Traditional and Newer Pathologic Factors

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There has long been a pressing clinical need to identify prognostic and predictive factors for patients with breast cancer. Although numerous candidate biological and molecular markers have been identified during the last two decades, traditional factors such as lymph node status, tumor size, histologic type, histologic grade, and hormone receptor status remain the most useful indicators of prognosis and therapeutic response. A major obstacle to the translation of research advances into clinically useful prognostic and predictive markers has been the considerable methodologic variability used in the evaluation of the newer markers. It is now generally accepted that, to be useful in patient management, a putative prognostic or predictive marker must have clinical importance, independence, significance, and standardization with regard to methods, interpretation, and reporting. It is hoped that recognition and adoption of these criteria will serve to clarify the value of newer biologic and molecular markers. [J Natl Cancer Inst Monogr 2001;30:22–6]

The identification of new biologic and molecular indicators of clinical outcome and response to therapy in patients with breast cancer has been an area of active investigation during the last two decades. Unfortunately, studies of these new markers have often yielded contradictory results and clinical confusion. Furthermore, even the results of studies of the same marker are often difficult to compare because of differences in treatment, study design, patient selection, methodology, and statistical analysis. In fact, despite intensive efforts to identify new prognostic and predictive factors, traditional pathologic factors such as lymph node status, tumor size, histologic type, histologic grade, and hormone receptor status remain the most useful indicators of prognosis in patients with breast cancer (1–6).

LYMPH NODE STATUS

Uniform agreement exists that the status of the axillary lymph nodes is the single most important prognostic factor for patients with breast cancer and that disease-free survival and overall survival decrease as the number of positive lymph nodes increases. Nevertheless, a number of important issues regarding axillary lymph node evaluation need to be addressed. First, methods for the pathologic examination of these lymph nodes are not standardized. For example, although some pathologists submit grossly uninvolved lymph nodes in their entirety for histologic examination, others subject only a single section from such lymph nodes to microscopic scrutiny (6). This difference in method could result in the misclassification of some lymph node-negative patients as lymph node negative. In addition, although sentinel lymph node biopsy is now widely used to evaluate the status of the axilla, methods for examination of sentinel lymph nodes are also highly variable. Another unanswered clinical question concerns the significance of axillary lymph node micrometastases, particularly those identified exclusively by the use of immunohistochemistry. Approximately 10%–20% of patients considered to be lymph node negative by conventional examination of the axillary lymph nodes are found to have identifiable tumor cells in these lymph nodes when these lymph nodes are examined by serial sectioning, by immunohistochemical staining, or by both methods. Studies that have sought to evaluate the significance of axillary micrometastases have differed with regard to patient population, treatment, methods to detect tumor cells, and length of follow-up. Virtually all studies with more than 100 patients have shown that the presence of micrometastases detected by serial sectioning, immunohistochemistry, or both methods is associated with a small but significant decrease in disease-free survival, overall survival, or both (7,8). However, most of these studies have been retrospective and were not initially designed to address this question. Furthermore, in some of these studies, it is not clear whether the prognostic significance of micrometastases is independent of other factors such as tumor size or lymphatic vessel invasion. The clinical significance of axillary lymph node micrometastases detected by immunohistochemistry is currently being evaluated in a number of randomized clinical trials, and it is likely that these trials will provide important information about this issue. Until then, most experts agree that it is premature to recommend the routine use of immunohistochemistry to evaluate either sentinel or nonsentinel lymph nodes (9).

TUMOR SIZE

After lymph node status, tumor size is the most important prognostic factor for patients with breast cancer. Even among patients with breast cancers 1 cm and smaller (T1a and T1b), size represents an important prognostic factor for axillary lymph node involvement and outcome (10). It should be noted, however, that the manner in which the pathologic tumor size is reported has not yet been standardized. Some pathologists report the size of the macroscopically identified tumor, some report a microscopic size that includes both the invasive and in situ components, and still others report the microscopic size of the invasive component only. Prior studies have shown that, particularly for small breast cancers, a poor correlation often exists between the tumor size determined by gross pathologic examination and the size of the tumor’s invasive component as determined by measurement from the histologic sections (11). Moreover, some studies suggest that the size of the invasive component is the most clinically significant determinant of outcome. This was recently recognized in the fifth edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (12), which states that “the pathologic tumor size for classification (T) is a measurement of only the invasive component.” Therefore, in the case of a discrepancy between the gross tumor size and the...
microscopic size of the invasive component of small breast cancers, the microscopic size should take precedence and the microscopic size of the invasive component of the tumor should be indicated in the pathology report and used for pathologic staging.

