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Breast Cancer

Prognostic and Predictive Factors in Early-Stage Breast Cancer

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Key Words. *Breast cancer · Prognostic factors · Predictive factors · Adjuvant therapy*

LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Differentiate between prognostic and predictive factors in early-stage breast cancer.
2. Identify prognostic factors used to determine the risk of recurrence and death for a patient with early-stage breast cancer.
3. Identify predictive factors used to determine the optimal therapy for a patient with early-stage breast cancer.

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ABSTRACT

Breast cancer is the most common malignancy among American women. Due to increased screening, the majority of patients present with early-stage breast cancer. The Oxford Overview Analysis demonstrates that adjuvant hormonal therapy and polychemotherapy reduce the risk of recurrence and death from breast cancer. Adjuvant systemic therapy, however, has associated risks and it would be useful to be able to optimally select patients most likely to benefit. The purpose of adjuvant systemic therapy is to eradicate distant micrometastatic deposits. It is essential therefore to be able to estimate an individual patient's risk of harboring clinically silent micrometastatic disease using established prognostic factors. It is also beneficial to be able to select the optimal adjuvant therapy for an individual patient based on established predictive factors. It is standard practice to administer systemic therapy to all patients with lymph node-positive disease. However, there are clearly differences among node-positive women that may warrant a more aggressive therapeutic approach. Furthermore, there are many node-negative women who would also benefit from adjuvant systemic therapy. Prognostic factors therefore must be differentiated from predictive factors. A prognostic factor is any measurement available at the time of surgery that correlates with disease-free or overall survival in the absence of systemic adjuvant therapy and, as a result, is able to correlate with the natural history of the disease. In contrast, a predictive factor is any measurement associated with response to a given therapy. Some factors, such as hormone receptors and HER2/*neu* overexpression, are both prognostic and predictive. ***The Oncologist*** *2004;9:606-616*

INTRODUCTION

Breast cancer is the most common malignancy among American women, with more than 200,000 new cases diagnosed each year [1]. In recent years mortality from breast cancer has declined in the U.S., likely as a result of more widespread screening resulting in earlier detection as

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well as advances in the adjuvant treatment of early-stage disease [2]. As a result of increased screening, the majority of patients now present with early-stage breast cancer. In 1995, 56.2% of all breast cancer cases were stage 0 or 1 compared with 42.5% in 1985 [3]. The Oxford Overview Analysis demonstrates that adjuvant hormonal therapy and polychemotherapy reduce the risk of both recurrence and death from breast cancer [4, 5]. Adjuvant systemic therapy, however, does have associated risks, and it would therefore be useful to be able to optimally select patients who are most likely to benefit. Prognostic factors may select patients most likely to recur without adjuvant therapy and therefore potentially benefit from therapy. In addition, predictive factors may identify the appropriate therapy for an individual patient.

Assessment of Recurrence Risk

In the late 1800s, *Halsted* popularized the radical mastectomy based on his belief that breast cancer spreads in an organized fashion, initially via the skin and regional lymphatics and then, at a later stage, hematogenously to other organs. Unfortunately, however, only 12% of patients treated with a radical mastectomy survived 10 years. The poor outcome with the Halstedian approach, as well as the observation that 20%-30% of node-negative patients ultimately develop metastatic disease, led to the currently held micrometastatic paradigm. This paradigm asserts that many patients with early-stage disease have distant micrometastatic disease present at the time of diagnosis, putting them at risk for the later development of overt metastatic disease [6].

The purpose of adjuvant systemic therapy is to eradicate these distant micrometastatic deposits. It is essential therefore to be able to estimate an individual patient's risk of harboring clinically silent micrometastatic disease using established prognostic factors. It is also beneficial to be able to select the optimal adjuvant therapy for an individual patient based on established predictive factors. Prognostic factors therefore must be differentiated from predictive factors. A prognostic factor is any measurement available at the time of surgery that correlates with disease-free or overall survival in the absence of systemic adjuvant therapy and, as a result, is able to correlate with the natural history of the disease. In contrast, a predictive factor is any measurement associated with response to a given therapy. Some factors, such as hormone receptors and HER2/*neu* overexpression, are both prognostic and predictive.

