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Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up

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Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up

Morphological assessment of the degree of differentiation has been shown in numerous studies to provide useful prognostic information in breast cancer, but until recently histological grading has not been accepted as a routine procedure, mainly because of perceived problems with reproducibility and consistency. In the Nottingham/Tenovus Primary Breast Cancer Study the most commonly used method, described by Bloom & Richardson, has been modified in order to make the criteria more objective. The revised technique involves semiquantitative evaluation of three morphological features—the percentage of tubule formation, the degree of nuclear pleomorphism and an accurate mitotic count using a defined field area. A numerical scoring system is used and the overall grade is derived from a summation of individual scores for the three variables; three grades of differentiation are used. Since 1973, over 2200 patients with primary operable breast cancer have been entered into a study of multiple prognostic factors. Histological grade, assessed in 1831 patients, shows a very strong correlation with prognosis; patients with grade I tumours have a significantly better survival than those with grade II and III tumours ($P<0.0001$). These results demonstrate that this method for histological grading provides important prognostic information and, if the grading protocol is followed consistently, reproducible results can be obtained. Histological grade forms part of the multifactorial Nottingham prognostic index, together with tumour size and lymph node stage, which is used to stratify individual patients for appropriate therapy.

Keywords: breast, breast cancer, histological differentiation, histological grade, prognostic factors

Introduction

As the range of options for the treatment of patients with breast cancer widens, so it becomes increasingly important that the clinician is provided with accurate prognostic information on which to base therapeutic decisions. A fundamental aspect of histopathology has been the recognition that the morphological appearances of tumours can be correlated with the degree of malignancy[1–3]. Since Greenough[4] undertook the first formal assessment of the grading of morphological features in breast cancer, a large number of studies have been carried out, and these data have recently been reviewed by Elston[5]. Briefly, two main types of method have arisen from Greenhough's original study, based either on a combination of cellular factors[6–10] or on predominantly nuclear features[11–13].

The Nottingham/Tenovus Primary Breast Cancer Study was established in 1973 with the aim of assessing the relative importance of a range of potential prognostic factors in breast cancer. Because one of us (C.W.E.) had previously modified the Bloom & Richardson method[9] and applied it successfully in the CRC Trial for Early Breast Cancer[14], it was decided to evaluate histological grade in the Nottingham/Tenovus study. We report here our further modification of the method devised by

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Elston[10], and demonstrate its value as an independent prognostic factor based on the study of a large number of patients with long-term follow-up.

Materials and methods

This work is based on patients entered into the Nottingham/Tenovus Primary Breast Cancer Study. To date, over 2200 patients with primary operable breast cancer have been treated consecutively under the care of a single surgeon (Professor R.W.Blamey) by mastectomy or local excision and radiotherapy, with loco-regional lymph node sampling. Long-term clinical follow-up has been maintained by regular visits to the clinic. Major recurrences are recorded as loco-regional (recurrences requiring some form of major treatment such as radiotherapy) or distant (confirmed radiologically by isotope scan or liver function tests). Mortality data are recorded on all patients when available.

A total of 1951 patients presenting with primary operable breast carcinoma in the study period 1973–1989 were entered, and histological grading performed where appropriate.

TISSUE PREPARATION

To obtain the best results it is very important that careful attention is paid to specimen preparation. Fixation should be prompt. We use 10% phosphate buffered formalin which gives good preservation of cytological detail. Autolytic artifacts are kept to a minimum by slicing specimens in the fresh state immediately after resection. This may require special arrangements, the simplest of which is to ensure that specimens are sent to the laboratory fresh. The practice of immersion of the whole breast unsliced into formalin should be discouraged. In the Nottingham/Tenovus study a skilled laboratory technician has been specially trained to sample tumours immediately after excision in the operating theatre laboratory. The tumour is sliced in a cruciate manner and segments are removed and snap frozen (for receptor assay, immunochemistry and archival storage), leaving quadrant leaves of tissue for routine histology (Figure 1). Blocks obtained in this way give a good representation of the whole tumour from central core to periphery. The number of blocks which can be obtained depends on overall tumour size but, bearing in mind the potential value of archival material, no upper limit should be set. Careful processing of paraffin blocks is required and 4–6 μm-thick sections are cut; nuclear detail is obscured if sections are too thick. Conventional staining with haematoxylin and eosin is adequate and no special stains are required routinely.

