Tumor-Related Prognostic Factors for Breast Cancer

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Introduction

The number of tumor-related features available to predict the prognosis of patients with breast cancer has grown impressively in recent years. Histology, tumor stage, and lymph-node status are now supplemented with measurements of steroid hormone receptors, ploidy, S-phase fractions, growth factors, oncogenes, and oncoprotein products. Cellular and molecular biology have not only advanced the understanding of carcinogenesis, but have provided a host of new biologic measures potentially related to clinical outcome.

Interest in prognostic factors has been stimulated by the success of systemic adjuvant therapy for early-stage, operable cancer of the breast. Any feature of a tumor, or combination of features, that accurately indicates which patients are destined for recurrence and which are not is of considerable importance. Patients destined for recurrence can be selected for systemic adjuvant therapy, while patients who will not have a recurrence can be spared the morbidity of a treatment that offers no benefit. In addition, refinement of prognostic information facilitates improved clinical testing by ensuring comparability of treatment groups and providing markers to measure the success or failure of specific therapies.

The literature devoted to prognostic factors for breast cancer is extensive. Scientific reports are supplemented by a multitude of letters, reviews, and meta-analyses. Univariate and multivariate analyses are basic techniques. Variables are individually compared with measures of outcome, and those that are significantly related to outcome are used in multivariate analyses to determine if they have independent predictive value. These are then combined to form new prognostic categories. The mix of individual variables changes, however, and interrelationships are not always consistent.

Measures of outcome are multiple, and relationships to outcome are subject to change with duration of follow-up. Confirmation of projected outcomes with prospective studies is largely lacking for newer variables. The complexities are such that computer models are needed for integration of information. Computerized neural networks that are designed to learn from new data and predict individual patient outcome are under development to assist clinicians in making decisions about clinical management. The purpose of this article is to review tumor-related biologic factors of current interest and relate them to prognosis and treatment objectives.

Histologic Type

Ductal Carcinoma in Situ

Ductal carcinoma in situ (DCIS) represents a small, but important, group of preinvasive breast cancers that can almost always be cured by local-regional therapy. DCIS made up 6.3 percent of 169,260 carcinomas (4.7 percent if lobular carcinoma in situ is excluded) reported to the National Cancer Institute’s Surveillance, Epidemiology, and End Results
(SEER) national registry between 1973-1987. Only one percent of these early cancers are associated with metastasis to axillary nodes, and almost all (98 percent) are cured by local-regional therapy regardless of their size. Local recurrence, particularly after breast-conserving therapy, is associated with poor nuclear grade and comedo-type necrosis, but because dissemination is infrequent, systemic adjuvant therapy is unnecessary.

MICROINVASIVE CARCINOMA

Invasive carcinoma carries with it the clear potential for metastasis and a diminished opportunity for cure. The term “microinvasive” is loosely used to describe the process of invasion in its earliest beginnings and to suggest that a tumor is still highly curable, possibly as curable as noninvasive carcinoma. No consensus exists on the definition of such tumors, and no consensus exists for the prognosis, other than that it is favorable. Distinguishing among in situ cancers, cancers with minimal signs of invasion, and those with a few millimeters of invasion is often difficult, and judgments among pathologists are not uniform. As a group, invasive carcinomas 5 mm or less in diameter have widely divergent rates of metastasis to regional lymph nodes, ranging from three to 28 percent in various reports. These rates are far higher than for noninvasive carcinomas and imply a poorer prognosis.

SPECIAL HISTOLOGIC TYPES OF INVASIVE CARCINOMA

A few special histologic types of invasive carcinoma pose a smaller risk of dissemination and death than other types of invasive ductal carcinoma. These types are pure mucinous, pure tubular, pure medullary, and pure papillary carcinoma. Mucinous carcinomas tend to occur later in life than the other three types. Typical medullary carcinoma is noteworthy because of the disparity between its aggressive histologic appearance and its less aggressive clinical behavior. However, it may be more aggressive than the other types mentioned.

These special types form a small group, representing less than six percent of all invasive carcinomas. Most special types are localized when diagnosed. When nodes are involved, the number of involved nodes is usually three or less. In a large study, Rosen et al found that patients with special histologic types measuring 1.0 cm or less in diameter had a 10-year recurrence-free survival of 100 percent. The 10-year survival for all patients with tumors measuring 3.0 cm or less was 91 percent. Five-year relative survivals (survival corrected for normal mortality) of patients in the SEER registry treated for each of these special forms of invasive carcinoma are about 95 percent compared with 80 percent for all other types of invasive ductal carcinoma.

Histologic and Nuclear Grade

A number of additional histologic features of invasive ductal and lobular carcinomas have prognostic value when considered in isolation. They include histologic grade, nuclear grade, tumor borders as stellate or circumscribed, peritumoral lymphatic and blood vessel invasion, and necrosis within the tumor. Many of these lose relevance in multivariate analyses.

Particular attention has been given to tumor differentiation as a morphologic indicator of tumor aggressiveness. Grading of differentiation can be performed without special equipment and is to some degree independent of axillary node status.

Histologic grade is currently based on the degree of tubule formation, number of mitoses, and nuclear pleomorphism in routine sections. These are combined as the Bloom-Richardson (B-R) grade or Scarff-Bloom-Richardson grade. Grades from 1 to 3 indicate progression.
from well differentiated (low or good grade) to poorly differentiated (high or poor grade). In general, well-differentiated tumors are a distinct minority. Histologic grade is found to increase with tumor size and with advancing anatomic stage. Histologic and nuclear grade are subordinate to node status and tumor size as prognostic features, but both are significant predictors of overall mortality for node-positive and node-negative patients. In a multivariate analysis by Fisher et al, neither histologic grade nor any of nine additional pathologic variables related univariately to prognosis had independent predictive value for 15-year survival of 620 stage I and II patients treated with radical mastectomy after the number of positive axillary nodes, tumor size, and nipple involvement were taken into account. In a similar analysis of 859 cases, Shek and Godolphin found that histologic type and degree of histologic differentiation had no incremental value for predicting death rates after the number of involved axillary nodes, tumor size, and nipple involvement were taken into account. In a similar analysis of 859 cases, Shek and Godolphin found that histologic type and degree of histologic differentiation had no incremental value for predicting death rates after the number of involved axillary nodes, tumor size, and nipple involvement were taken into account.