**Histologic Type**

Some histologic types of breast cancer are associated with a particularly favorable clinical outcome (13,14). Special-type tumors that consistently have been shown to have an excellent prognosis include tubular, mucinous, adenoid cystic, and invasive cribriform carcinomas. Some authors also place tubulolobular carcinomas and papillary carcinomas in this group. Moreover, Rosen et al. (14) have shown that the 20-year recurrence-free survival of special-type tumors 1.1–3.0 cm in size is similar to that of invasive ductal carcinomas both 1 cm and smaller (87% and 86%, respectively). However, strict diagnostic criteria must be employed in order to observe the favorable outcome reported for these lesions.

**Histologic Grade**

The importance of tumor grading as a prognostic factor in patients with breast cancer has been clearly demonstrated in numerous clinical outcome studies. These studies (1–6,15–24) have repeatedly shown higher rates of distant metastasis and poorer survival in patients with higher grade (poorly differentiated) tumors, independent of lymph node status and tumor size. In fact, tumor grading has been shown to be of prognostic value even in patients with breast cancers 1 cm and smaller (10). Although a variety of nuclear and histologic grading methods have been used in these studies, the grading method used most often at present is the histologic grading system of Elston and Ellis (21). These authors advocate the use of histologic grading for all types of invasive breast cancer; they acknowledge, however, that histologic grade partially defines some of these histologic types (e.g., tubular carcinomas are by definition grade 1 and medullary carcinomas are grade 3 lesions). However, these authors have also pointed out that the combination of histologic type and grade provides a more accurate assessment of prognosis than does histologic type alone (25).

Some studies have suggested that histologic grade may provide useful information with regard to response to chemotherapy and may, therefore, be of value as a predictive factor in addition to its role as a prognostic indicator. The results of several studies suggest that the presence of high histologic grade is associated with a better response to certain chemotherapy regimens than is the presence of low histologic grade (26,27). However, additional studies are required to define more clearly the relationship between histologic grade and response to chemotherapy.

A frequent criticism of the use of histologic grading is that this assessment is subjective and, as a consequence, prone to considerable interobserver variability (28–30). Most of the studies that have suggested this used grading systems lacking precisely defined criteria or did not attempt to educate the participating pathologists in the use of the system evaluated. Recent studies have indicated that the use of strict criteria and guidelines for histologic grading can result in acceptable levels of interobserver agreement and also identify areas that might benefit from refinement. In one of these studies (31), six pathologists each graded 75 invasive ductal carcinomas using the Elston and Ellis grading system. Moderate to substantial agreement was found for the overall histologic grade. There was substantial agreement with regard to tubule formation, moderate agreement with regard to mitotic count, and near moderate agreement for nuclear pleomorphism as determined by generalized $k$ statistics. The authors (31) concluded that this grading system is suitable for use in clinical practice and suggested that efforts to improve agreement on nuclear grading would be of value in further fostering agreement in histologic grading. In another study (32), a substantial level of agreement ($k$ statistic, 0.70) was found among 25 pathologists who used the Elston and Ellis grading system, albeit in a small number of cases.