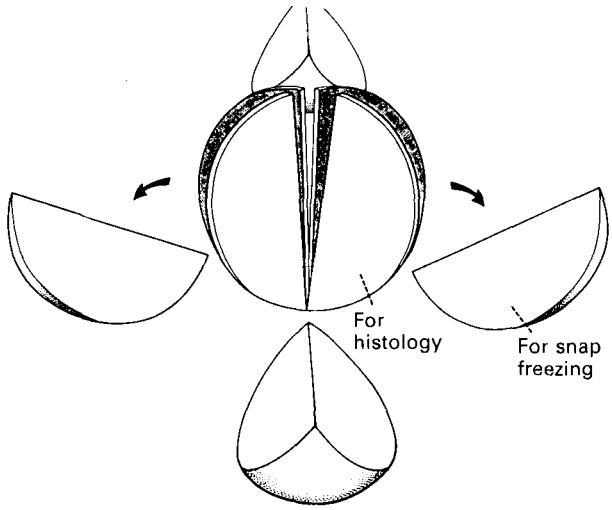

Figure 1. Method for incision and sampling of tumour specimens.

GRADING TECHNIQUE

Histological grading is directed principally at invasive adenocarcinomas; tumours of other types or those which are completely or predominantly of *in situ* type are not suitable for the particular method we describe. It is the policy in our laboratory to grade all histological types of invasive adenocarcinoma; assessment is not restricted to tumours of no special histological type and special types are not excluded. The grade for an individual tumour is derived from an assessment of three morphological features, each of which is scored 1–3 (Table 1).

Tubule formation

All parts of each block are scanned and the proportion of

Table 1. Summary of semiquantitative method for assessing histological grade in breast carcinoma

Feature	Score
Tubule formation	
Majority of tumour (>75%)	1
Moderate degree (10–75%)	2
Little or none (<10%)	3
Nuclear pleomorphism	
Small, regular uniform cells	1
Moderate increase in size and variability	2
Marked variation	3
Mitotic counts	
Dependent on microscope field area (see Table 2)	1–3

tumour displaying tubular structures is assessed. Clear lumina must be visible, and care should be taken not to mistake clefts induced by shrinkage artifact for tubular structures (this problem is diminished with good fixation). When more than 75% of the tumour area is composed of definite tubules a score of 1 point is given. Two points are appropriate for tumours in which between 10 and 75% of the area shows tubule formation. Where tubules occupy 10% or less of the tumour 3 points are assigned.

Nuclear pleomorphism

In this feature both a quantitative and a qualitative judgement is made. When the nuclei are small, with little increase in size in comparison with normal breast epithelial cells, have regular outlines and uniformity of nuclear chromatin and vary little in size, 1 point is appropriate. A score of 2 points is given when the cells appear larger than normal, have open, vesicular nuclei with visible nucleoli, and there is moderate variability in both size and shape. A marked variation in size and shape, especially when very large and bizarre nuclei are present, scores 3 points. In this group nuclei are vesicular with prominent, often multiple nucleoli.

Mitotic counts

Mitotic activity is best assessed at the periphery of the tumour where active growth is most likely. A minimum of 10 fields is assessed. Strict criteria for the identification of mitotic figures must be employed, and only nuclei in which clear morphological features of metaphase, anaphase and telophase are counted. Hyperchromatic and apoptotic nuclei are ignored and care is taken to avoid mistaking lymphocytes within a tumour for mitoses. The assignment of points was originally carried out using a Leitz Ortholux microscope with wide angle eyepieces and × 25 objective. This gives a field area of 0.274 mm^2. Up to 9 mitoses per 10 fields scores 1 point, 10–19 scores 2 points and more than 20 scores 3 points. The point scoring system can be adapted for use with other microscopes after the field area is calculated, and comparative examples are given in Table 2.