Histologic grade was second only to tumor diameter as a predictor of disease-free survival among aneuploid tumors according to Winchester et al. These investigators reported a disease-free survival of 100 percent for a small group of node-negative patients with nuclear grade 1 tumors or with histologic grade 1 aneuploid tumors.

An estimate of tumor grade is often included in a pathology report, but despite the clear influence of histologic and nuclear grading on prognosis and their ready availability, problems with tumor heterogeneity and with interobserver inconsistency continue to generate uncertainty about their role as routine prognostic indicators.

Tumor Angiogenesis

Tumor angiogenesis as a prognostic assay is predicated on the evidence that ingrowth of blood vessels is a necessity for sustained tumor growth and metastasis. Interest in neovascularity as a prognostic factor was stimulated by the work of Judah Folkman on tumor angiogenesis and by the potential for treatment with antiangiogenic agents. The prognostic relevance of tumor angiogenesis was first reported by Weidner et al, who counted microvessels (veins and arteries) in the most densely vascularized areas of 49 invasive carcinomas and found their number and density significantly increased in
cases with nodal and distant metastasis. The frequency of distant metastasis increased with increase in the vessel count.

Subsequently, other investigators have produced varying results. In a carefully performed, blinded study of 220 cases of invasive breast carcinoma, Axelsson et al found considerable variability in microvessel counts in different parts of the same tumor and between the readings of two evaluators. These investigators found no significant correlation between microvessel count and other tumor factors of prognostic value and no significant correlation between microvessel count and survival or metastasis-free survival. They concluded that microvessel assay in its present state was unsuited for general application in the management of patients.

**Anatomic Staging**

The anatomic extent of a cancer determined clinically or histologically is a classic and reliable indicator of prognosis, but an imprecise one. The general staging categories include localized (confined to...
breast tissue), regional (direct invasion to extramammary tissues or metastasis to regional lymph nodes), and distant (metastasis beyond regional tissues). These categories identify three general groups with distinctly different probabilities for survival after diagnosis and treatment but allocate potentially curable cases into only two categories. In both of these categories, a substantial proportion of cases are not cured. According to a statistical report from the NCI, the five-year relative survivals of localized and regional cases diagnosed during the years 1960 through 1964 were 84 and 53 percent, respectively, a difference of only 31 percent. For the three stages, Gardner and Feldman reported ten-year survival rates of 54, 28, and five percent for 1,024 patients diagnosed before 1981.

The five-stage TNM staging system is an improvement over general staging. It categorizes noninvasive carcinomas, which are highly curable, as stage 0. For staging invasive carcinomas, it places emphasis on size of the primary tumor and the extent of nodal metastasis. Excluding the incurable cases with distant metastasis (stage IV), the remaining stages of invasive carcinoma (stages I, IIA, IIB, IIIA, and IIIB) have an increasing likelihood of treatment failure and death. Five-year survivals for stages I to IIIB are 90, 80, 65, 50, and 40 percent, respectively.

Staging based on clinical or pathologic information is limited to providing a static picture of the disease. Within each stage are cases with differing biologic potential and speed of progression and a broad spectrum of prognoses. The most important components of anatomic staging are the size of the primary tumor and the extent to which regional lymph nodes are involved. These two variables are independent, but they are closely related. The probability of metastasis to regional nodes (both axillary and internal mammary) and to distant sites increases progressively as tumors enlarge. As continuous variables the number of nodal metastases and tumor size allow more precision for assessment of prognosis than does anatomic staging.

Metastasis to Regional Lymph Nodes

Axillary Lymph Nodes

Clinical examination of axillary lymph nodes is notoriously inaccurate for determining the presence of metastases (unless they are large and advanced) and provides a poor criterion for staging. In a representative study, examinations yielded false-negative results in 38.6 percent of cases and false-positive results in 27.3 percent.

The presence of metastasis to axillary lymph nodes on histologic examination of the nodes provides proof that a tumor with the capacity to metastasize has done so and may have metastasized to distant sites as well. Multivariate analyses regularly indicate that the presence or absence of metastasis to axillary lymph nodes is the single most influential predictor of posttreatment recurrence and death (Table 1). In the absence of systemic adjuvant therapy, the chance of recurrence within 10 years is 24 percent for patients without nodal metastasis on histologic examination and 76 percent for patients with nodal metastasis. Rotter’s interpectoral nodes, a subgroup of axillary nodes, contain metastases when all other axillary nodes are normal in only 0.5 percent of cases, and their contribution to prognosis is considered of little importance. While axillary metastasis is the most important determinant of prognosis in operable cases, the fact that a quarter of patients without axillary metastasis are not cured by local-regional therapy and some patients with metastasis are alive and well after many years (at 10 years, 30 percent overall and 17 percent for patients with metastasis to four or more nodes) indicate that they are an imperfect sign of systemic disease.
More prognostic information is derived from axillary lymph nodes than the fact of involvement alone. The most important information is the number of involved nodes. The absolute number of involved nodes provides a prognostic continuum that is directly related to the prospects for recurrence and indirectly related to survival.15,29,59-63 In one large study of 1,741 cases, the 10-year survival of patients with 0, 1 to 3, 4 to 9, and 10 or more involved nodes was 75, 62, 42, and 20 percent, respectively.29 The total number of nodes examined, and by inference the percentage of nodes involved, does not alter the prognostic importance of the absolute number of nodes that contain metastases, provided that sampling is sufficient to detect all positive nodes. Surgical removal or pathologic examination that is too limited is likely to provide misleading information.64 A level II axillary dissection is considered necessary to obtain reasonably accurate information.56

The size of metastases, the growth of metastases through the capsule of the lymph nodes, and the highest axillary level reached by the metastases can be related individually to prognosis, but all are interrelated in a complex manner and generally tend to be a function of the total number of involved nodes. Metastasis to increasing numbers of nodes results in larger metastases, extracapsular growth of metastases, and a higher axillary level of involvement.62 For example, extracapsular growth is seen only with macrometastases and influences prognosis adversely only when three or more nodes are involved.65,66 When the number of nodes with metastases is constant, the level of axillary involvement has no additional predictive value.62,67 Micrometastases (≤2 mm in diameter) are more favorable than macrometastases (>2 mm), and micrometastases rarely involve more than three nodes.68,69

