysis, however, it was not significant.\(^\text{36}\) More recently, Camplejohn et al,\(^\text{37}\) in a study that included patients receiving adjuvant therapy, indicated that S-phase fraction was a significant prognostic marker for overall and disease-free survival.

The present study is similar to that of Stal et al\(^\text{34}\) except that we controlled for surgical treatments (mastectomies only). Our finding that neither ploidy nor S-phase fraction contributes significant prognostic information to overall or disease-free survival agrees with the conclusions reached by Meyer and Province\(^\text{35}\) but contrasts with the findings of Camplejohn et al,\(^\text{37}\) suggesting an independent role for S-phase fraction in stage I breast cancer. This might be because of the effect of patients who received perioperative adjuvant chemotherapy in the latter study. In a patient population whose treatment was as carefully controlled as ours, Witzig’s group\(^\text{36}\) reached conclusions similar to ours.

In an analysis of essentially all the known possible risk factors for recurrence of breast cancer, Wood\(^\text{38}\) summarizes the problem as follows: a woman younger than 50 years with a tumor less than 1 cm has a 6% chance of recurrence. With systemic chemotherapy, we would expect a 30% reduction in risk of recurrence (2% overall). For dropping this recurrence rate to 4%, the cost in many trials would be 1% mortality, representing a net gain of 1%. Giuliano and colleagues\(^\text{39}\) looked at predictors of axillary lymph node metastasis in T1 carcinomas of the breast and found that tumor size was the only accurate predictor. Although they did not specifically look at recurrence in their study, using the known likelihood of recurrence when lymph nodes are involved, they analyzed various factors for prediction of lymphatic involvement—neither ploidy nor S phase was statistically reliable in such a prediction (\(P > .99\) and \(P = .29\), respectively).\(^\text{39}\)

Using definitions of S phase greater than 5% and DNA index greater than 2 being high, in 1997 Velanovich\(^\text{40}\) found high S-phase fraction to be the only independent predictor for onologists administering chemotherapy, but he was not specifically looking at recurrence of breast cancer. Continuing the theme on prediction of lymphatic involvement, Barth and colleagues\(^\text{41}\) published their results in 1997 for the prediction of lymph node involvement and also found no statistically significant association with ploidy status or percentage of S phase. Two recent articles\(^\text{42,43}\) have addressed the impact of axillary lymph node dissection on recommendations for therapy, the costs of axillary lymph node dissection, lack of S-phase fraction or DNA index being able to predict axillary lymph node involvement (and hence be a reliable tool to use instead of axillary lymph node dissection), and the possible advantages of sentinel lymph node biopsy.
A prognostic factor can be useful only if it provides clear and compelling evidence of better or worse prognosis. The preponderance of the available research, including the present study, suggests that ploidy status, DNA index, and S-phase fraction cannot be used to predict recurrence of small (T1 or T2) node-negative breast cancers. Furthermore, it seems that DNA analysis has been most commonly used to justify adjuvant therapy of T1a,b N0 breast cancer, not to eliminate adjuvant therapy for some T1c and T2 N0 breast cancers. Therefore, most treatment decisions are made without DNA analysis. These findings significantly limit the clinical utility of DNA analysis for early breast cancer. Certainly, sentinel node biopsy promises to more accurately determine which patients are truly node positive or node negative, and this will likely affect adjuvant therapy decisions.

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REFERENCES