Table 2. Assignment of points for mitotic counts according to the field area, using several microscopes

	Microscope		
	Leitz Ortholux	Nikon Labophot	Leitz Diaplan
Objective	×25	×40	×40
Field diameter (mm)	0.59	0.44	0.63
Field area (mm^2)	0.274	0.152	0.312
Mitotic count*			
1 point	0–9	0–5	0–11
2 points	10–19	6–10	12–22
3 points	>20	>11	>23

* Assessed as number of mitoses per 10 fields at the tumour periphery.

ALLOCATION AND VALIDATION OF GRADE

To obtain the overall tumour grade the scores for each category are added together, giving a possible total of 3–9. Tumour grade is then allocated on the following basis:

3–5 points: grade I —well-differentiated
6–7 points: grade II —moderately differentiated
8–9 points: grade III—poorly differentiated

This method of assessing tumour differentiation (along with most other methods) is based essentially on a subjective assessment of morphological features. For this reason it is advisable, when feasible, to validate the results. In our study the tumours are graded independently by two histopathologists. In cases where assessment of grade differs, the disagreements are resolved by consensus after joint review using a conference microscope.

Results

Histological grade has been assessed in over 2200 cases of primary operable carcinoma of the breast in the Nottingham/Tenovus study since 1973. To allow adequate follow-up, patients presenting up to 1989 were studied. During this period 1951 consecutive patients were entered into the study; 103 were found to have pure ductal carcinoma *in situ* with no invasive element, in seven the histological material was not considered suitable for assessment of grading, and 11 patients had incomplete follow-up. A total of 1830 evaluable patients remained, and the relationship between grade and prognosis was analysed: 342 cases (19%) were grade I, 631 (34%) grade II and 857 (47%) grade III. Recurrence-free and survival curves were constructed by the life table method and differences analysed by the log rank test. There was a highly significant correlation between histological grade and prognosis; both recurrence-free interval and overall survival were worse in patients with poorly differentiated tumours compared with those with well-differentiated tumours (Figures 2 & 3).