Micrometastases found on routine sections of nodes or as occult metastases on multiple recuts of nodes initially considered free of metastasis are of interest because they potentially identify the subgroup of node-negative cases likely to relapse. A number of retrospective studies have found little or no difference in survival of patients with micrometastases compared with patients without metastases,70-72 but a recent prospective study by the International (Ludwig) Breast Cancer Study Group using multiple step sections of nodes showed a significantly poorer prognosis of patients with micrometastases.73

With this encouragement, more sensitive methods of detecting micrometastases in axillary nodes are being investigated in a continuing attempt to identify high- and low-risk populations of node-negative cases. The techniques being used include immunohistochemical methods with tumor-seeking monoclonal antibodies and polymerase chain reaction technology, which can detect minute amounts of messenger RNA related to human breast cancer.74 Models predict that monoclonal antibody techniques can detect one cancer cell among one million normal cells.72 Flow cytometry may be equally or even more sensitive. Monoclonal antibodies are also being used to detect micrometastasis in bone marrow samples of patients with early breast cancer, which may prove even more relevant to early distant relapse.75

INTERNAL MAMMARY LYMPH NODES

Internal mammary lymph nodes are also a primary lymphatic drainage basin of the breast. They are not routinely examined for pathologic staging, but they are involved in nine percent of cases when no metastases are found in axillary nodes, unmasking a high-risk group of “node-negative” cases.54 For this reason, histologic proof that they are free of metastasis provides additional evidence that a tumor is locally confined. Metastasis to these nodes has the same overall prog-
nostic importance as metastasis to axillary nodes. However, they are less accessible for examination, and their small number provides less potential for quantifying prognosis.

Internal mammary node metastasis is second only to axillary node metastasis as a prognostic variable, and it has been found more important than DNA ploidy and ERBB2 (c-erb B-2) expression. Internal mammary node metastasis indicates a worse prognosis than metastasis to either axillary nodes or internal mammary nodes alone, reducing ten-year survival from about 55 percent when either group is involved down to 30 percent when both are involved. It is likely that metastasis to internal mammary nodes simply indicates more widespread disease. Five involved regional nodes may have the same prognostic significance whether they are found only in the axilla or represent the total from both sites.

**SUPRACLAVICULAR LYMPH NODES**

Metastasis to supraclavicular nodes implies extensive involvement of axillary nodes, but it can occur in the absence of axillary involvement, suggesting passage through internal mammary nodes or blood-borne passage. The prognosis for patients with metastasis to this site is equated in the current TNM staging system with general dissemination of cancer (i.e., stage IV). While patients with supraclavicular metastasis regularly de-

Nodal status and primary tumor size are independent influences on survival of 24,740 patients with breast cancer. These two variables identify prognostic groups with five-year relative survivals from 99.2 to 45.5 percent. Relative survival is actuarial survival adjusted for age- and race-related natural mortality. Adapted from Carter et al.15

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![Graph showing five-year relative survival (%) vs. tumor size (cm) for different node statuses.](image)
velop distant dissemination, their survival is not always as poor as that of patients with distant metastasis at the outset. The five-year survival of the latter rarely exceeds 18 percent. The five-year survival rates for patients found initially with ipsilateral supraclavicular metastasis in two reports were 30 and 34 percent, suggesting a prognosis intermediate between TNM stages III and IV.

Tumor Size

The importance of tumor size as a prognostic variable in cases of invasive carcinoma is robust enough to survive measurements derived variously from clinical estimates, mammograms, and gross and histologic sections. In many analyses it is second only to axillary node status as an independent prognostic factor. Tumor size is directly related to an increasing probability of regional metastasis, an increasing average number of involved axillary lymph nodes, and an increasing probability of recurrence and death.

The favorable prognosis of nonpalpable invasive carcinomas relative to palpable ones and of screening-detected versus nonscreening-detected cancers is easily explained by their smaller size. In one report cancers 0.1 to 5 mm and 6 to 10 mm in diameter produced axillary metastasis in only 7.7 and 12.5 percent of cases, respectively. However, the incidence of positive nodes can range up to 21 percent for both of these size groups. Tumors of equal size are prognostically similar whether they are palpable or not and regardless of how they are detected.

The influence of primary tumor size on prognosis can be appreciated in both node-negative and node-positive cases. This relationship probably reflects increasing vascular and lymphatic dissemination with progressive tumor growth. Of particular interest are node-negative cases, where tumor size provides a readily available means for identifying patients at low and high risk for recurrence.

Tumors 1.0 cm or less in diameter have an especially low risk of recurrence. The five-year disease-free survival of node-negative patients with tumors 1.0 cm or less in diameter is 92 to 96 percent. The 10-year relapse-free survival of 47 cases of invasive carcinoma measuring 1.0 cm or less on gross section or on mammograms reported by Tinnemans et al. exceeded 90 percent. A large study at Memorial Sloan-Kettering Cancer Center found a 10-year relapse-free survival of 91 percent.

Only 12 percent of 171 patients with tumors 1.0 cm or less in diameter had recurrence within 20 years of their primary treatment in a study by Rosen et al. Survival was significantly superior to that of patients with tumors 1.1 to 2.0 cm in diameter, and it was estimated that 80 percent of patients with tumors 1 cm or less were cured at 20 years. Tumor sizes were taken from the gross description of the specimen or from measurement on histologic sections.

These and other studies support the contention that patients with the combination of node-negative disease and a tumor diameter of 1 cm or less represent a favorable subset of patients who would not benefit significantly from systemic adjuvant therapy. As failure rates are high enough in any case with macrometastasis to axillary lymph nodes to justify systemic adjuvant therapy, investigations have largely focused on the ability of other biologic variables to further define the prognosis of node-negative individuals, a group that is increasing due to more widespread use of screening mammography.