It is currently standard practice to administer systemic therapy to all patients with lymph node-positive disease. However, there are clearly differences among node-positive women that may warrant a more aggressive therapeutic approach. Furthermore, there are many node-negative women who would benefit from adjuvant systemic therapy. *Clark* addressed the issue of prognostic and predictive factors and suggested three main reasons to justify their use [7]. The first reason is to identify patients with good prognoses for whom adjuvant systemic therapy would not provide a large enough benefit to warrant the risks. The second is to identify patients whose prognosis is poor enough to justify a more aggressive adjuvant approach, and the third is to select patients whose tumors are more or less likely to benefit from different forms of therapy. Prognostic factors that are considered to be independent variables include lymph node status, tumor size, and estrogen/progesterone receptor (ER/PR) status. Additional factors include grade, presence of lymphovascular invasion, age, and ethnicity. Certain biologic factors, including ER/PR and HER2/*neu*, are both prognostic and predictive.

Prognostic Factors

Axillary Nodal Status

The most significant prognostic indicator for patients with early-stage breast cancer is the presence or absence of axillary lymph node involvement. Furthermore, there is a direct relationship between the number of involved axillary nodes and the risk for distant recurrence [8, 9]. For simplicity, however, most clinical trials stratify patients based on four nodal groups that are based on National Surgical Adjuvant Breast and Bowel Project (NSABP) data: negative nodes, 1-3 positive nodes, 4-9 positive nodes, and 10 or more positive nodes. The 5-year survival for patients with node-negative disease is 82.8% compared with 73% for 1-3 positive nodes, 45.7% for 4-12 positive nodes, and 28.4% for ≥13 positive nodes [10]. These data demonstrate that the risk of recurrence is significant enough with lymph node-positive disease to warrant adjuvant systemic therapy since, generally, a future risk of distant recurrence of 20% or greater is regarded significant enough to consider the risks of therapy. For lower-risk patients, especially those who are node negative, an individualized assessment utilizing other prognostic factors must be performed.

Traditionally, the status of the axilla has been assessed by a standard axillary dissection in which level I and level II lymph nodes were removed. Recently, the use of sentinel node (SN) biopsy has become more common. SN biopsy was first used to stage malignant melanoma [11]. The initial study of this technique in breast cancer was reported by *Giuliano et al*. using the blue dye method [12]. SN were identified in 65% of patients and accurately staged the axilla in 96% of those patients. More recent studies using a combination of blue dye and radiolabeled colloid have achieved detection rates of greater than 95% [13].

Although the ability of an experienced surgeon to accurately stage the axilla with SN biopsy is accepted, multiple questions remain, including the most suitable method to identify the SN as well as the optimal pathologic method to assess the SN for involvement. Serial sectioning of each SN increases the sensitivity as does the use of immunohistochemistry (IHC) for histologically negative lymph nodes. The significance, however, of occult micrometastases found by IHC alone remains controversial [14-16]. A recent retrospective review with long-term follow-up demonstrated an increased risk of recurrence and breast cancer-related death in women who had occult or micrometastatic tumor deposits in their axillary lymph nodes [17]. Similar results were observed in the International Breast Cancer Study Group Trial V of 1,275 node-negative women randomly assigned to a single cycle of perioperative cyclophosphamide, methotrexate, and 5-flourouracil (CMF) versus no chemotherapy [18]. The axillary nodes of 736 participants on this trial were later examined by serial sectioning and IHC. Occult nodal metastases were found in 7% by serial sectioning and in 20% by IHC. These metastases, detected by either method, were associated with a higher risk of recurrence.

A prospective evaluation of the survival impact of IHC metastases has been reported by *Hansen et al*. [19]. The SN of 696 patients were examined by hematoxylin and eosin (H&E) and IHC. The patients were divided into four groups: Group I, SN-negative; Group II, SN IHC-positive/H&E negative or equivocal; Group III, SN micrometastases ≤2 mm; and Group IV, SN H&E macrometastases >2 mm. At a median follow-up of 38 months, the size of the SN metastasis was a significant predictor of disease-free survival (DFS) ($p = 0.0001$) but not overall survival (OS) ($p = 0.0520$). There was no significant difference in DFS or OS between the SN-negative and the SN IHC-positive patients The authors concluded therefore that treatment decisions should not be made on the basis of SN IHC positivity.