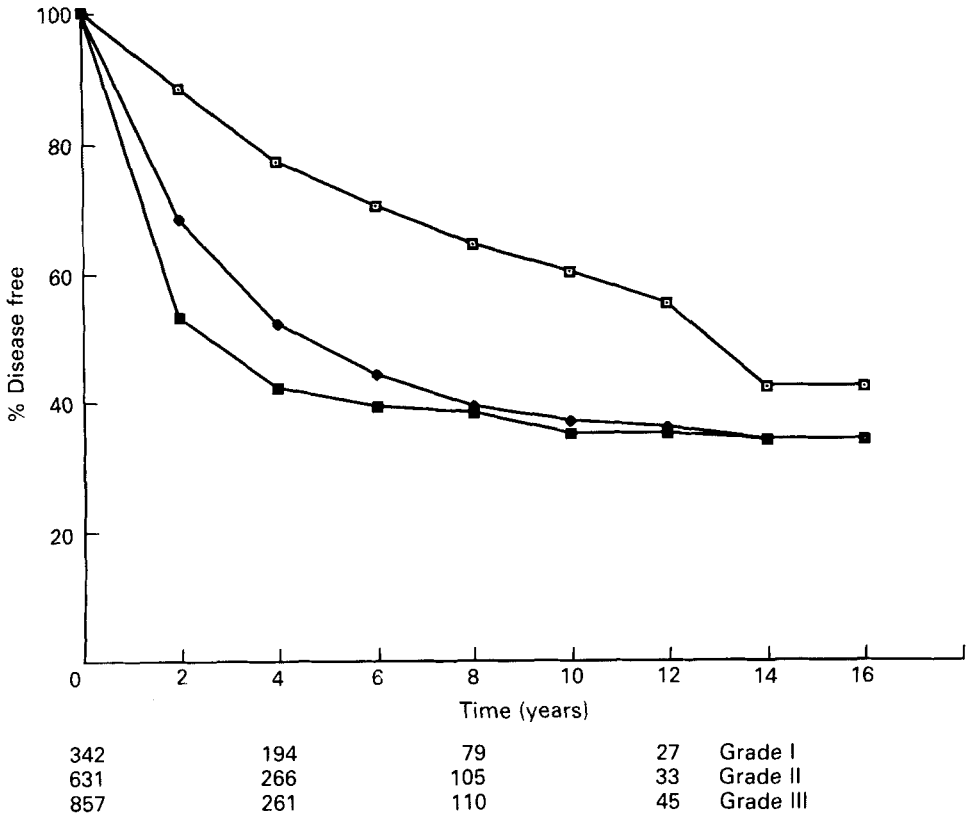

Figure 2. Relationship between histological grade (□ = I; ◆ = II; ■ = III) and recurrence-free interval in 1830 patients with primary carcinoma of the breast; $\chi^2 = 133.7$, 2 *d.f.*; $P < 0.0001$.

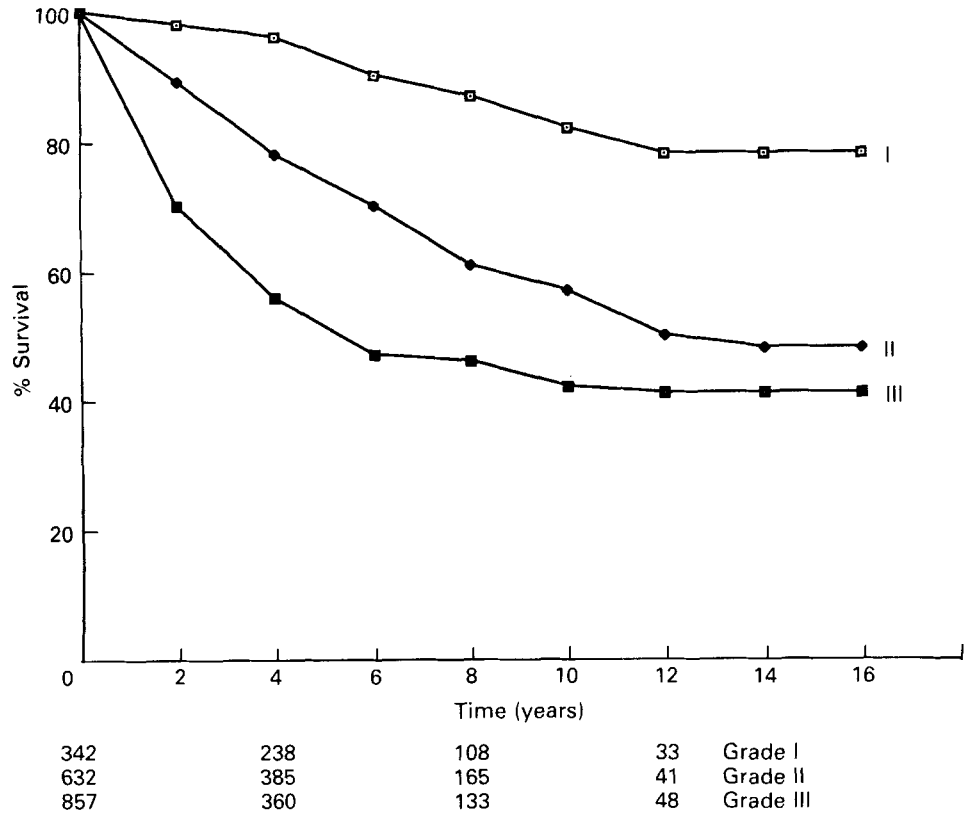

Figure 3. Relationship between histological grade (□ = I; ◆ = II; ■ = III) and overall survival in 1830 patients with primary carcinoma of the breast; $\chi^2 = 198.06$, 2 *d.f.*; $P < 0.0001$.