Steroid-Hormone Receptor Proteins

Intracellular steroid-hormone receptor proteins, primarily estrogen receptor (ER) and progesterone receptor (PR),
have received intensive study both as indicators of prognosis and as guides to hormone and endocrine therapy. About 50 to 85 percent of breast cancers contain measurable amounts of ER. The frequency with which tumors contain ER and the concentration of ER increase with patient age, both reaching their highest levels in postmenopausal patients. Concentrations of 10 fmol/mg or more of cytosol protein are generally considered positive for clinical purposes, and upper levels can reach more than 1,000 fmol/mg.

The presence of ER implies that normal cellular mechanisms for processing estrogen have been maintained despite malignant change, particularly if PR is present. PR is expressed only after transcriptional activation of its gene by a functional ER-estrogen complex. The clinical importance of ER relates principally to the fact that its presence identifies hormone-sensitive tumors. About 50 to 60 percent of patients with significant amounts of ER in their tumors respond favorably to hormone or endocrine therapy. A higher percentage respond if ER levels are high and if both ER and PR are positive.

Patients with ER-positive tumors have prolonged disease-free survival after primary treatment, superior overall survival, and longer survival after recurrence compared with patients with ER-negative tumors, and this advantage is independent of axillary node status. However, the value of ER status as an independent prognostic variable is diminished by its association with other established indicators of favorable prognosis and by its relationship to successful hormone therapy. ER-positive cancers generally have low-grade histology, favorable nuclear grade, a low S-phase fraction, a normal complement of DNA, a low proliferative index, and a low thymidine labeling index.

The influence of therapy on prognosis is difficult to exclude because ER-positive patients so regularly receive and benefit from either adjuvant or palliative hormone therapy. In some studies the lengthened disease-free survival and survival of patients with ER-positive tumors are seen only in the presence of hormone therapy. Often the effect of ER-positive status as a discriminant is lost after several years, further suggesting a temporary influence of treatment. When node-positive patients not receiving adjuvant hormone therapy were studied, five-year disease-free survival was 20 percent higher for ER-positive patients than ER-negative patients. However, the five-year survival of the most favorable subgroup, patients with one to three positive nodes and ER-positive tumors, did not exceed 60 percent.

Among node-negative patients, hormone therapy is less of a confounding factor, and small but statistically significant differences in disease-free survival and overall survival from eight to 12 percent have been found between ER-positive and ER-negative cases after various periods of follow-up. A multivariate analysis of prognostic factors by McGuire that included ER and PR status for more than 3,000 cases shows ER status to be more important for prognosis than tumor size in node-negative cases but not in node-positive cases. Fisher et al found ER status to be less important for the prognosis of disease-free survival or overall survival than number of positive nodes and nuclear grade.

The indications are that ER status is a weak prognostic indicator and that PR status provides no important advantage. Both are probably more reflective of growth rate than of metastatic potential. ER status alone or in combination with axillary status fails to identify a node-negative or a node-positive subset that has a rate of recurrence low enough to exclude systemic adjuvant therapy.

Enzyme immunoassay and immunohistochemical methods of measuring ER in tissues depend on colorimetric reactions or on scoring systems based on visu-
al estimates of the number and intensity of stained cells. These semiquantitative methods show high correlations with the results of biochemical ligand-binding assays. Similar correlations have been demonstrated with response to hormone therapy and with prognosis.

The pS2 protein appears to identify a subset of ER-positive tumors with a particularly favorable outlook. pS2 protein is an estrogen-regulated secretory protein of unknown function, but it probably indicates a more intact cellular estrogen-processing mechanism. It is expressed predominantly by ER-positive tumors. When found in ER-negative tumors, it is in much lower concentrations. It is not expressed in other normal human tissue (except stomach). Values above 11 ng/mg are associated with increases in disease-free survival and overall survival of patients with ER-positive tumors.

After adjustment for tumor size, lymph node status, and ER status, negative pS2 status is still associated with early recurrence and death. Positive pS2 status in ER-positive patients indicates improved prognosis in both node-negative and node-positive patients. In patients with ER-positive/PR-positive tumors, positive pS2 status was associated with a five-year survival of 97 percent versus 54 percent for negative pS2 status.

In node-negative patients, pS2-positive and pS2-negative cases had overall survivals of 89 and 58 percent, respectively. In node-positive patients, pS2-positive and pS2-negative patients had overall survivals of 88 and 34 percent, respectively. pS2 status may be an even stronger predictor of adjuvant hormone responsiveness than ER status.

Ploidy and S-Phase Fraction

Flow cytometry with laser-stimulated DNA fluorescence makes automated measurement of the DNA content of individual cells and the number of cells in each phase of the cell cycle readily available. It is possible to determine whether the DNA of each cell is normal (diploid versus nondiploid) compared with a control and to determine the fraction of cells actively synthesizing DNA.

Normally, diploid cells are in the resting phase (G₀) or in the first gap phase of the cell cycle (G₁). Cells with twice the normal DNA content are in either the G₂ or early mitotic phase (M), and cells with intermediate amounts of DNA are in the synthesis phase (S). About 32 to 51 percent of tumors are diploid. The remainder are aneuploid to various degrees. The degree of departure from normal DNA content is calculated as the DNA index (i.e., the DNA content of the predominant cell population divided by diploid DNA content). By definition, a diploid tumor has a DNA index of 1.0.

Standardization of S-phase fraction (SPF) and ploidy has been a continuing challenge. It is not possible to obtain measurements in 10 to 20 percent of tumors, and contaminating debris and non-neoplastic cells are potential sources of error. The definition of high and low SPF varies between laboratories and is often adjusted to provide optimum separation of prognostic groups. Computer programs for determining SPF vary, and ploidy determined with flow cytometry differs from that determined from cell image analysis.

To obtain lengthy follow-up, archival fixed or frozen tissue is often used, producing histograms with more debris and variability than those prepared from fresh frozen tissues. For reliable results it has been recommended that fresh frozen tissue samples be used for SPF when possible; tissue samples contain at least 20 percent tumor cells; and results be segregated into three rather than two risk categories to avoid misclassifying cases with borderline values.

