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*Perspectives in Cancer Research*

# Tumor Grade as a Prognostic Factor in Primary Breast Cancer

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## INTRODUCTION

THE VAST MAJORITY of women with breast cancer are operable according to conventional criteria and about half of them remain alive and disease-free at 10 years, with no difference between pre- and post-menopausal patients. Which indicators suggest that the outcome of local–regional therapy will be poor? Met-analyses have recently confirmed that the 5-year tumor mortality has been significantly reduced in given patient subsets following adjuvant systemic therapy [1, 2]. Then the other important question is: which are the most simple and accessible methods to properly select women who are candidates for adjuvant chemotherapy or endocrine therapy?

For many years, clinicians have been aware of the prognostic importance of axillary nodal status and its relation to the size of primary tumor: limited to about 5% in women with minimal breast cancer and maximum in women with locally advanced neoplasms [3]. Retrospective analyses of prospective surgical trials have also emphasized the inverse relationship between the number of pathologically involved lymph nodes and both relapse-free and total survival rates [4, 5]. Thus, the number of positive nodes, i.e. an anatomical finding, has been utilized as one of the stratification parameters for the initial trials with cyclical adjuvant chemotherapy [6, 7]. Also the evaluation of all subsequent adjuvant trials has stressed the prognostic relevance of the number of positive axillary nodes [3, 8], and this histologic parameter is still being utilized as the single most important and reproducible prognostic factor in resectable breast cancer. At the Consensus Development Conference for Adjuvant Chemotherapy in Breast Cancer [8], the Panel has recommended that the nodal status following complete axillary dissection should be expressed as follows: negative, positive 1 to 3 nodes, 4 to 10, and more than 10.

During the past decade, particularly since the wide application of adjuvant drug therapy, the search for prognostic variables other than those represented by nodal status has become more systematic. The increased interest in the biology of breast cancer and the desire to avoid side-effects from postoperative cytotoxic drugs to low-risk patients have in fact multiplied the efforts of research physicians and basic scientists. Today, several indicators have been explored, both retrospectively and prospectively, and their relative merits for predicting tumor recurrence are being compared versus the gold standard represented by axillary lymph node involvement. The first indicator which was examined was tumor grade.

## TUMOR GRADE

Pathologists have known for about a century [9] that the degree of anaplasia and the number of mitotic figures in a surgical specimen were often associated with aggressive tumor behavior. For this reason, over the years several types of histologic grading were proposed and utilized [10–12]. In addition to the overall growth pattern (histologic grade, degree of anaplasia, degree of differentiation), cytologic differentiation of nuclei (nuclear

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grade) was also determined [13] and the medical literature abounds with various forms of grading, at times attempting to make comparisons between histologic and nuclear grade [14]. Pathologists were also attracted by the interrelationships between tumor cells and the host, and in particular by certain types of stroma: an inflammatory reaction, or elastosis, was usually regarded as a favorable prognostic indicator [15, 16] while tumor necrosis was associated with poor prognostic outcome [17].

In recent years, a number of pathologic studies carried out first by the National Surgical Adjuvant Breast Project (NSABP) group revealed a significantly greater number of well differentiated breast cancers in older ($\geq$50 years) than in younger ($\leq$49 years) women. This finding correlated with the well recognized observation of a greater incidence of estrogen receptors (ER)-positive tumors in older women [18]. Indeed, a strong association between ER status and degree of tumor differentiation has been confirmed. This information, along with the finding of low responsiveness to adjuvant chemotherapy in older patients [1, 8, 19], has revitalized the importance of exploring the value of certain histopathologic characteristics of breast cancer, namely tumor grade, in predicting responsiveness to adjuvant systemic therapy.

Before considering the essential details of recent case series evaluated with multivariate analysis, it is of practical importance to briefly state the terms of reference utilized in this review. What is designated 'tumor grade' in the medical literature actually covers the analysis of three types of morphologic characteristics in coded form. The first is the *histologic grade* (HG) which takes into account tubule formation by neoplastic cells (code 1: well-developed tubules throughout; code 2: occasional tubule formation; code 3: no tubule formation seen). The *nuclear grade* (NG) takes into account the degree of anysonucleosis, i.e. irregularity and hyperchromatism of the cancer cell nuclei. In the initial coding system [13], code 1 was assigned to tumors having a high degree of atypia in size and shape while code 3 or 4 was assigned to nuclei which are very uniform. Currently, this order is reversed [20]. In fact, in code 1 nuclei are small and relatively uniform in size, shape and chromatin pattern and have small or inconspicuous nucleoli; code 2 nuclei are somewhat less consistent in size with more irregular nuclear contours, more coarse chromatin and often prominent nucleoli; code 3 nuclei are large, often bizarre and pleomorphic. The *mitotic grade* is calculated in a different way according to the various authors. Certain pathologists calculate the maximum number of mitoses within a single high-power field (code 1: occasional, i.e. one mitotic figure; code 2: two mitotic figures; code 3: three or more mitotic figures). Other pathologists calculate the total number of mitoses seen in examining 10 or more high-power fields.