In conclusion, axillary node status is the most consistent prognostic factor used in adjuvant therapy decision making. It is standard practice to administer adjuvant therapy to patients with lymph nodes that are positive using H&E staining. There is, however, increasing use of SN biopsies to stage the axilla. Patients with lymph nodes that are positive using H&E staining are offered adjuvant therapy. Therapy for patients that have SN positive by IHC only is a more complex decision, and other factors, such as tumor size, grade, hormone receptor status, and age become more influential.

Tumor Size

Tumor size correlates with the presence and number of involved axillary lymph nodes and is also an independent prognostic factor, with distant recurrence rates increasing with larger tumor size. The SEER database includes 13,464 women with node-negative breast cancer. Patients with tumors <1 cm had a 5-year OS of close to 99% compared with 89% for tumors between 1 cm and 3 cm and 86% for tumors between 3 cm and 5 cm [20]. This association persists with longer follow-up. *Rosen et al*. examined the relationship between tumor size and 20-year recurrence-free survival and found a significant association, with a 20-year recurrence-free survival of 88% for tumors ≤1 cm, 72% for tumors 1.1 cm to 3 cm, and 59% for tumors between 3.1 cm and 5 cm [21]. Furthermore, median time to the development of metastatic disease also shortens as tumor size increases [20-23].

For node-negative patients, tumor size is the most powerful prognostic factor and is routinely used to make adjuvant treatment decisions. In general, patients with a tumor size of >1-2 cm warrant consideration of adjuvant therapy since they may have a distant recurrence risk of ≥20%.

Tumor Type/Grade

The pathologic characteristics of the tumor have prognostic significance. Certain subtypes such as tubular, mucinous, and medullary have a more favorable prognosis than unspecified breast cancer [24-26].

In an attempt to improve interobserver variability, multiple grading systems have been proposed, with the most widely accepted being the Scarff-Bloom-Richardson (SBR) classification [27]. Mitotic index, differentiation, and pleomorphism are scored from 1 to 3 and the scores from each category are totaled. Tumors with scores from 3 to 5 are well differentiated (grade 1), from 6 to 7 are moderately differentiated (grade 2), and 8 to 9 are poorly differentiated (grade 3). A correlation between histologic grade as determined by SBR and 5-year DFS has been demonstrated in a study of 1,262 women [28]. Patients with an SBR score of 3 had a relative risk of recurrence of 4.4 compared with those with an SBR of 1. In conclusion, tumor grade does have prognostic significance and is primarily used to make decisions for lymph node-negative patients with borderline tumor sizes.

Lymphatic and Vascular Invasion

Peritumoral lymphatic vessel and vascular invasion (LVI) has been demonstrated to have prognostic significance for the risk of local and distant recurrence. At 20 years of follow-up, *Rosen et al*. noted a correlation between lymphovascular invasion and the risk of recurrence and death [29]. The recurrence rate for women with LVI-positive stage I disease was 38% compared with 22% for those with LVI-negative disease. In addition, the International Breast Cancer Study Group randomized 1,275 women with

node-negative breast cancer to a single cycle of perioperative chemotherapy or no systemic adjuvant therapy and demonstrated that the presence of LVI was associated with a 15% increase in the 5-year recurrence risk, and this effect was independent of whether or not they received adjuvant therapy [30]. Lymphatic and vascular invasion does have prognostic significance and is primarily used to make decisions for lymph node-negative patients with borderline tumor sizes.