Aneuploidy tends to be associated with large tumor size and with high nuclear grade. SPF is high in medullary
carcinomas, large tumors, tumors with poor histologic differentiation, ER-negative/PR-negative tumors, and tumors of young patients. SPF and ploidy are weakly associated with node status, if at all.\textsuperscript{113-116}

Results generally confirm that patients with diploid tumors or tumors with a low SPF have more favorable disease-free survival and observed survival than patients with aneuploid tumors or tumors with a high SPF.\textsuperscript{116-120} However, the differences may be small. In an analysis of 1,665 patients, Fisher et al\textsuperscript{112} found a nonsignificant difference in 10-year disease-free survival based on ploidy and only a difference of 13 percent in disease-free survival based on high or low SPF.\textsuperscript{112} This 13 percent difference dropped to 10 percent when adjustment was made for tumor size.

The results of a comprehensive evaluation of ploidy and SPF by Wenger et al\textsuperscript{116} involving 15,877 patients whose tumors were assayed in a large central laboratory help summarize the relationship between these two variables and their relationship to prognosis. SPF and ploidy were related because median SPFs were lower in diploid than aneuploid tumors. The medians were 3.4 percent for diploid tumors and 10.7 percent for aneuploid tumors. After SPFs were defined as high or low based on ploidy status (i.e., high, \textsuperscript{\textgreater}6.7 percent for diploid tumors; high, \textsuperscript{\textgreater}11 percent for aneuploid tumors), a multivariate analysis indicated that ploidy was not a significant prognostic variable for disease-free survival after node status, tumor size, SPF, and steroid receptors were taken into account. High and low SPFs separated both node-positive and node-negative patients into groups with high and low probabilities of disease-free survival. The four-year disease-free survivals of node-positive patients with high SPFs (2,222 patients) and low SPFs (3,919 patients) were 66 and 79 percent, respectively. The corresponding four-year disease-free survivals for node-negative patients were 86 and 92 percent.

In smaller studies of node-positive patients, other investigators have failed to find prognostic value for SPF after conventional indicators were considered. In a study of 167 node-positive patients, Witzig et al\textsuperscript{115} reported no value for using ploidy or SPF to predict survival or time to recurrence after number of positive nodes and tumor size were taken into account. The same was true for 490 node-positive patients treated in the Ludwig Group adjuvant chemotherapy protocols after node number, menopausal status, tumor grade, ER status, PR status, and tumor size were considered.\textsuperscript{113} A similar result was obtained for 197 patients treated with adjuvant chemotherapy and tamoxifen.\textsuperscript{121}

Merkel et al\textsuperscript{87} summarized 13 studies of flow cytometry in node-negative patients and found that SPF was more predictive for treatment outcome than ploidy. Of the seven studies that analyzed SPF, all found it predictive of outcome, whereas only six of the 13 studies found ploidy predictive.

The results of multivariate analyses depend on several factors, including the particular measure of outcome. In a study of ploidy, SPF, mitotic grade, tumor diameter, histologic grade, and nuclear grade in node-negative patients, Merkel et al\textsuperscript{87} found histologic grade to be the only independent predictor of relapse, while tumor diameter and SPF were the only independent predictors of cancer-specific survival. Patients with histologic grade 1 tumors had a five-year recurrence rate of only five percent, which was not influenced by SPF. Evaluation of SPF did allow for stratification of high-grade tumors into more and less favorable groups. Patients with tumors with a high grade and high SPF had a five-year recurrence rate of 36 percent. In a Cox analysis of 10-year disease-free survival, only SPF was significantly correlated with distant recurrence when tumor size and ER status were included as the other variables.\textsuperscript{122} In a study that did not include SPF, Lewis\textsuperscript{110} found that ploidy was a more important
predictor of recurrence than histologic grade or tumor size.

SPF evaluation allows for stratification of node-negative patients with diploid tumors into high- and low-risk groups. Clark et al. reported that patients with diploid tumors with a low SPF (<6.7 percent) had a five-year disease-free survival of 90 percent. Patients with diploid tumors with a high SPF had a poor prognosis comparable to patients with aneuploid tumors. These same investigators initially found no influence of SPF on aneuploid tumors, but later came to the opposite conclusion in an analysis of patients with small, ER-positive, node-negative tumors.

In multivariate analyses, tumor size and SPF often emerge as independent determinants of prognosis in node-negative patients, permitting these prognostic factors to be combined to advantage. Bosari et al. were able to identify three discrete prognostic groups based on tumor size, SPF, and ploidy. Only 12 percent of patients with small (≤2.0 cm), diploid tumors with a low SPF (<5 percent) had recurrence within nine years. O’Reilly et al. found that patients with tumors 1.0 cm or less had a disease-free survival of 96 percent and placed them in a separate group. The remaining patients with tumors more than 1.0 cm in diameter with SPFs less than 10 percent or greater than 10 percent had five-year disease-free survivals of 78 and 52 percent, respectively. Indications are that SPF may be a useful discriminant for tumors even less than 1.0 cm in diameter. Stal et al. found that five out of six patients with tumors 1.0 cm or less who relapsed had tumors with high SPFs.

An international consensus group met in 1992 to consider the clinical use of DNA cytometry in carcinoma of the breast. The group concluded that operable breast cancers that were diploid, even up to a DNA index of 1.3, had a favorable prognosis compared with aneuploid tumors but that the advantage was small and tended not to survive as a prognostic determinant in multivariate analyses because of the correlation of ploidy with more powerful prognostic factors. SPF, however, was believed to have an important association with recurrence and mortality for both node-positive and node-negative breast cancers. This association was generally independent of other prognostic factors, although its strong correlation with tumor grade often caused it to lose significance as an independent prognostic variable, particularly when grading was expertly performed. Its practical utility lay in being less subjective than grading.

**Mitotic Index and Thymidine Labeling Index**

Mitotic index (MI) and thymidine labeling index (TLI) measure cellular proliferative activity directly on histologic sections. The MI is measured as the number of mitoses per specified number of high-power microscopic fields (usually 10 fields) in routine sections. It requires no special technology but varies with field selection. Baak et al. were able to correlate increasing MI with decreasing cancer-specific survival. The association was as significant as number of axillary metastases and more significant than tumor size or histologic grade. MI of 0 to 81 per 10 high-power microscopic fields produced a span of five-year cancer-specific survivals from 90 to 10 percent and 10-year cancer-specific survivals from 80 to 0 percent.