**Lymphatic Vessel Invasion**

Lymphatic vessel invasion has been shown in numerous studies to be an important and independent prognostic factor. Its major clinical value at this time is as an aid in identifying lymph node-negative patients at increased risk for axillary lymph node involvement (34–41) and adverse outcome (19,36,37,42,43). The identification of lymphatic vessel invasion may be of particular importance in patients with T1, lymph node-negative breast cancers, since this finding may permit the identification of a subset of patients at increased risk for axillary lymph node involvement and distant metastasis. For example, in one study (10), lymphatic vessel invasion was the only clinical or pathologic factor associated with lymph node metastasis in patients with tumors 1 cm and smaller (T1a and T1b). In that study (10), lymph node involvement was present in four of seven patients whose tumors showed lymphatic vessel invasion (57%), compared with only one of 100 patients without lymphatic vessel invasion. In another study of 461 patients with T1, lymph node-negative breast cancer (14), patients with tumors lacking lymphatic vessel invasion had a 20-year overall survival rate of 81%, compared with 64% overall survival rate for those whose tumors exhibited lymphatic vessel invasion. Similar findings have been reported by others (43–46), even when the analysis was restricted to the subset of T1 breast cancers that were 1 cm and smaller (43,44).

As with histologic grade, the ability of pathologists to reproducibly identify lymphatic vessel invasion has been challenged. For example, in one study (47), three pathologists concurred on the presence or absence of lymphatic vessel invasion in only 12 of 35 cases. However, a higher level of interobserver agreement has been noted in other studies (34–37,46). In one of these studies in which stringent criteria were employed (34), an 85% level of overall agreement between two pathologists was found for the presence or absence of lymphatic vessel invasion. The use of strict criteria for the identification of lymphatic vessel invasion is, therefore, imperative.

**Hormone Receptor Status**

The presence of steroid hormone receptors (estrogen receptor [ER] and progesterone receptor [PR]) represents a relatively weak prognostic factor for patients with breast cancer, but these receptors are the strongest predictive factors for response to hormonal therapy (1–6). In recent years, immunohistochemical staining has replaced the ligand-binding biochemical assay for assessment of ER and PR status. In fact, the immunohistochemical method is easier to perform and has been shown to be equal to or better than the biochemical assay in predicting the response to adjuvant endocrine therapy (48). However, issues related to
the standardization of methodology, interpretation, and reporting remain to be resolved (6).

**Newer Factors**

Numerous biologic and molecular markers have been reported to have prognostic or predictive value (or both) in patients with breast cancer. These markers include apoptosis, oncogenes, suppressor genes, proteases, adhesion molecules, angiogenesis, proliferative rate, and DNA content (ploidy) (1–3, 6). Some of the factors initially reported to be significant independent prognostic markers subsequently have been shown to have little or no independent prognostic value (e.g., ploidy and cathepsin D). Among most of the other reported factors, variations in study design, methodology, and statistical analysis have led to conflicting and contradictory data (49). A comprehensive review of biologic and molecular prognostic and predictive factors is beyond the scope of this presentation. However, a few of these factors merit specific comment.

**HER2/neu**

HER2/neu is reviewed in references (50, 51). In 1987, Slamon et al. (52) first reported that amplification of the HER2 gene was an important prognostic factor for patients with breast cancer. Since this initial report, many studies have attempted to assess the prognostic significance and predictive value of HER2 gene amplification or protein overexpression. The role of HER2 as a predictive factor is discussed elsewhere. With regard to HER2 as a prognostic factor, amplification or overexpression (or both) of this gene has been associated with an adverse clinical outcome in most studies of lymph node-positive patients. Its role as an independent prognostic marker in lymph node-negative patients, however, remains an unresolved issue. Substantial methodologic variability has made it difficult to reconcile the results of these studies.

**p53**

Mutations in the p53 tumor suppressor gene or an accumulation of p53 protein has been reported in approximately 20%–50% of human breast cancers. These phenomena are more often seen in patients with familial/hereditary breast cancer syndromes (such as the familial breast and ovarian cancer and Li–Fraumeni syndromes) than in those with sporadic breast cancer. Recent immunohistochemical studies suggest that p53 protein accumulation is associated with several other adverse prognostic factors such as high tumor grade, high proliferative rate, and ER and PR negativity. The results of several follow-up studies (1, 53–57) suggest that p53 may be an independent predictor of decreased disease-free and overall survival in both lymph node-positive and lymph node-negative patients. However, not all studies have found p53 expression to be a significant, independent prognostic factor (1, 58, 59). Furthermore, not all cases in which p53 expression is detected by immunohistochemistry show p53 mutations (60). Aside from their potential value as a prognostic factor, p53 mutations may be associated with drug or radiation resistance, or both, and may, therefore, be a predictive factor as well (61).

