Biochemical Markers as Prognostic Indices in Breast Cancer

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Traditional prognostic markers in breast cancer include histological variables such as tumor size, grade, and axillary node status. In recent years some new potential prognostic markers of a biochemical nature have been described: estradiol receptors, progesterone receptors, epidermal growth factor receptors, erbB-2 proto-oncogene, and certain proteolytic enzymes. None of these new markers excels axillary node status as a prognostic marker. Biochemical markers can, however, be evaluated with use of minimal surgery and may help distinguish the minority of aggressive axillary-node-negative breast cancers.

Additional Keyphrases: estradiol receptors, progesterone receptors, epidermal growth factor receptors, erbB-2 proto-oncogene, proteolytic enzymes

Once a diagnosis of breast cancer is established, the next major question in patient management is: Should the patient receive adjuvant treatment? Clearly, if the patient has aggressive disease it should be treated. On the other hand, patients with non-aggressive malignancy could be followed up without adjuvant treatment. This latter approach would have the following advantages: (a) the patient would avoid drug-induced side effects, (b) a greater range of treatment options would be available if disease recurs, and (c) there would be a substantial saving in drug costs.

The main problem, however, is how to differentiate tumors with good prognosis from those with poor prognosis. Traditionally, the main prognostic markers have been histological variables such as the presence or absence of axillary node metastases, tumor size, and tumor grade. There are problems in using each of these as a prognostic index.

The principal problem with tumor size as a prognostic marker is that size is probably a reflection of chronological age of the tumor rather than an indicator of tumor biology. In addition, it exhibits no relationship to tumor proliferative rates, at least as measured by thymidine labeling (1). Unlike tumor size, tumor grade—especially nuclear grade—may be related to aggressiveness, because it appears to correlate with proliferation rate (2). However, determining grade is time consuming, subjective, and not very reproducible. Moreover, there is no uniform grading system.

The best and most frequently used prognostic marker in breast cancer is the presence or absence of metastases to the axillary nodes. Generally, patients with nodal metastases have a worse prognosis than those who do not. However, about 25–35% of the so-called axillary-node-negative patients die from their disease within 10 years (3). There is a particularly urgent need for markers to identify these high-risk, node-negative patients. A further problem in using nodal status as a prognostic marker is that it involves extensive surgery. Because this major and mutilating surgery apparently does not enhance survival, more patients are opting for conservative treatment.

Clearly, the traditional prognostic markers in breast cancer are far from satisfactory. A good prognostic marker should provide information on tumor cell proliferation rates and metastatic potential, and it also should indicate what therapy is appropriate. Finally, it should be measurable by using a minimum of surgery—preferably, fine-needle aspirates.

In recent years, many potential new prognostic markers of a biochemical nature have been described for breast cancer. These include steroid-hormone receptors, growth-factor receptors, activated proto-oncogenes, and proteolytic enzymes. The aim of this presentation is to review the use of these markers as prognostic indices in breast cancer. Biological prognostic indicators such as ploidy and thymidine labeling index will not be discussed except in a comparative context.

Estradiol Receptors

In 1977, Knight et al. (4) were among the first to show that the absence of estradiol receptors (ERs) in breast tumors was associated with early recurrent disease. The effect of ERs on recurrence was found to be independent of other prognostic indices such as tumor size and axillary lymph node status. Although a significant relation was found between the absence of ERs and a shortened disease-free interval, this study of Knight et al. had some shortcomings. For example, the number of patients in the study was only 145, and only 33 of them received no adjuvant treatment. Moreover, the follow-up periods were short; the median follow-up for ER-negative patients was 16 months; for ER-positive patients, 18 months. Despite these limitations, this study stimulated a number of further investigations to assess the prognostic role of ERs in breast cancer.

In 1980, it was shown that ER-negative tumors had a poorer survival pattern than ER-positive cancers (5). In general, these early reports have been confirmed; i.e., most such studies show that patients with tumors containing high concentrations of ERs have a better prognosis than do patients with cancers lacking ERs (6–9). In a few studies, however, no difference was found in disease-free interval between ER-positive and ER-negative patients (10, 11).

As with the study of Knight et al. (4), most of these early attempts to relate ER-status to prognosis involved relatively small numbers of patients and short-term follow-up.
With larger studies and longer follow-up periods, the generalization that ER-containing tumors had better prognosis than did tumors lacking ERs has had to be modified. For example, some workers (12, 18) found that the beneficial effect of ER-positivity may only last for short periods, two to three years. Other findings show that the beneficial effect of the presence of ERs is confined to certain subgroups of patients.

Some recent studies in which large numbers of patients were used illustrate this last point. In one of these involving over 800 axillary-node-negative patients with a median follow-up time of 50 months, Thorpe et al. (14) found that ER-positive patients had only a marginally significantly better relapse-free survival than did ER-negative patients (P = 0.07). However, when the patients were categorized by menopausal status, the presence of ERs was found to be a significant prognostic marker for premenopausal patients but not for peri- or postmenopausal women. In contrast to these findings for node-negative patients, ER status was found to be a significant prognostic marker in high-risk postmenopausal patients—i.e., those with axillary node involvement or tumor diameters >5 cm (14).