TLI is a direct measure of cells in S phase of the cell cycle. Slices of fresh tumor tissue are incubated with tritiated thymidine, and cells actively synthesizing DNA incorporate this radioactive label. The tissue section is then coated with photographic emulsion, allowed to develop autoradiographically for one week, and developed. Silver grains are found in the emulsion over cells that incorporated the radioactive thymidine. Two thousand cells are counted, and the TLI is ex-
pressed as the percentage of cancer cells that were labeled.

More conveniently, 5-bromo-2-deoxyuridine (BrdU) is used as the label and identified in S-phase cells with a specific antibody. In vivo measurement of TLI can be performed by intravenous injection of BrdU prior to excision of tissue. The percentage of tumor cells in S phase measured by these means varies from 0.1 to 36 percent.\textsuperscript{127,128} Some investigators have found close correlation between the TLI of primaries and their metastases.\textsuperscript{129} Others find considerable intratumoral heterogeneity of TLI.\textsuperscript{130} The correlation between high TLI and poor prognosis does not apply to medullary carcinomas, where biologic behavior is not commensurate with the high TLIs.

For histologic types other than medullary carcinomas, TLI is strongly predictive of outcome and is independent of nodal status, ploidy, and ER/PR status. High values are associated with high MIs, poor histologic differentiation, and young age. In a large, multi-

| Prognostic Factor                           | Favorable         | Unfavorable          |
|---------------------------------------------|-------------------|----------------------|
| Axillary lymph nodes                        | No metastasis     | Metastasis present   |
| Positive axillary nodes                     | 1-3               | 4 or more            |
| IM lymph nodes                              | No metastasis     | Metastasis present   |
| Tumor size                                  | Small             | Large                |
| Histologic grade                            | I (well differentiated) | III (poorly differentiated) |
| Nuclear grade                               | I (well differentiated) | III (poorly differentiated) |
| Estrogen receptor                           | ≥10 fmol/mg protein | <10 fmol/mg protein        |
| Progesterone receptor                        | ≥10 fmol/mg protein | <10 fmol/mg protein        |
| pS2 protein                                 | High (>11ng/mg)   | Low (<11 ng/mg)       |
| S-phase fraction                            | Low               | High                 |
| Ploidy                                      | Diploid           | Aneuploid            |
| Mitotic index                               | Low               | High                 |
| TLI                                         | Low               | High                 |
| HER-2/neu (c-erbB-2)                         | Absent            | Present              |
| p53                                         | Absent            | Present              |
| Ki-67                                       | Low               | High                 |
| PCNA/cyclin                                 | Low               | High                 |
| Cathepsin-D                                 | Low               | High                 |
| uPA                                         | Low               | High                 |

IM = internal mammary, TLI = thymidine labeling index, uPA = urokinase plasminogen activator
variate analysis, TLI ranked fourth behind nodal status, tumor size, and nuclear size as an indicator of relapse-free survival. Among node-negative patients, it is superior to tumor size but not to ER status. Node-negative patients with a low TLI have five-year disease-free survivals of 85 to 89 percent. Meyer and Province reported that node-negative patients with tumors 2.0 cm or less in diameter that had a low TLI and were ER-positive had a five-year disease-free survival of 91 percent. For small tumors, however, TLI is not a strong discriminant.

Analysis of MI does not require any special preparations and is often found in reports from pathologists. However, TLI has not been adopted in practice, largely because the method is cumbersome, expert histologic interpretation is needed, and SPF determinations are available more conveniently with automated methods. Meyer et al, who have done most of the work with TLI, found no clear superiority of SPF determined by TLI or by flow cytometry for prediction of disease-specific relapse-free survival.

**Ki-67 and PCNA/Cyclin**

Ki-67 is a monoclonal antibody that identifies a nuclear antigen found in cells in the proliferative phases of the cell cycle (i.e., G1, S, G2, and M). It does not identify cells in the resting phase (i.e., G0). It is measured by an immunohistochemical assay. Until recently only fresh or frozen tissues could be used, but new antibodies permit the assay on fixed tissues. Fractions are larger than the SPF of tumors but have similar relationships to relapse and death. High Ki-67 scores are associated with poor histologic differentiation and with lymph node metastasis. Using 20 percent labeled cells as the cutoff to define high and low proliferation indices, Veronesi et al found that Ki-67 predicted four-year survival independently of node and ER status. Among node-positive patients, Railo et al found a significant difference in disease-free survival favoring Ki-67-positive/ER-negative patients over Ki-67-negative/ER-positive patients.

Proliferating cell nuclear antigen (PCNA)/cyclin is a nuclear antigen associated with proliferation that has promise as a prognostic marker. PCNA/cyclin level is correlated with SPF, TLI, and Ki-67 and has similar implications.

**Proteases**

**CATHEPSIN-D**

Cathepsin-D (CD) is an estrogen-dependent lysosomal protease that is synthesized by normal tissues and overexpressed and secreted by some breast cancers. Its secreted 52-kilodalton protein precursor (pro-cathepsin-D) has mitogenic activity and in an acid environment is proteolytic for basement membranes. CD is suspected of facilitating invasion and metastasis of breast cancer, and indeed, levels of CD tend to be higher in node-positive cases of breast cancer. The enzyme is measured with Western blot analysis, radioimmunoassay, or immunohistochemical methods. High levels are found in one third of breast cancers.

Overexpression of CD in breast cancer is associated with high risk of recurrence and poor survival, largely because of its relationship with node status. Whether it has any further implication is not settled, as inconsistent results have been obtained by investigators. Most studies suggest that CD has some significance for prognosis in patients with positive lymph nodes. Among node-negative patients results have conflicted, even in studies by the same investigators. In a multivariate analysis, Isola et al found that CD, tumor size, and SPF each had independent predictive value for disease-free survival of node-negative patients. But early indications that
CD may discriminate among node-negative patients were not confirmed in a much larger study of 1,489 patients at the University of Texas, San Antonio. Lack of uniform assay techniques among investigators may contribute to such inconsistencies. Because of strong linkage with node status and conflicting results of analyses, perhaps due to lack of standardization, the role of CD and pro-cathepsin-D as independent prognosticators remains uncertain.

