DNA Flow Cytometry in Early Breast Cancer: A Step in the Right Direction

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Dramatic changes occurred in the management of patients with primary breast cancer (stages I–IIIa) after physicians recognized that many such patients already had established, clinically occult micrometastases by the time of initial diagnosis. Trials of adjuvant, systemic, cytotoxic chemotherapy or endocrine therapy were initiated by investigators in an attempt to eradicate these micrometastases and improve survival. Long-term results of many of these trials are now available, and they conclusively demonstrate a moderate therapeutic advantage for the addition of systemic therapy compared with that for local therapy alone (1). In patients with positive axillary lymph nodes, who have a relatively high rate of relapse, approximately one-third of the early deaths in premenopausal patients can be avoided or delayed by optimal combination chemotherapy. Similarly, about one-fifth of early deaths of postmenopausal patients are avoided or delayed by the administration of tamoxifen. Although follow-up time is much shorter, a similar pattern of results is now emerging from studies in patients with negative axillary lymph nodes (2–5).

However, these node-negative patients present a unique challenge. Although adjuvant therapy may benefit some of them, most (70%) survive disease-free for 10 years after local therapy alone; they stand to suffer the costs, morbidity, and possible mortality related to systemic treatment with no possible benefit. Furthermore, the absolute benefit with adjuvant therapy is only modest, and many patients fail to benefit despite optimal therapy. These considerations underscore our need to develop methods of identifying those patients who (a) have a high risk for distant relapse, (b) will be cured by local modalities, and (c) will or will not respond well to a systemic therapy.

Data are accumulating that indicate assays of the proliferative rate of a patient’s tumor cells may help to resolve these therapeutic dilemmas. Both laboratory and clinical experience demonstrate that rapidly proliferating tumors, such as acute leukemia, high grade lymphomas, and germ cell cancers, are generally more responsive to chemotherapy. It would not be surprising if this same pattern applied to human breast cancer, a heterogeneous neoplasm that can be rapidly proliferating in some patients and unusually indolent in others. A study published 10 years ago (6) indeed demonstrated a relationship between the percentage of tumor cells in the DNA synthesis or S phase of the cell cycle [determined by the [3H]thymidine labeling index (TLI)] and responsiveness of metastatic breast cancer to chemotherapy; none of the nine patients with a TLI less than 9% responded to chemotherapy, whereas 11 of 16 with a TLI greater than 9% did respond. Similar reasoning suggests that slowly proliferating tumors or those with a normal DNA content are more responsive to endocrine therapy (7).

Other data indicate the potential usefulness of proliferative indices in predicting the rate of recurrence and survival. Intuitively, patients whose tumors have a high proliferative rate might have more rapid recurrence and poorer survival. Several researchers (8,9) now suggest that proliferative activity, whether determined by the TLI or by calculating the S-phase fraction (SPF) by DNA flow cytometry, has prognostic importance for patients with early breast cancer. SPF and DNA content (ploidy status) may be the most powerful prognostic factors yet identified in node-negative breast cancer. On the one hand, patients with tumors with a high proliferative rate may have relatively poor prognoses with local therapy alone; on the other, they may be the subset most likely to benefit from adjuvant chemotherapy.

Elsewhere in this issue, Remvikos et al. present additional data on the potential role of flow cytometry in early breast cancer. They demonstrate a relationship between SPF determined on fine needle samples by flow cytometry and response to chemotherapy in a group of patients with stages II–IIIa breast cancer treated by neoadjuvant chemotherapy. Tumors with a low SPF, arbitrarily defined as less than 5%, had a response rate of 46% (six of 13, one complete response). Those tumors with an SPF between 5% and 10% had an intermediate response rate of 84% (21 of 25, six complete responses). All 12 patients with an SPF of 10% or more responded (six complete responses). Although patients achieving complete response had a higher median tumor SPF of 9.7%, a wide range of values with considerable overlap with the nonresponding groups was found; seven of 13 patients achieving complete response had an SPF of less than 10%, one as low as 4%. Possible relationships between chemotherapy response and other variables including DNA ploidy, tumor histologic grade, and estrogen receptor status were also explored in this study. The authors concluded that none of these parameters correlated significantly with response, although important trends that are clearly evident would likely have reached statistical significance in a larger study. Whereas this study would have provided more interpretable information with a larger sample size and the inclusion of additional patient variables, such as tumor diameter,
nevertheless, the relationship between SPF and chemotherapy response is convincing.

A major question remains. Is the information obtained by DNA flow cytometry useful to physicians today to individualize their treatment decisions for patients with early breast cancer? Although a growing body of evidence supports the value of flow cytometry as a prognostic factor for recurrence and survival that may be clinically useful today, data on its use to predict tumor sensitivity to chemotherapy are still relatively meager. The report by Remvikos et al. is a step in the right direction, but their results are inconclusive and raise additional questions. Most important, despite the statistically significant correlations, the differences observed were quantitative, not qualitative, and their use of SPF failed to identify a totally chemotherapy-resistant subset. In fact, the least responsive subset still had a response rate of nearly 50%, and it would be difficult for physicians to withhold chemotherapy on this basis alone. Combinations of factors, such as SPF, ploidy status, receptor status, and histologic variables, may help us to identify more accurately chemotherapy-sensitive or -resistant groups, but a larger prospective trial is necessary.

Furthermore, the Remvikos study correlated SPFs of the primary breast tumor with response of the primary tumor to neoadjuvant chemotherapy. However, because the primary tumor can easily be removed surgically, the target cells of interest are the occult micrometastases that are the real determinants of survival. Micrometastases may well have different cell-cycle kinetics, especially after removal of the primary neoplasm; thus, SPFs of the primary tumor may or may not predict its chemosensitivity at these distant sites. Additional prospective studies correlating SPF with disease-free and overall survival benefit in patients receiving adjuvant chemotherapy are needed if investigators are to confirm the provocative data from Milan. The results of this study (2) suggest that the major impact of adjuvant chemotherapy in delaying recurrence in node-negative breast cancer occurs in patients with tumors displaying a high TLI, whereas there is little benefit in those whose tumors have a low TLI. Trials are now in progress that will answer this question more definitively.

Finally, standardization of DNA flow cytometry methodology and internal as well as external quality control are critical issues that need to be addressed before this technology can be used routinely on a wide scale. Only a handful of researchers have published clinical correlations demonstrating the significance of the ploidy and SPF values generated by their equipment and analytical methods. Although ploidy status on most breast cancer specimens can be obtained relatively easily and should be reproducible in different laboratories most of the time, calculation of SPF is much more difficult. Even under optimal conditions determination of SPF is not possible in up to 20% or more of the patients. Furthermore, SPF calculations vary depending on the instrument selected, assay methodology, and, most critically, on the computer analysis program used. Until these factors are standardized, an SPF value generated in one laboratory may not be equivalent to that obtained in another, despite the fact that both may correlate with the clinical variable of interest. Thus although the present study of DNA flow cytometry is a step in the right direction, much work remains to be done before the full potential of this new technology will be available to the patients.

References