Table 3. Comparison of relative percentage of cases in each grade in different series

	Histological grade (% of cases)		
	I	II	III
Bloom & Richardson[9]	26	45	29
Wolff[16]	33	33	34
Tough *et al.*[18]	11	51	38
Champion *et al.*[19]	23	52	25
Fisher *et al.*[23]	11	23	66
Elston[5]	18	37	45
Contesso *et al.*[24]	21	50	29

Discussion

There is little merit in histopathologists attempting to assess the morphological differentiation of breast carcinoma unless it provides useful information which will be of practical value in patient management. Since the first, rather crude, study carried out by Greenhough[4], large numbers of reports have emphasized the strong significant correlation between histological grade as a measure of differentiation, and prognosis[6-9,11-24]. We have previously reported similar results from the Nottingham/Tenovus Primary Breast Cancer Study[5,10,25,26] and our current results, which form one of the largest single studies ever reported, amply confirm the value of histological grade as a prognostic factor.

Despite this considerable body of supportive evidence and the adoption of the Bloom & Richardson method by the World Health Organization[27], histological grading has not been widely accepted and is still not regarded as an important procedure in routine diagnostic histopathology. There are two main reasons for this; first, the method is subjective in nature and this is associated with perceived problems in consistency and reproducibility; secondly, for a method to become established as routine practice clinical demand is required, and this is dependent on the availability of a range of therapeutic options. Until comparatively recently this did not apply to breast cancer.

The subjective nature of histological grading is reflected in the varying proportion of each grade in different series (Table 3). It is unfortunate that many of the earlier studies were, in fact, carried out by clinicians[9,15,17,18] who, with respect, cannot have had the depth of experience of a trained histopathologist. No information was given in these publications concerning the verification of results by double- or cross-checking, essential in a subjective method. To achieve internal consistency in the Nottingham/Tenovus study, tumours are graded independently by two experienced histopathologists, who obtain over 90% agreement on first assessment. The remaining cases are resolved by joint examination on a conference microscope, i.e. agreement is reached by consensus. A similar degree of consistency was obtained by Fisher and colleagues within one centre[28], and they also achieved a discrepancy of only 6% by the same reviewer on different occasions[19].

Reproducibility between different centres is more difficult to achieve. Cutler *et al.*[29], using the nuclear grading method devised by Black[11,12], reported that only 60% agreement was reached between an experienced observer and a pathologist who had not previously graded tumours. Stenkvist *et al.*[30] analysed the WHO[27], Black[11,12] and Hartveit[13] methods and concluded that all had a low inter- and intra-observer variability. Interpretation of this study is difficult, since the authors give few details of their methodology (e.g. whether grading protocols were provided and *used* by each participant), and no reference is made to their level of experience in breast cancer histology. More encouraging results have recently been reported by Hopton and co-workers[31]. They evaluated the WHO system on a regional basis: the sections from 10 centres were graded independently by the contributing pathologist and a coordinating pathologist. The observer variation of 21.9% for 874 tumours was considered satisfactory. Experience and dedication are essential requirements for accurate histological grading, and the method should only be undertaken by trained histopathologists. Results should be double-checked, preferably by a second pathologist, but if this is not possible, by the same pathologist on a separate occasion. In addition, we believe that it is essential to have a defined protocol appropriate for the conditions (corrected microscopic field size) which is adhered to strictly by the observers.

Whilst recognizing that histological grading of breast cancer will always have an underlying subjective element, we have devised modifications to the Bloom & Richardson method[9] in order to introduce greater objectivity. In fact, the need for this was appreciated by Bloom & Richardson themselves when they improved upon the method used by Bloom[7,8] by adding a numerical scoring system. In our method we have taken this process a stage further by introducing more precise definitions for the assignment of points within each category of morphological features assessed.

Thus, for tubule formation Bloom & Richardson[9] made a subjective judgement as to whether tubular structures were 'well-marked', 'moderately well-formed' or present in only a 'slight' degree. In our method 1 point is given when 75% or more of the tumour is composed of

clear tubular structures exhibiting central lumina. This is an arbitrary cut-off point, but it was chosen to correspond with the percentage used by McDivitt[32] in his definition of tubular carcinoma. At the other end of the range 3 points are assigned if less than 10% of the tumour shows tubule formation. We have previously investigated the prognostic significance of the proportion of tubular structures in a pilot study which supports the use of these criteria[33].