### Table 3

**Recommendations for Systemic Adjuvant Therapy for Node-Negative and Node-Positive Patients**

| Category                     | Node Negative | Node Positive |
|------------------------------|---------------|---------------|
| **Minimal/low risk** (all ages) |               |               |
| Ductal Carcinoma in situ ≤1cm, ER positive, and Grade I† | No treatment | ER negative   |
| **Good risk** (all ages)     |               |               |
| 1-2 cm, ER positive, and Grade I-II† | Tamoxifen     | ER positive   |
| **High risk** (one or more of) |               |               |
| ≥2 cm or ER negative or Grade II-III† |               | Postmenopausal |
| Premenopausal                |               |               |
| ER negative                  | Chemotherapy  | ER negative   |
| ER positive                  | Chemotherapy  | ER positive   |
| Postmenopausal               | Chemotherapy  |                |
| ER negative                  | Tamoxifen     | Chemotherapy  |
| ER positive                  |                | Tamoxifen     |
| >70 years old                | Chemotherapy† |               |
| and ER negative              |               |               |
| >70 years old and ER positive| Tamoxifen     |               |

*For routine use or for baselines in clinical trials.
†Grade = histologic or nuclear grade
‡For selected patients in good condition.
Data from Goldhirsch et al.174
ER = estrogen receptor

For premenopausal women the most successful systemic adjuvant is combination chemotherapy. To date the addition of oophorectomy or tamoxifen to chemotherapy in ER-positive cases has not convincingly improved results compared with chemotherapy alone and is investigational.

For postmenopausal women with ER-positive tumors, tamoxifen is the preferred systemic adjuvant. The addition of chemotherapy to tamoxifen in some trials has had incremental value in ER-positive cases at high risk of recurrence. In ER-negative cases chemotherapy offers some benefit for postmenopausal women; the addition of tamoxifen in such cases is not of established value.
UROKINASE PLASMINOGEN ACTIVATOR
Urokinase plasminogen activator (uPA) is one of several proteases that have been implicated in the process of invasion and metastasis. It is a broad-spectrum serine endopeptidase that catalyzes conversion of plasminogen to plasmin. Plasmin can degrade various substrates in the extracellular matrix and can activate collagenses. Activity of uPA correlates with metastatic potential in animal tumor systems. The level of uPA can be measured with an enzyme-linked immunosorbent assay.

Investigations in human breast cancer indicate that high levels of uPA are correlated with a short disease-free interval and poor survival. As a prognostic marker, uPA level is independent of tumor size, node status, and ER status. The level of uPA has proved to be a discriminant for disease-free survival and observed survival in patients with positive nodes and those who are ER positive. It is also a discriminant for disease-free survival in patients with negative nodes. In node-negative patients and in patients with ER-positive tumors, low levels have been associated with five-year disease-free survivals of about 90 percent and 95 percent, respectively. This promising work awaits more general confirmation.

Proto-oncogenes, Oncogenes, and Tumor-Suppressor Genes
ERBB2 (HER-2/NEU, c-ERB B-2)
A number of proto-oncogenes and oncogenes have been investigated for their prognostic value. Proto-oncogenes are normal genes involved with cell growth and proliferation whose mutated forms promote neoplastic transformation. Examples include ERBB2, c-MYC, c-RAS, and RB1. ERBB2 has perhaps received the most attention in breast cancer. The encoded product of ERBB2 is a transmembrane glycoprotein receptor structurally similar to epidermal growth factor receptor (EGFR). Amplification results in overexpression of the cellular membrane receptor. Marks et al. found overexpression of ERBB2 and p53 to be independent markers of prognosis.

About one quarter of breast cancers overexpress ERBB2. It is not expressed in lobular carcinomas, and it is more often found in the in situ component of ductal carcinomas than in the invasive component. As a single variable, overexpression of ERBB2 is associated with poor prognosis, but the prognostic discrimination is almost entirely confined to node-positive patients. In node-negative patients, the influence on prognosis has been inconsistent and not clearly independent of other prognostic factors. The weakness of ERBB2 as a prognostic factor is evident in the fact that less than half of recurrent cancers show amplification.

As the prognostic influence of ERBB2 relates predominantly to node-positive patients, who usually receive systemic adjuvant therapy, it raises the possibility that ERBB2 is a marker for drug resistance. A similar influence, however, has been reported in node-positive patients who do not receive adjuvant chemotherapy. Amplification of ERBB2 has been related variously to increased sensitivity to doxorubicin adjuvant therapy, resistance to cyclophosphamide, methotrexate, 5-fluorouracil (CMF) combination chemotherapy, and resistance to hormone therapy. Its strong association with DCIS suggests it may be a marker for breast recurrence after breast-conserving therapy.

Less extensively studied proto-oncogenes in breast cancer include c-RAS, c-MYC, and INT2. The c-RAS gene encodes a p21 protein. Mutant overexpression of this gene can result in oncogenicity. Most breast cancers overexpress the p21 protein. High levels are associated with nodal involvement, advanced anatomic stage, and recurrence, suggesting a role in tumor progression. RB1 is a tumor suppressor gene. Loss of expression, which is found in 17 to 46 percent of
breast cancers, is associated with large tumor size and poor differentiation.159

**EPIDERMAL GROWTH FACTOR RECEPTOR**

Epidermal growth factor is structurally similar to transforming growth factor-α. EGFR is a large, transmembrane tyrosine kinase cell surface receptor that binds epidermal growth factor and transforming growth factor-α. EGFR is the product of the proto-oncogene c-ERBB. EGFR is measurable in most breast cancers, and overexpression or overstimulation of EGFR could result in unrestrained cell proliferation. Correlations between its expression in breast cancers and prognosis have been the subject of a number of studies, but the results have been inconsistent.160 High levels of EGFR are associated with ER-negative tumors, suggesting that it might identify tumors less likely to respond to hormone therapy, but this has not been shown convincingly.