In another study, involving >1000 patients, McGuire (9) has shown that for Stage 1 (axillary-node-negative) breast-cancer patients, ER status and tumor size were the two most important prognostic factors with respect to both disease-free interval and overall survival. Other factors such as progesterone receptors, age, and type of treatment were less important if ER status and size were known. In Stage 2 breast-cancer patients, however, progesterone receptor (PR) status appeared to be a better prognostic marker than did ER status for predicting disease-free interval. For predicting overall survival in Stage 2 patients, both ER and PR status were significant prognostic factors.

Progesterone Receptors

Compared with ERs, less information is available on PRs as a prognostic marker. As with ERs, most but not all investigators find that patients with PR-positive tumors have a better prognosis than those who are PR-negative. In a recent review paper, Hawkins (15) showed that in six of nine studies PR-positive patients had a better relapse-free interval, and in five of seven studies a better overall survival, than did PR-negative patients.

Some reports show that PR status is a stronger prognostic marker than is ER status, at least for certain groups of patients (9). As mentioned above, McGuire (9) found that for Stage 2 patients, PR status was a better prognostic marker than was ER status for predicting disease-free interval. In contrast, Thorpe and Rose (16) did not find that PR status was a significant prognostic indicator in postmenopausal patients irrespective of risk group. These workers did, however, find that PR status was a stronger prognostic marker than was ER status in node-negative patients (16). In another study on axillary-node-negative patients, Clark et al. (17) showed, using multivariate analysis, that PR status was the strongest of a group of prognostic markers in predicting overall survival. The markers investigated in this study were ploidy, tumor size, PR, ER, and patient's age. However, for predicting disease-free survival, only ploidy was a significant marker.

Clearly, there is considerable controversy on the precise contribution of both ERs and PRs to prognosis in breast cancer. Possible explanations for the conflicting results are as follows:

- different methodologies for receptor assays, and different cutoff points used
- different patient follow-up times
- different patient populations with respect to menopausal status, disease stage, and adjuvant treatment
- different statistical tests used to analyze the data
- the small numbers of patients used in some studies
- nonstandard criteria used to assess date of first recurrence

Of these various possibilities, different patient populations, different cutoff points for receptor assays, and different follow-up periods are likely to be the main contributors to these conflicting results.

Before leaving this section on steroid receptors, I must point out that all of the above findings were generated by using receptor assays based on radioligand binding. In recent years, immunohistosays (both biochemical and immunocytochemical) have been described for ERs and PRs. Generally, results of these immunohistosays not only correlate well with those by the standard binding assays (18, 19), they also have the potential to introduce greater uniformity into steroid receptor assays and thus might help minimize some of the variability found with these assays. This in turn might lessen the number of conflicting reports on the prognostic value of steroid receptor assays in breast cancer. Preliminary data (19, 20) indicate that ERs determined by immunohistosay can have prognostic value. In a study comparing an estradiol-binding assay and an immunocytochemical assay, Kinsel et al. (20) found that only the immunocytochemical results related significantly to survival after five years of follow-up. This finding clearly needs confirmation.

Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) mediates the action of the growth factors EGF and TGF-α. Unlike ERs and PRs, which are both intracellular receptors, the EGFR is found on the cell membrane. A high-molecular-mass protein (Mr = 170 000–180 000), it is divided into three main domains: (a) an external domain, where the binding of EGF occurs; (b) a transmembrane domain; and (c) a cytoplasmic domain, which possesses protein-tyrosine kinase (EC 2.7.1.112) activity. The cytoplasmic region of the EGFR is homologous to the protein product of the v-erbB1 oncogene.

EGFRs are present in a variety of different tumors and in certain normal cells such as keratinocytes. In breast cancer, EGFR concentrations have been found in two studies (21, 22) to correlate inversely with ERs and, in another (23), to show no significant relation to ERs. Recently, Sainsbury et al. (24) showed that both relapse-free survival and overall survival were significantly worse for patients with EGFR-positive breast tumors than for those whose tumors were EGFR-negative. Using multivariate analysis, they found that only axillary node status and EGFR status were significant prognostic markers for predicting relapse-free survival. However, based on characteristics of the primary tumor, the only significant marker for disease-free survival was EGFR. For death, nodal status was the only significant factor (P = 0.007). EGFR status was the second most important prognostic indicator, but this was not significant (P = 0.1). As with relapse-free survival, EGFR status was the only primary tumor characteristic that
correlated significantly with overall survival. Finally, EGFR status was a valid criterion for dividing ER-negative tumors into those with good and poor prognosis; i.e., ER-negative/EGFR-positive patients had a significantly worse prognosis than did patients negative for both receptors (24).

c-erbB-2/HER-2/neu

The c-erbB-2 gene, also known as HER-2 or neu, encodes a protein with properties very similar to EGFR. Both are membrane-bound proteins possessing protein kinase activity. Furthermore, each has an extracellular domain, a transmembrane domain, and an intracellular domain. These findings suggest that the c-erbB-2 protein is likely to be a receptor for a ligand that is yet to be identified.