Evaluation of nuclear pleomorphism is the least satisfactory element of any tumour grading system, and the only way in which differences can be identified accurately is by use of morphometry or image analysis, both very time-consuming procedures. In order to introduce a degree of objectivity we have suggested that tumour cell size and nuclear characteristics are compared with normal epithelial cells in adjacent breast tissue. The definition and number of nucleoli is also a useful feature.

It is in the assessment of mitotic counts that our method differs most from that described by Bloom & Richardson[9]. They analysed the relative numbers of both hyperchromatic nuclei and mitotic figures. It is now believed that hyperchromicity implies individual cell death or apoptosis rather than mitotic activity, and hyperchromatic or pyknotic nuclei should therefore be excluded. It is our practice only to include figures which clearly fulfil the morphological criteria for the various stages of mitosis, metaphase, anaphase and telophase. By excluding cells which may be in prophase, possible confusion with apoptotic cells and intratumoural lymphocytes is avoided. Bloom & Richardson were also imprecise in their numerical allocation for point scoring; phrases such as 'an occasional mitotic figure per high-power field' and 'about two or three figures per high-power field' were used, and the high-power field was not further defined. Since the size of fields varies from microscope to microscope[34], and this may have a significant effect on the reproducibility of a method, we have standardized our counts to a defined field area. The allocation of points for the number of mitoses per field was based on a detailed analysis of mitotic counting[35] which produced arbitrary but prognostically significant subdivisions. Using this standardization any microscope can be calibrated to produce comparable data (Table 2). To our knowledge, in only one other method, that of Contesso *et al.* [23], is reference made to expression of mitotic counts per defined field area. Unfortunately, in their method, which is also based on that described by Bloom & Richardson, no attempt is made to increase precision in the assessment of tubule formation and nuclear pleomorphism, and so the overall evaluation retains a considerable element of subjectivity. We believe that with the modifications referred to above we have reduced problems of consistency and reproducibility to a minimum.

The correlation with prognosis, especially long-term survival, supports the concept that histological grade provides a measure of tumour differentiation. Further evidence is supplied by the relationship between histological grade and more objective criteria of tumour differentiation and proliferation. Cell kinetic studies, using [^{3}H]-thymidine uptake, have shown that tumours with a high labelling index, indicating rapid replication, have a greater early relapse rate than those with a low index[36,37], and are more likely to be of poor histological grade. Similarly, a good correlation has also been found between histological grade and proliferation index demonstrated by immunostaining for the Ki-67 antigen[38–40]. Well-differentiated tumours tend to have a low proliferation index, whilst poorly differentiated tumours have a high index. Assessment of DNA content, using both static[41] and flow cytometry[42–44] has demonstrated a significant correlation between DNA ploidy and histological grade. Tumours which are DNA diploid are more likely to be well-differentiated, whilst DNA aneuploid tumours are usually poorly differentiated.

Associations between histological grade, growth factor receptors and oncogene products have also been demonstrated. Tumour expression of epidermal growth factor receptor confers a poorer prognosis and is more frequently observed in grade III tumours[45]. Similarly, the presence of certain oncogene products such as c-erbB2 shows a relationship to tumour behaviour and to histological grade. Tumour cell membrane immunoreactivity for c-erbB2 is associated with poorer survival and is more frequently seen in grade III tumours[46]. A relationship between cell morphology and amplification of certain genes has been proposed[47]. This is supported by the evidence emerging on *c-erbB2* amplification which is associated with large cell size in ductal carcinoma *in situ*[48]. Histological grading combines detail of cell morphology (nuclear pleomorphism) with a measurement of differentiation (tubule formation) and an assessment of proliferation (mitotic frequency). By extrapolating further, one could suggest that, potentially, histological grade provides an overview of a number of molecular events which are reflected in histological morphology. Measurement of one single molecular event is unlikely to provide powerful information in a high proportion of patients. We believe the future clinical application of molecular measures of prognosis will be in combination, providing information analogous to histological grade.