In certain studies some investigators have taken into consideration one or other of the above mentioned coded characteristics while other investigators have utilized a numeric system which included two or three characteristics and then assessed a final grading classification. Thus grade I (G1) usually corresponds to tumors with the best prognosis and grade III (G3) to tumors with poor prognosis, respectively.

A major criticism of tumor grading systems has always been the issue of interobserver reproducibility [21] which can be reflected in interseries comparison in terms of proportion of cases classified as G1, G2 and G3 tumors. However, besides its inherent subjectivity, the limit of tumor grading may be due to a number of factors, i.e. lack of a considerable degree of practice on large sample sizes [22], the use of different grading systems and the fact that other variables not assessed by grading contribute independent prognostic information. If we examine from Table 1 the comparative frequency of tumor grades in five recent large case series with stage II (node-positive, N+) breast cancer, we observe that, for instance, G1 tumors range from 9 to 22.3%. As summarized in Table 2, there may be a valid reason for the reported variation simply because the authors have utilized different grading systems. The NSABP group has adopted personal grading methods, i.e. old and revised [23, 24], which take into consideration histologic differentiation (tubule formation) and nuclear grade but not mitotic activity. The Memorial Hospital Group [25] has evaluated both histologic differentiation and nuclear grades through a personal method where type and grade of differentiation were designated as well, moderate, and poor rather than G1, G2 and G3, respectively. Differentiation, when analyzed in terms of nuclear grade, is more evenly distributed in the three categories, whereas histologic grading resulted in relatively fewer well differentiated tumors. As can be noticed from Table 1, both the American studies, which do not include mitotic activity in the tumor grade assessment, actually present comparable distribution among the three categories, i.e. G1, G2 and G3.

Two research groups, namely the Ludwig Group [26] and the Institut Gustave Roussy [27], have utilized the Scarff, Bloom and Richardson (SBR) method [12], following the WHO critieria [28]. The criteria consist of a numerical coded system which evaluates mitotic grade besides degree of tubule formation and anysonucleosis, and formally expresses the final grade in three numbered categ-

*Table* 1. *Comparative frequency of tumor grades in some recent case series*

| Authors | Year | No. of cases | Node + (%) | Grade(%) | | |
|---|---|---|---|---|---|---|
| | | | | G1 | G2 | G3 |
| Fisher *et al.* [23, 24] | 1984 | 614 | 54.6 | 11 | 37 | 52 |
| Rosen *et al.* [25] | 1986 | 573 | 46 | 9 | 45 | 46 |
| Davis *et al.* [26] | 1986 | 1537 | 100 | 22.3 | 48.7 | 29 |
| Contesso *et al.* [27] | 1987 | 612 | 56.2 | 21 | 50 | 29 |
| Russo *et al.* [29] | 1987 | 646 | 51.4 | 10.4 | 71.1 | 18.6 |

*Table* 2. *Morphologic features and systems utilized to classify patients*

| Authors | Tubule formation (HG) | Anyso-nucleosis (NG) | Mitotic Max/1HPF* (MG) | figures No./10HPF (MG) | Classification system |
|---|---|---|---|---|---|
| Fisher *et al.* | HG | NG | | | Personal |
| Rosen *et al.* | HG | NG | | | Personal |
| Davis *et al.* | HG | NG | MG | | Modified SBR† |
| Contesso *et al.* | HG | NG | MG | | Modified SBR |
| Russo *et al.* | HG | NG | | MG | Personal ± SBR |

*HPF = high-power field.
†SBR = Scarff, Bloom and Richardson [12].

ories (G1, G2 and G3). It is clear from Table 1 that when very similar methods of classification are employed, the distribution of grade categories becomes almost superimposable.

Russo *et al.* [29], from the Michigan Cancer Foundation, calculated the mitotic index according to the largest number of mitoses per 10 high-power fields (HPF). When singly comparing the three grading systems, these investigators concluded that the mitotic grade had the most important influence on the risk of recurrence and breast cancer death. However, although the authors mention a final grade similar to that of Bloom and Richardson [12], they have not compared simultaneously all three types of coded characteristics with the course of breast cancer.