Proliferation Markers

Various methods of measuring the proliferative rate of tumors have been evaluated in an attempt to correlate them with prognosis. These putative markers include the S-phase fraction (SPF), thymidine labeling index, mitotic index, and IHC analyses using antibodies directed against proliferation antigens such as Ki-67 and proliferating-cell nuclear antigen [31-33]. Many studies are limited by a lack of consistent methodology as well as a lack of information regarding systemic therapy and other prognostic variables. Several trials, however, have demonstrated an association between SPF and prognosis. After adjusting for tumor size, lymph node status, ploidy, age, and adjuvant systemic therapy, analysis of the San Antonio Breast Cancer Data Base revealed that a high SPF was associated with a relative risk of death of 1.29 ($p < 0.0001$) [34]. Similar results were observed on the NSABP B-14 trial in which women with ER-positive, node-negative tumors were randomized to receive 5 years of tamoxifen or placebo [35]. After adjusting for tumor size, age, and treatment, patients with high SPF tumors had a higher risk of both recurrence and death compared with those with low SPF tumors. Furthermore, an intergroup trial evaluated the natural history of 1,208 node-negative patients who were determined to be low risk based on tumor size, hormone receptor status, and SPF and were followed without adjuvant therapy. Low-risk patients either had hormone receptor-positive tumors that were <2 cm with a low SPF or had tumors that were too small for receptor analysis. High-risk patients had a tumor size ≥2 cm or tumors that were ER and PR negative. Low-risk patients were observed without adjuvant systemic therapy, while high-risk patients were randomized to receive CMF or cyclophosphamide, adriamycin, and 5-flourouracil (CAF). At 5 years of follow-up, the low-risk women had a DFS of 88%-89% and an OS of 96%-97%, regardless of whether they were classified as being low risk by tumor size alone or by a low SPF [36]. This finding suggests that a low SPF may be used in conjunction with other prognostic factors to identify a group of node-negative patients that are sufficiently low risk to not warrant a recommendation of adjuvant systemic therapy.

In conclusion, proliferation factors, such as SPF, do have prognostic significance. An elevated SPF is primarily used as justification to administer adjuvant therapy to lymph node-negative patients with borderline tumor sizes. A low SPF, however, may be used to identify a group of lymph node-negative patients who may not require adjuvant therapy.

Ethnicity and Patient Age at Diagnosis

African American and Hispanic women have a decreased survival from breast cancer compared with white women [37-39]. The source of this disparity is likely multifactorial, including issues such as lack of access to care resulting in a higher stage at diagnosis. There are data, however, to suggest that survival may be worse for African American women, even after adjusting for disease stage [40].

Many studies evaluating the influence of age on outcome in breast cancer have been small and have had conflicting results [41-45]. Two relatively large trials have, however, demonstrated a worse prognosis for patients younger than 35 years of age, even after adjustment for other prognostic factors [46, 47].

Ethnicity and age at diagnosis may be used to identify a group of patients who have a higher risk recurrence. They should be used, however, as an adjunct to other prognostic factors that are better validated such as tumor size.

Prognostic and Predictive Factors

ER/PR Status

The presence of estrogen and progesterone receptors in an invasive breast carcinoma is both prognostic and predictive. Its prognostic effect is difficult to evaluate in that it must be assessed in the absence of adjuvant tamoxifen. The NSABP B-06 trial randomized women with early-stage breast cancer to mastectomy, lumpectomy alone, or lumpectomy followed by radiation therapy [48]. No adjuvant systemic therapy was administered. The women with ER-positive tumors had a 5-year DFS of 74% and OS of 92%, while the women with ER-negative tumors had a 5-year DFS and OS of 66% and 82%, respectively.

Studies with longer follow-up, however, suggest that the prognostic significance of hormone receptors may not persist long-term. *Hilsenbeck et al*. demonstrated an improved prognosis for ER-positive tumors during the first 3 years of follow-up but not after 3 years [49]. It is possible that the presence of estrogen or progesterone receptors merely predicts for a more indolent, slower growing tumor with longer times to disease recurrence.

The presence of estrogen or progesterone receptors is, however, a powerful predictive factor for the likelihood of benefit from adjuvant tamoxifen. The most recent Early Breast

Table 1. HER2/*neu* overexpression and resistance to adjuvant CMF

Author	Trial	Design	*n* of ER$^+$ patients	Does HER2 predict resistance?
Gusterson et al. [58]	IBCSG	LN$^-$: PeCT versus Nil LN$^+$: PeCT versus CMFP	1,506	Maybe
Allred et al. [59]	Intergroup 001	CMF versus Nil	306	Maybe
Menard et al. [61]	Istituto Nazionale Tumori	CMF versus Nil	337	No

Abbreviations: IBCSG = International Breast Cancer Study Group; LN = lymph node; PeCT = perioperative chemotherapy; CMFP = CMF plus prednisone

Cancer Trialists' Collaborative Group update of randomized trials using adjuvant tamoxifen, published in 1998, included 37,000 women [4]. These data demonstrated that 5 years of adjuvant tamoxifen led to proportional reductions in the risk of recurrence and mortality of 47% and 26%, respectively, of patients with ER-positive tumors. This proportional reduction in mortality was similar for node-negative and node-positive patients. This translated to absolute mortality reductions of 5.6% for those with node-negative disease and 10.9% for those with node-positive disease. Five years of adjuvant tamoxifen also led to a proportional reduction of 47% in the risk of contralateral breast cancer, which was the rationale for the NSABP P-1 tamoxifen prevention trial [50]. There was no benefit for tamoxifen in hormone receptor-negative women [4].