Although histological grade functions well as an independent prognostic factor, Bloom[8] showed that its

predictive value is improved by combination with histological lymph node stage. In the Nottingham/Tenovus study a composite prognostic index has been produced, following an analysis of a number of potential factors, using the multiple regression technique of Cox[49]. The only factors found to give a significant correlation with prognosis were tumour size (measured pathologically), histological lymph node stage and histological grade[26]. Using the coefficients of significance produced in the Cox analysis a simple numerical prognostic index (PI) has been devised as follows:

$$PI = 0.2 \times \text{tumour size} + \text{lymph node stage } (1-3) + \text{histological grade } (1-3).$$

Prognosis worsens as the prognostic index increases, and by using cut-off points of 3.4 and 5.4 patients may be stratified into good, moderate and poor prognostic groups having an annual mortality of 3, 7 and 30% respectively.[50]

In order to conform with the requirements of the Nottingham prognostic index, histological grading is carried out in all cases of invasive breast carcinoma, regardless of tumour type. It is recognized that special types such as pure tubular, pure mucinous and invasive cribriform carcinomas carry an excellent prognosis, whilst pure infiltrating lobular and medullary carcinomas appear to carry an intermediate prognosis between the first group and the common infiltrating duct carcinoma. It could be argued that histological grading should only be applied to the latter group of tumours, but a number of practical points are pertinent. When grade is assessed on tumours of particular histological types it is usually found to be appropriate. For example, infiltrating lobular carcinomas are usually designated grade II and the overall survival curve of lobular carcinomas overlaps that of all grade II tumours; similarly the special tumour types such as tubular or invasive cribriform carcinoma invariably have an excellent prognosis comparable with their grade I status. Medullary carcinoma of the breast is perhaps the only tumour which would contravene this approach. By definition, these tumours have grade III histology but are generally considered to have a prognosis more favourable than this grade would imply. In our series, however, despite use of very strict criteria, we have been unable to demonstrate a significant survival advantage for patients with medullary carcinoma when compared with other grade III tumours[33]. Typing of breast cancer is equally as subjective as grading, as demonstrated by the wide variation in recorded frequencies for special types[51]. Furthermore, up to a quarter of tumours in a series may be of combined or mixed type[28], and the prognostic significance of this group is unclear. If grading is restricted to the infiltrating ductal group, then in a symptomatic series at best 75%[52] and at worst only 53%[28] of cases will be assessed, and important prognostic information lost.

Methods for histological grading in breast cancer were first described over 50 years ago. Despite the clear correlation with survival which has been demonstrated in many studies, there is still a great reluctance to use such prognostic information in patient management. In Nottingham the prognostic index is included routinely on histological reports in breast cancer, and patients are counselled for appropriate therapy according to their prognostic group. Provided that grading is carried out by experienced pathologists and the guidelines described above are followed, consistent and reproducible results can be obtained. The importance of establishing histological grade in breast screening has been recognized by the inclusion of this method in the guidelines drawn up by the Royal College of Pathologists Working Group[53]. There is good evidence that grade compares favourably with kinetic and biochemical indices of differentiation, and until a more accurate predictor of prognosis becomes available it should be used as part of a standard prognostic index in all patients with breast cancer.

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References

1. Hansemann D von. Ueber assymetrische Zelltheilung in Epithelkrebsen und deren biologische Bedeutung. *Virchows Arch. Pathol. Anat.* 1890; **119**; 299–326.
2. Broders AC. Squamous-cell epithelioma of the lip. *JAMA* 1920; **74**; 656–664.
3. Broders AC. Squamous-cell epithelioma of the skin. *Ann. Surg.* 1921; **73**; 141–160.
4. Greenhough RB. Varying degrees of malignancy in cancer of the breast. *J. Cancer Res.* 1925; **9**; 452–463.
5. Elston CW. Grading of invasive carcinoma of the breast. In: Page DL, Anderson TJ, eds. *Diagnostic Histopathology of the Breast.* Edinburgh: Churchill Livingstone, 1987; 300–311.
6. Patey DH, Scarff RW. The position of histology in the prognosis of carcinoma of the breast. *Lancet* 1928; **i**; 801–804.
7. Bloom HJG. Prognosis in carcinoma of the breast. *Br. J. Cancer* 1950; **4**; 259–288.
8. Bloom HJG. Further studies on prognosis of breast carcinoma. *Br. J. Cancer* 1950; **4**; 347–367.
9. Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. *Br. J. Cancer* 1957; **11**; 359–377.