In spite of the different methods utilized to assess tubule structures of neoplastic cells, irregularity of the cancer cell nuclei, hyperchromatism of the nuclei and the number of cells in mitosis, the common finding reported in all above mentioned case series is that 'tumor grade', no matter how assessed, significantly affected the 5- and 10-year relapse-free and total survival rates. Thus, the overall results point to one direction and indicate that G1 tumors exhibit the best prognosis while G3 tumors are associated with a more aggressive clinical course. Furthermore, tumor grading is more discriminating than the use of histologic subtypes, which are numerous and endlessly expanded [27].

Recently, tumor grade was also utilized in the retrospective evaluation of prognostic variables in node-positive patients subjected to systemic adjuvant therapy. As reported in detail by the Ludwig Group [26], tumor grade remained a statistically prognostic factor for the 6-year relapse-free and overall survival in a multivariate analysis controlling for nodal status, tumor size, receptor and menopausal status, age, peritumoral vessel invasion and treatment assigned. In postmenopausal patients, where adjuvant treatment (endocrine therapy alone or combined with chemotherapy) was compared with no adjuvant therapy, the prognostic significance of tumor grade was modified by the effect of treatment. The NSABP Group [19, 24] related the response to adjuvant chemotherapy to nuclear grade. These investigators observed a highly significant improvement in both relapse-free and total survival through 12 years of follow-up for all patients having tumors with marked nuclear pleomorphism (G3) who received adjuvant chemotherapy. Of great importance was the observed benefit in those women $\leq 49$ years as well as in those $\geq 50$ years having G3 tumors. In contrast, the benefit from adjuvant tamoxifen when added to chemotherapy occurred in patients with tumors with good nuclear grade (G1).

At this point, the crucial question is whether tumor grade represents a significant prognostic indicator alone or in conjunction with other variables. In the experience of the Institut Gustave Roussy [27], the SBR method retained its individual long-term prognostic value which was independent of the number of histologically involved axil-

lary lymph nodes. In 612 women with resectable breast cancer both tumor grade and axillary nodes were the only two significant prognostic factors in the multivariate analysis. It is also important to point out that in the French Study the SBR method also retained its individual and independent prognostic value in women with inoperable breast cancer where it was assessed through core needle biopsies.

## OTHER PROGNOSTIC INDICATORS

In 1977, Knight *et al.* [30] were the first investigators to show that ER measurements could identify women at high risk for recurrence after local–regional therapy. This finding was confirmed by most of subsequent studies related to case series with stage II (axillary node positive) and stage I (axillary node negative) disease treated by operation alone [31–35]. The magnitude of differences in the relapse-free survival between ER-positve tumors and ER-negative tumors as well as some of the inconsistencies in the reported findings could in part be attributed to the heterogeneity of tumors and methodologies for receptor determination [35]. In stage II breast cancer, a subsequent observation from the San Antonio Group involved PgR whose status was found superior to other measurement parameters in a multivariate analysis and appeared equivalent to the number of axillary lymph nodes for predicting recurrence [36, 37].

The prognostic value of steroid receptors in patients subjected to adjuvant chemotherapy has been so far reported by a limited number of investigators. Following adjuvant CMF (cyclophosphamide, methotrexate and fluorouracil), no significant difference between ER-positive and ER-negative tumors was observed at 5 and 10 years by the Milan Group [2, 3]; similar 5-year results were recently published by the Danish Breast Cooperative Group [38]. On the contrary, utilizing less intensive chemotherapy, the NSABP and Danish groups have observed a more favorable outcome at 5 years in women with ER-positive compared to ER-negative tumors [24, 38]. Most probably, CMF chemotherapy was more effective in selectively improving those subsets with undifferentiated tumors having low receptor content, thus leaving no difference between well and poorly differentiated tumors.

Results from separate studies have confirmed that the higher the proliferative rate of a tumor, as measured on fresh tissue by the thymidine labeling index, the more likely a patient is to have a breast cancer recurrence after local–regional therapy [39–41]. Of particular importance is the observation made by Silvestrini *et al.* [40] who, in a multivariate analysis of 215 stage I patients, showed that the thymidine labeling index was an independent prognostic factor when compared to tumor size and ER status. Flow cytometric methods on fresh pulverized tissue have been utilized by the San Antonio Group to measure the percentage of tumor cells in S-phase, and also to determine ploidy [37, 42, 43]. The investigators observed that the median S-phase of diploid tumors was significantly lower compared to the aneuploid tumors with a wide range in both populations.