The prognostic significance of estrogen or progesterone receptors is limited. Its optimal use is as a predictive factor for the benefit of adjuvant tamoxifen therapy. As a result of the data discussed above, all hormone receptor-positive women who warrant adjuvant systemic therapy should receive hormonal therapy unless otherwise contraindicated.

HER2/*neu*

The c-erbB-2 (HER2/*neu*) proto-oncogene is located on 17q21 and encodes an Mr 185,000 transmembrane glycoprotein, p185^{HER2}, with intrinsic tyrosine kinase activity homologous to the epidermal growth factor receptor [51]. It is amplified and/or overexpressed in approximately 30% of human breast tumors [52]. Overexpression is associated with increased tumor aggressiveness, increased rates of recurrence, and increased mortality in node-positive patients, while the influence in node-negative patients is more variable [53-56]. Interpretation of many studies of HER2, however, is limited by variability in the methods used to detect overexpression, definition of positivity, and that most are retrospective subset analyses.

Retrospective studies have suggested that HER2/*neu* overexpression may also have a predictive role for response to chemotherapy and endocrine therapy. A Southwest Oncology Group study, for example, randomized patients to tamoxifen alone or tamoxifen plus CAF and found that CAF improved the outcomes of the HER2/*neu*-positive women [57].

Several studies have suggested that HER2/*neu* overexpression may be associated with resistance to alkylator-based chemotherapy (Table 1). The International (Ludwig) Breast Cancer Study Group Trial V randomized node-negative patients to receive either no adjuvant therapy or a single cycle of perioperative chemotherapy with CMF, and node-positive patients to either prolonged chemotherapy (a perioperative cycle and six postoperative cycles of CMF) or a single perioperative cycle. HER2/*neu* overexpression was seen in 16% of the node-negative patients and 19% of the node-positive patients. For both node-positive and node-negative patients, the benefit of chemotherapy was greater for the HER2/*neu*-negative patients [58]. Similarly, *Allred et al*. demonstrated that HER2/*neu*-negative patients who received adjuvant CMF plus prednisone had an improved DFS compared with those with HER2/*neu*-positive tumors [59]. *Miles et al*. examined the relationship between HER2/*neu* status and outcome in 274 node-positive women who were randomized to receive six cycles of adjuvant CMF or no adjuvant therapy [60]. Although all of the treated women appeared to benefit from adjuvant CMF, the improvement in survival was less in the HER2/*neu*-positive patients. Results from the first CMF randomized trial with a 20-year follow-up, however, do not support a negative relationship between HER2/*neu* overexpression and response to adjuvant CMF [61].

HER2/*neu* expression may also predict benefit from adjuvant anthracyclines (Table 2). A retrospective analysis of 141 breast tumors included in a multicenter randomized, phase III trial comparing adjuvant CMF to 5-fluorouracil, doxorubicin, cyclophosphamide (FAC) was performed, demonstrating that the survival for the FAC arm was similar for HER2/*neu*-positive and HER2/*neu*-negative patients ($p = 0.1$) [62]. In contrast, the survival for the CMF arm was significantly worse for HER2/*neu*-positive compared with HER2/*neu*-negative patients ($p = 0.006$), supporting the concept that anthracycline-based chemotherapy offers a

Table 2. HER2/*neu* overexpression and benefit from adjuvant anthracyclines

Author	Trial	Design	*n* of HER2+ patients	Does HER2 predict benefit?
Vera et al. [62]	Multicenter phase III	FAC versus CMF	18	Yes
Paik et al. [63]	NSABP B-11	PAF versus PF	239	Yes
Pritchard et al. [64]	NCIC MA-5			
		CEF versus CMF	145	Yes
Muss et al. [65]	CALGB 8541	High-, moderate-, and low-dose CAF	455	Yes, for high dose