**Angiogenesis**

A number of studies (1, 62–65) have reported an association between the density of microvessels in the tumor stroma (as detected by immunohistochemical stains for endothelial cells, such as factor VIII-related antigen, CD34, or CD31) and prognosis in patients with breast cancer. These studies have indicated that tumors with numerous microvessels are associated with a poor prognosis. Although these studies also have shown that high microvessel density is associated with larger tumor size, poor tumor differentiation, and lymph node-positive status, the prognostic information provided by microvessel density appears to be independent of these other factors. However, a significant association between high microvessel density and poor prognosis has not been observed in all studies evaluating this relationship (66, 67). Regardless of its potential role as a prognostic factor, assessment of microvessel density may ultimately be a useful marker for tumors that might respond to anti-angiogenic therapy (1).

**Current Status of Pathologic Factors**

Tumor size, histologic type, nuclear grade, proliferative rate, and hormone receptors were considered to be the major useful prognostic factors in patients with lymph node-negative breast cancer at the 1990 National Institutes of Health Consensus Development Conference on the Treatment of Early-Stage Breast Cancer (4). At the 1998 St. Gallen Conference on Adjuvant Therapy of Primary Breast Cancer, lymph node status was recognized as the most important breast cancer prognostic factor. Among patients with lymph node-negative disease, tumor size, histologic or nuclear grade, hormone receptor status, and lymphatic vessel invasion were considered to be the most relevant prognostic factors (5). Most recently, at a 1999 Consensus Conference held under the auspices of the College of American Pathologists (CAP) (6), a multidisciplinary group of pathologists, clinicians, and statisticians reviewed prognostic and predictive factors of breast cancer and categorized them into three groups based on the strength of the published data:

- **Category I:** Well supported by the literature. Generally used in patient management (size, lymph node status, histologic type, histologic grade, mitotic figure count, and hormone receptor status).
- **Category II:** Extensively studied biologically, clinically, or both; tested in clinical trials: Biological and correlative studies were done, as were a few clinical outcome studies (HER2, p53, lymphatic vessel invasion, and other proliferation markers such as MIB-1).
- **Category III:** Currently does not meet criteria for category I or category II (ploidy, cathepsin D, angiogenesis, and others).

Of note, the factors considered most relevant at the 1999 CAP Consensus Conference (category I) are virtually identical to those considered most important at the 1990 National Institutes of Health Consensus Development Conference. The fact that little has changed in this area during the last 10 years serves to emphasize two points: 1) that traditional prognostic and predictive pathologic factors are clinically valuable; and 2) that, despite dramatic advances in our understanding of the molecular biology of breast cancer during the last decade, it is difficult to translate research advances into prognostic and predictive markers that are useful in clinical management. In large part, this is a result of the considerable methodologic variability used in evaluating these newer factors.

The AJCC and the International Union Against Cancer have developed criteria to assess the value of putative prognostic and predictive factors (49). The criteria include clinical importance...
(the factor is a powerful predictor that can be used in patient management), independence (the factor retains its prognostic or predictive value when other factors are combined with it), and significance (the prognostic or predictive accuracy of the factor rarely occurs by chance). In addition, there must be standardization with regard to methods, interpretation, and reporting. It is hoped that the recognition and widespread adoption of these criteria will result in greater clarity of the value of the newer putative prognostic and predictive factors.

Finally, although the evaluation of individual prognostic and predictive factors has value, a pressing clinical need exists to develop a comprehensive profile of the biologic and molecular characteristics of tumors that may aid in the assessment of prognosis and the prediction of response to various therapeutic modalities. The tools of modern molecular biology, such as microarray technology, may ultimately provide such an assessment by permitting high through-put and parallel analysis of hundreds or thousands of parameters (68).

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**NOTE**

Dr. Schnitt is a member of the Genentech HER2 Advisory Board.