Amplification of the erbB-2 gene is found in 20–30% of primary breast cancers (21, 22). In 1987, Slamon et al. (25) showed that in breast-cancer patients with axillary-node metastases, the degree of gene amplification correlated significantly with both disease-free interval and survival. Univariate analysis of amplification of this gene was equivalent to number of axillary-node metastases as a prognostic marker and that it was a stronger prognostic marker than either PR status, tumor size, or ER status for both disease-free interval and survival. In multivariate analysis, c-erbB-2 gene amplification was also a significant prognostic index and independent of number of axillary-node metastases (25). These results of Slamon et al. were recently confirmed by the same workers, in an expanded study involving a greater number of patients (26). Similar results on the relationship between erbB-2 gene amplification and poor prognosis were obtained by Varley et al. (27).

Measurement of gene amplification is relatively demanding and not suitable for the routine pathology laboratory. Detection of the protein product of the erbB-2 gene, particularly if it could be carried out on paraffin-embedded sections and yet give similar information to gene amplification, would have great appeal. In one study, carried out by Wright et al. (28), erbB-2 protein staining was found to correlate inversely with ER and positively with histological grade. No significant association was seen between erbB-2 concentrations and either lymph-node status, EGFR, or tumor size. Positive staining correlated significantly with earlier relapse, shorter post-relapse survival, and shorter overall survival. According to multivariate analysis, only lymph-node status was a stronger predictor of both relapse-free and overall survival than the erbB-2 staining. Neither ER status, EGFR status, or tumor grade were significantly associated with prognosis (28). These results, if confirmed, would make erbB-2 a useful prognostic marker in breast cancer. However, two other groups (29, 30) using the same antibody as Wright et al. (27) were unable to confirm the above findings. In a further study, in which a different erbB-2 antibody was used, no correlation was found between increased expression of erbB-2 and disease-free interval in Stage II breast cancer. Increased concentrations of erbB-2 did correlate with overall survival, but this relationship was not significant after adjustment for tumor size (31). These discrepant results with c-erbB-2 are probably ascribable to factors similar to those discussed above for ER and PR.

Preliminary data suggest that other oncogenes/oncoproteins can be used to provide prognostic information in breast cancer. Clair et al. (32) have shown that breast cancers with high concentrations of the ras protein have a greater likelihood of developing recurrent disease than do tumors containing low concentrations of this protein. Similarly, amplification of both the int-2 and c-myc oncogenes have been associated with aggressive breast cancer (33).

Proteolytic Enzymes

Considerable indirect evidence from animal cancers suggests that certain proteases are involved in cancer invasion and metastasis (for a review, see ref. 34). The proteases implicated in these processes include plasminogen activator (EC 3.4.21.31), cathepsin B (EC 3.4.22.1), and collagenase IV (EC 3.4.24.3). In certain model systems, each of these proteases has been shown to correlate with tumor metastatic potential (34). If proteases are also involved in the spread of human cancers, their measurement in these tumors might also correlate with metastatic potential. Preliminary evidence shows that high concentrations of at least two proteases correlate with bad prognosis in breast cancer. One of these proteases is the urokinase form of plasminogen activator. Duffy et al. (35) have shown that patients whose primary breast cancer contains high amounts of this enzyme have a significantly worse disease-free interval than do patients with low amounts. Similarly, the presence of high amounts of a cathepsin D-related protein correlates with poor prognosis in breast cancer (36). But not all proteases are associated with aggressive breast cancers. High concentrations of tissue-type plasminogen activator correlate with good prognosis in breast cancer (37).

Conclusion

From the above it is clear that the number of potential biochemical markers becoming available as prognostic indices for breast cancer is growing. However, results for many of the markers mentioned above are still preliminary. Many of these results need to be confirmed with larger numbers of patients and longer follow-up. We also need studies with multiple markers carried out on the same specimens, to see which marker (or combination of markers) gives the best information. In addition, it has to be established if biochemical markers give information independent of that provided by the traditional histological indices. So far, no biochemical marker has been shown to be superior to axillary-node status.

Other aspects of the biochemical markers that will have to be evaluated include analytical aspects such as assay reproducibility, stability of analyte, whether the marker can be assayed immunocytochemically, and, if so, whether paraffin-embedded sections can be used. Finally, if the preliminary results I have described are confirmed, it should mean that more information is available in planning the management of individual breast-cancer patients. This should result in better quality of life for certain patients and reduced demands on health budgets.

References

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