**p53**

The p53 tumor suppressor gene, located on the short (p) arm of chromosome 17, is a negative regulator of cell proliferation. It is believed to function by blocking cells in G1, or by programing cell death (apoptosis). Its normally encoded nuclear protein has a life span too brief to be detected in cells, but the protein produced by mutant p53 is longer lasting and can be detected with immunohistochemical methods. Expression of mutant p53 is the most common genetic defect found in human cancers. It can be demonstrated in 14 to 26 percent of in situ and invasive breast cancers, depending on the criteria for positivity.161,162 Mutant p53 protein is more common in familial cases of breast cancer than in sporadic cases. It has been found in up to 52 percent of cases of breast and ovarian cancer syndrome and in all cases of Li-Fraumeni syndrome.162 It is strongly associated with other markers of high tumor proliferation rate (i.e., poor nuclear grade, ERBB2 protein overexpression, aneuploidy, high SPF, and ER-negative status), but it is independent of age, node status, and tumor size.162-164

Expression of mutant p53 has a negative influence on both overall survival and disease-free survival of breast cancer patients. In node-negative cases, some investigators have found that testing for expression of mutant p53 to predict disease-free survival is superior to testing for TLI, tumor size, and ER status. Silvestrini et al165 examined 256 node-negative cases and reported six-year disease-free survivals for cases with and without p53 mutant protein as about 60 percent and 90 percent, respectively.

In other reports the disease-free survival of node-negative patients without mutant p53 expression has been considerably lower.166 Allred et al166 found that 52 percent of the tumors of 700 node-negative patients showed some nuclear staining for mutant p53 protein. Increased staining correlated with progressive decreases in overall and disease-free survival. Disease-free survival at five years fell from 80 percent in negative cases to 58 percent in those with the most intense staining. In this study the prognostic importance of p53 positivity was independent of SPF, but this has not been found by other investigators.161

The close association between mutant p53 protein expression and other indicators of rapid cell proliferation establishes its link to prognosis, but often prevents it from surviving as an independent prognostic factor in multivariate analyses. Lack of uniformity in the method for assay of p53 protein is also evident among reports.

**Discussion**

Despite the deluge of information about prognostic factors, predicting specifically
which individuals are cured and which are not remains elusive; outcome continues to be attended by measures of doubt. Clinicians are well aware that some patients predicted to have recurrence and to die do not, and some with every indication of cure succumb to the disease. At best, prognostic indicators serve as guides for clinical decisions, which continue to require considerable judgment. These decisions are made easier by information that identifies patients whose chances of recurrence appear too small to justify the morbidity of adjuvant treatment (less than 10 percent according to the NCI Consensus Panel of 1990) and others whose risk is so great that extraordinary therapy may be justified.

Traditional pathologic features (i.e., nodal status, tumor size, and tumor differentiation) continue to provide guides for prognosis and are information that is routinely available. ER and PR are also important, but are more important for guiding selection of hormone treatment than for determining prognosis. Newer prognostic indicators relating to the proliferative rates of tumors are increasingly available and are potentially helpful, but for the most part their role is uncertain.

The lack of consensus among investigators about methods of measurement and about results and the absence of large, prospective studies justify reservations about their immediate application. How they relate to established parameters and to each other is still incompletely understood. Many may well prove redundant. Also unclear is how to properly evaluate combinations of good and bad indicators that are so often found in a single case. Table 2 shows the complexity of the information that is potentially available. Means for more completely integrating this information would be valuable.

ER and PR are the prime examples of prognostic indicators capable of identifying patients likely to respond to a particular form of therapy (i.e., hormone therapy). Poor histologic grade may indicate a higher potential for response to chemotherapy. The overexpression of c-ERBB2 may be a potential indicator of resistance to chemotherapy and hormone therapy. It is possible that reliable markers for resistance or sensitivity to specific chemotherapeutic agents will be forthcoming, information that could have a constructive influence on treatment planning.

With respect to decisions about systemic adjuvant therapy, prognostic factors provide important guides. Using information currently available, patients can be identified who have risks of recurrence and death less than 10 percent after potentially curative therapy. As adjuvant therapy can be expected to reduce the risk of recurrence by 30 percent, the potential benefit of an absolute reduction in recurrence of only two to three percent (10 X .30) may not justify the risks of adjuvant therapy in such cases.

Patients with an excellent prognosis include women with DCIS and women with negative axillary nodes whose invasive carcinomas are less than 1.0 cm in diameter or who have special histologic types of carcinoma less than 3.0 cm in diameter. On the other hand, patients with any number of metastases to regional lymph nodes and node-negative patients with tumors more than 2.0 cm in maximum diameter have recurrence rates high enough to derive a substantial benefit from systemic therapy.

Node-negative patients with tumors 1.0 to 2.0 cm in diameter have an intermediate prognosis with average five-year disease-free survivals of about 85 percent. It is in this group that measures of proliferation such as histologic or nuclear grade, SPF, and ER status may have the most value in deciding for or against systemic therapy.

The 1990 NIH Consensus Conference on treatment of early-stage breast cancer recognized emerging prognostic
factors but emphasized node status and tumor size in recommending no routine adjuvant treatment for node-negative patients with tumors 1.0 cm or less in diameter. At the 1992 and 1995 international St. Gallen consensus conferences, prognostic groups were based on node status, tumor size, ER status, and histologic or nuclear grade. Table 3 shows the recommendations for adjuvant systemic therapy outside of clinical trials that resulted from the second St. Gallen conference.

While prognosis can be estimated from available data, decisions for systemic adjuvant therapy are not based solely on risks of recurrence and survival. The patient’s general condition and tolerance for therapy are important considerations. Menopausal status and age influence the type of treatment that would be appropriate. The short- and long-term morbidities of adjuvant therapy vary considerably for hormonal and chemotherapeutic programs. Intensive chemotherapy protocols, often reserved for those with the highest risk of treatment failure, involve considerable morbidity and more than a negligible risk of mortality. It is paramount that patients understand the potential risks and the expected gains from treatment, as ultimately, they make the final decision.

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