greater survival benefit than CMF in HER2/*neu*-positive patients. Further support is provided by a retrospective review of the tumors included in NSABP B-11, in which lymph node-positive, hormone receptor-negative patients were randomized to receive L-phenylalanine mustard plus 5-fluorouracil (PF) or L-phenylalanine mustard plus 5-fluorouracil and doxorubicin (PAF) [63]. A statistically significant benefit in DFS and OS in favor of PAF compared with PF was demonstrated for the HER2/*neu*-positive patients but not for the HER2/*neu*-negative patients. Similarly, *Pritchard et al*. examined the prognostic and predictive value of HER2/*neu* in the National Cancer Institute of Canada (NCIC) trial of CEF (cyclophosphamide, epirubicin, and 5-flourouracil) versus CMF in 710 premenopausal women with node-positive breast cancer [64]. HER2/*neu* was amplified by fluorescence in situ hybridization (FISH) testing in 24% of the women. DFS and OS were significantly lower for the women with HER2/*neu* overexpression. Furthermore, the women with HER2/*neu* overexpression had a nonsignificant trend toward greater differential benefit from CEF compared with CMF than women without HER2/*neu* overexpression.

HER2/*neu* overexpression may also identify patients who are likely to benefit from higher doses of adjuvant chemotherapy. The Cancer and Leukemia Group B (CALGB) 8541 randomized women with node-positive breast cancer to three doses (high, moderate, and low) of CAF [65]. A significant DFS and OS benefit in favor of the high-dose regimen was observed for the HER2/*neu*-positive women. There was no evidence of a dose-response effect in the HER2/*neu*-negative patients.

The influence of HER2/*neu* overexpression on response to taxanes was also evaluated in the adjuvant trial comparing TAC (docetaxel, doxorubicin, and cyclophosphamide) to FAC in node-positive breast cancer [66]. Administration of TAC compared with FAC resulted in a DFS risk ratio of 0.74 ($p = 0.02$) in HER2/*neu*-negative patients and 0.62 in HER2/*neu*-positive patients ($p = 0.06$). This trial was not powered, however, to detect efficacy differences based on HER2/*neu* positivity, and therefore HER2/*neu* status should not be used to direct the use of adjuvant taxanes until further prospective data are obtained.

There are also data to suggest that HER2/*neu* overexpression may predict response to endocrine therapy (Table 3). Results from retrospective evaluations of the influence of HER2/*neu* on response to tamoxifen in the adjuvant and metastatic settings have been conflicting [67-76]. An Italian trial randomizing 173 lymph node-negative women to 2 years of adjuvant tamoxifen versus no adjuvant therapy retrospectively performed HER2/*neu* testing on tumor samples from 145 of the patients, 43 of whom were found to be HER2/*neu* positive [69]. Among these HER2/*neu*-positive women, the adjuvant use of tamoxifen had a detrimental effect on overall survival. A subsequent Italian trial, however, evaluating adjuvant CMF with and without adjuvant tamoxifen in premenopausal lymph node-positive women, failed to demonstrate a predictive effect for HER2/*neu* [75]. Furthermore, NSABP B-14, which randomized 2,661 lymph node-negative women to tamoxifen or placebo, did not show any significant difference in DFS or OS based on HER2/*neu* status [76]. Given the fact that ER expression and HER2/*neu* are

Table 3. HER2/*neu* overexpression and tamoxifen resistance

Author	Trial	Design	*n* of ER+ patients	Does HER2 predict resistance?
Constantino et al.[76]	NSABP B-14	Tamoxifen versus placebo	937	No
Borg et al. [67]	4 Trials		400	Yes
Carlomagno et al. [69]	GUN1	Tamoxifen × 2 years versus Nil	74	Yes
Sjogren et al. [70]		Tamoxifen × 2 years versus 5 years	435	Maybe
Ellis et al. [77]	Neoadjuvant	Letrozole versus tamoxifen		Yes

inversely related, most endocrine therapy trials have limited numbers of HER2/*neu*-positive patients, thereby limiting their interpretation.

Prospective data in the neoadjuvant setting are limited to a single trial that demonstrated a decreased response to tamoxifen in HER2/*neu*-positive women [77]. Postmenopausal women with hormone receptor-positive, operable breast cancer were randomized to receive preoperative therapy with 4 months of tamoxifen or letrozole. Among the HER2/*neu*-positive women, response rates were 88% for the letrozole arm compared with 21% for the tamoxifen arm ($p = 0.0004$). While this trial is significant, it is limited by its small size and further trials are needed before HER2/*neu* may routinely be used to select adjuvant endocrine therapy.