In recent years, monoclonal antibodies were studied as markers of tumor cell proliferation. A recent example is represented by the Ki-67 monoclonal antibody which reacts with a human nuclear antigen associated with cell proliferation that is expressed only in continuously proliferating cycling cells, and therefore offers the opportunity to determine the growth fraction of tumors by immunostaining of fresh tissue. In 154 invasive carcinomas of the breast, Lellé *et al.* [44] have noticed a correlation between growth fractions and histologic grading as well as a significantly higher growth fraction between node-positive and node-negative tumors. However, the results of this interesting study do not yet provide evidence of whether Ki-67 may represent a potentially useful predictor of the clinical course of breast cancer.

Monoclonal antibodies have been also actively investigated to detect occult tumor cells. Most pertinent to the subject of this review is the recent update of British investigators [45] on detection of occult tumor cells in the bone marrow by immunochemistry using an antiserum to epithelial membrane antigen. Both relapse-free and total survival times were significantly shorter for patients with micrometastases. The test, however, predicted bone metastases only and did not appear to be an independent prognostic factor.

The discovery of oncogenes is providing considerable insight into mechanisms of tumor growth [46–48]. Most important, the presence of oncogene amplification and/or expression in certain types, including breast cancer, is yielding significant prognostic information [49–51].

## OLD VERSUS NEW PROGNOSTIC INDICATORS

During the past decade, a constellation of findings have been considered to influence prognosis of resectable breast cancer. Table 3 outlines the most important variables which were either documented or claimed to represent useful indicators of primary treatment outcome. Although their importance is undeniable, we do not know yet the precise combination and the hierarchy of these factors that should be used in a clinical setting. Even more important, with the exception of nodal status, we are still unable to identify with certainty

*Table 3. Risk factors for relapse in resectable breast cancer*

| |
|---|
| Axillary nodal status, number of histologically positive lymph nodes, and vascular invasion |
| Tumor grade (histologic, nuclear, mitotic) |
| Tumor size |
| Steroid receptor status |
| Age (?) |
| Proliferative rate (thymidine labeling index, flow cytometry) |
| Aneuploidy |
| Oncogene amplification |
| Identification of occult bone marrow micrometastases by monoclonal antibodies |
| Low-dose adjuvant chemotherapy or short-term adjuvant tamoxifen |

the strong prognostic indicators upon which select proper adjuvant therapy. And here too, about 25% of node-positive women will not need postoperative chemotherapy or endocrine therapy while systemic adjuvant treatment will be required in about 25% of node-negative tumors.

Clinicians may be perplexed before the growing list of prognostic indicators to be taken into consideration. Nonetheless, they should become aware that old and new parameters expressing tumor cell proliferative activity will become more and more involved in assessing prognosis and treatment selection. As previously mentioned, recent studies have stressed the independent prognostic relevance of thymidine labeling index, flow cytometric analysis and over-expression of given oncogenes in predicting both relapse and survival. However, it is important to stress that many of these sophisticated and costly techniques will not soon be as accessible to all pathologists as are their microscopes. Therefore, the practical question is as follows: what test is now suitable for *routine* use? Besides nodal status, tumor grade was confirmed from recent retrospective evaluations to exert a significant influence on treatment outcome and to bear an important predictive value as positive axillary nodes [27] and steroid receptors [24]. Furthermore, at least in one large study group [17, 19, 52], tumor differentiation contributed to the discernment of significant responses in women having undifferentiated tumors and subjected to adjuvant systemic therapy. Therefore, it is highly recommended that the routine selection for adjuvant therapy as well as the design of new trials for node-positive and node-negative patients will systematically include tumor grade as well as other histopathologic discriminants to contribute to the definition of prognostic subsets. The prognostic importance of tumor differentiation will also be improved by its correlation with receptor status [53].

## CONCLUSION

Modern research for prognostic factors in primary breast cancer is attempting to improve, if not replace, the classical anatomic parameters represented by tumor size and nodal status with new biological indicators of tumor cell proliferation. Among the new markers of cell growth, the histoprognostic grade offers, for the time being, one of the most simple, inexpensive and (in experienced hands) reproducible methods to subclassify breast cancer into risk categories. The combination of more than one marker is expected to improve treatment selection and provide a more accurate assessment of treatment outcome. Sophisticated and potentially more promising methods to measure tumor cell proliferative activity should at present be further investigated within the context of research trials.

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