In the metastatic setting, the presence of HER2/*neu* overexpression also predicts for response to a humanized antibody, trastuzumab (Herceptin™). A trial by *Baselga et al*. treated patients with heavily pretreated, HER2/*neu*-overexpressing metastatic breast cancer with weekly trastuzumab [78]; 11.6% of the patients achieved a response with one complete remission, which was sustained without tumor progression for >24 months. An additional 37% of patients achieved minimal response or stable disease. In a large multinational trial of 213 women with HER2/*neu*-overexpressing metastatic breast cancer, single-agent trastuzumab resulted in complete responses in 4%, partial responses in 17%, minor response in 7%, stable disease in 30%, and progressive disease in 42% of patients [79]. Ongoing clinical trials are evaluating the role of trastuzumab in the adjuvant therapy of early-stage breast cancer.

The trastuzumab trials have, in general, included women who were 2+ or 3+ for HER2/*neu* by IHC. Recent data, however, suggest that HER2/*neu* amplification as determined by FISH may be a better predictor of response to trastuzumab. *Mass et al*. investigated the influence of HER2/*neu* amplification by FISH and response to trastuzumab in the pivotal trial of trastuzumab plus chemotherapy [80]. FISH results were obtained for 451 of 469 enrolled patients (96.2%). Amplification was detected in 76% of the total population, 89% of the 3+ patients, and 31% of the 2+ patients. Importantly, the addition of trastuzumab to chemotherapy improved the response rate in the FISH-positive group (54% versus 30.8%, $p < 0.0001$) but not in the FISH-negative group (38% versus 37.5%, $p =$ not significant). Similarly, the addition of trastuzumab to chemotherapy only resulted in a survival benefit for the FISH-positive patients. Similar results were demonstrated in the single-agent trastuzumab trials. A retrospective analysis was performed to determine the FISH status of the patients enrolled in HO650g, in which patients received trastuzumab as first-line nonhormonal therapy for metastatic disease, and HO649g, in which patients received trastuzumab as second- or third-line therapy for metastatic disease [81]. On HO650g, response rates were seen in 34% of the FISH-positive patients and in 7% of the FISH-negative patients. Similarly, on HO649g response rates for FISH-positive and FISH-negative patients were 19% and 0%, respectively.

HER2/*neu* overexpression is a prognostic factor that is associated with a more aggressive tumor. The data supporting the use of HER2/*neu* in selecting adjuvant therapy, however, are limited by the varying methods employed to detect overexpression. The optimal use of HER2/*neu* status may be as a predictive factor, especially in predicting response to trastuzumab in the metastatic setting. It is still premature to routinely use HER2/*neu* status in deciding between various adjuvant chemotherapy and endocrine therapy regimens since prospective data are still limited. Currently, the limited data trends toward selecting an adriamycin-based regimen for HER2/*neu*-overexpressing tumors.

Urokinase-Type Plasminogen Activator and Plasminogen Activator Inhibitor Type 1

Two invasion factors, urokinase-type plasminogen activator (uPA) and its inhibitor, plasminogen activator inhibitor type 1 (PAI-1), have prognostic and predictive significance. Node-negative patients with low uPA/PAI-1 have an excellent prognosis without systemic adjuvant therapy, with a 5-year DFS exceeding 90%. In contrast, node-negative patients with high uPA/PAI-1 have a higher risk for relapse [82-84]. Levels of uPA and PAI-1 have also been demonstrated to have predictive value in an analysis of 3,424 primary breast cancer patients [85]. Patients with high uPA and PAI-1 had an enhanced benefit from adjuvant chemotherapy compared with those with low levels. A significant interaction between chemotherapy and uPA/PAI-1 was seen for the entire group and within nodal subgroups. An assay for uPA/PAI-1 is now commercially available and may be used to better determine the potential benefit of chemotherapy for patients with small, node-negative tumors.

Genetic Profiling

Microarray analyses may be used to identify a gene-expression profile that yields prognostic and predictive information. Using oligonucleotide microarrays, *van de Vijver et al*. classified 295 patients with stage I or II breast cancer as having a poor prognosis or a good prognosis based on their gene-expression signatures [86]. At 10 years

of follow-up, DFS and OS rates were 50.6% and 54.6%, respectively, for the poor prognosis group, compared with 85.2% and 94.5%, respectively, for the good-prognosis group. The estimated hazard ratio for a distant recurrence in the poor-prognosis group compared with the good-prognosis group was 5.1 ($p < 0.001$).

More recently, the NSABP reported the results of a validation study of a multigene reverse transcription polymerase chain reaction (RT-PCR) assay using available tissue blocks from 668 node-negative, ER-positive, tamoxifen-treated patients enrolled on two trials: NSABP B-14 and B-20. Samples from patients enrolled on B-20 were initially studied to identify prognostic genes. RNA was extracted in 10-m sections and RT-PCR was used to measure expression of five reference and 185 cancer-related genes. Univariate analysis identified genes that were associated with relapse-free survival. A validation study was then performed with 668 samples from patients with ER-positive tumors enrolled on the tamoxifen arm of B-14. A 21-gene model was used to develop a recurrence score (RS) algorithm. Risk of distant recurrence at 10 years was 6.8% for those patients with a low RS (<18), 14.3% for those with an intermediate RS (18-30), and 30.5% for those with a high RS (≥31). The RS was an independent prognostic factor on multivariate analysis [87]. This 21-gene model was also evaluated in an MD Anderson Cancer Center study using tumor blocks from 149 node-negative, ER-positive and ER-negative patients who did not receive tamoxifen or chemotherapy. In this group of patients, however, the RS did not predict DFS [88].

In addition to providing prognostic information, genetic profiling may also be helpful in predicting response to therapy. A trial from MD Anderson utilized microarrays to predict response to neoadjuvant chemotherapy. The investigators performed microarray analyses on pretreatment fine needle aspirates and were able to identify a gene-expression profile that was predictive of a complete pathologic response to preoperative chemotherapy with FAC and paclitaxel [89].

Genetic profiling shows great promise in improving our prognostic and predictive accuracy. Other methodologies include the use of laser capture microdissection [90]. The ultimate clinical utility of these techniques will, hopefully, be better defined in the future as they are studied in prospectively randomized trials in defined subgroups of patients.

Summary and Future Directions

Table 4 summarizes the prognostic and predictive value of the factors discussed in this paper. Currently, it is standard practice to offer adjuvant systemic treatment to all patients with node-positive disease and many patients with node-negative disease and tumor sizes greater than 1 cm. Prognostic factors therefore are primarily used to detect a subset of node-negative patients who are likely to have a good outcome without further therapy. Tumor size, tumor grade, proliferation factors, and the presence or absence of hormone receptors are commonly used for this purpose. Hormone receptor status is also used to determine the appropriateness of adjuvant endocrine therapy. The role of HER2/*neu* overexpression in evaluating risk as well as determining optimal therapy is currently evolving. It is standard practice in the metastatic setting, however, to use HER2/*neu* status to identify patients who are likely to benefit from trastuzumab therapy. The use of trastuzumab in the adjuvant setting is currently being evaluated in controlled, prospective randomized trials. Because of the uncertain risk/benefit ratio in the adjuvant setting, trastuzumab is not recommended outside of a clinical trial for adjuvant therapy of HER2/*neu*-positive patients.

Table 4. Summary of prognostic and predictive factors

Factor	Prognostic	Predictive
Lymph node status	Yes	
Tumor size	Yes	
Lymphovascular invasion	Yes	
Proliferation markers	Yes	
Ethnicity	Maybe	
Age	Yes	
ER/PR status	Yes	Yes
HER2/*neu*	Yes	Yes
uPA/PAI	Yes	Yes
Genetic profiling	Yes	Yes

Unfortunately, our ability to accurately determine patients who are likely to develop metastatic disease without adjuvant therapy as well as our ability to individualize therapy for a given patient based on established prognostic and predictive factors is limited at the present time. We still treat many patients with toxic therapy who would have been destined to do well without therapy, and we still have many patients who subsequently develop metastatic disease despite adjuvant therapy. There are promising data to suggest a future role for factors such as cyclin E and gene-expression profiles [86, 91]. Many studies performed thus far are hampered by small sample sizes, varying assay methods, and retrospective methodology. In the future, it will be necessary to evaluate potentially useful factors in a prospective fashion using standardized assay and statistical methodology.

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