10. Elston CW. The assessment of histological differentiation in breast cancer. *Aust. N.Z. J. Surg.* 1984; **54**; 11–15.
11. Black MM, Speer FD. Nuclear structure in cancer tissues. *Surg. Gynecol. Obstet.* 1957; **105**; 97–102.
12. Black MM, Barclay THC, Hankey BF. Prognosis in breast cancer utilizing histologic characteristics of the primary tumor. *Cancer* 1975; **36**; 2048–2055.
13. Hartveit F. Prognostic typing in breast cancer. *Br. Med. J.* 1971; **4**; 253–257.
14. Elston CW, Gresham GA, Rao GS, Zebro T, Haybittle JL, Houghton J. The Cancer Research Campaign (Kings/Cambridge) trial for early breast cancer—pathological aspects. *Br. J. Cancer* 1982; **45**; 655–669.
15. Wolff B. Histological grading in carcinoma of breast. *Br. J. Cancer* 1966; **20**; 36–40.
16. Hamlin IME. Possible host resistance in carcinoma of the breast: a histological study. *Br. J. Cancer* 1968; **22**; 383–401.
17. Tough ICK, Carter DC, Fraser J, Bruce J. Histological grading in breast cancer. *Br. J. Cancer* 1969; **23**; 294–301.
18. Champion HR, Wallace IWJ, Prescott RJ. Histology in breast cancer prognosis. *Br. J. Cancer* 1972; **26**; 129–138.
19. Fisher ER, Redmond C, Fisher B. Histological grading of breast cancer. *Pathol. Annu.* 1980; **15**; 239–251.
20. Andersen JA, Fischermann K, Hou-Jensen K *et al.* Selection of high risk groups amongst prognostically favorable patients with breast cancer. *Ann. Surg.* 1981; **195**; 1–3.
21. Parl FF, Dupont WD. A retrospective cohort study of histologic risk factors in breast cancer patients. *Cancer* 1982; **50**; 2410–2416.
22. Fisher ER, Sass R, Fisher B *et al.* Pathologic findings from the National Surgical Adjuvant Project for breast cancer (protocol no. 4). Discriminants for tenth year treatment failure. *Cancer* 1984; **53**; 712–723.
23. Contesso G, Mouriesse H, Friedman S, Genin G, Sarrazin D, Rouesse J. The importance of histologic grade in long-term prognosis of breast cancer: a study of 1010 patients, uniformly treated at the Institut Gustave-Roussy. *J. Clin. Oncol.* 1987; **5**; 1378–1386.
24. Hopton DS, Thorogood J, Clayden AD, Mackinnon D. Histological grading of breast cancer; significance of grade on recurrence and mortality. *Eur. J. Surg. Oncol.* 1989; **15**; 25–31.
25. Blamey RW, Davies CJ, Elston CW, Johnson J, Haybittle JL, Maynard PV. Prognostic factors in breast cancer—the formation of a prognostic index. *Clin. Oncol.* 1979; **5**; 1–10.
26. Haybittle JL, Blamey RW, Elston CW *et al.* A prognostic index in primary breast cancer. *Br. J. Cancer* 1982; **45**; 361–366.
27. Scarff RW, Torloni H. Histological typing of breast tumours (international histological classification of tumours, no. 2). Geneva: World Health Organization, 1968.
28. Fisher ER, Gregorio RM, Fisher B. The pathology of invasive breast cancer. A syllabus derived from findings of the National Surgical Adjuvant Breast Project (protocol no.4). *Cancer* 1975; **36**; 1–85.
29. Cutler SJ, Black MM, Friedell GH, Vidone RA, Goldenberg IS. Prognostic factors in cancer of the female breast II. Reproducibility of histopathologic classification. *Cancer* 1966; **19**; 75–82.
30. Stenkvist B, Westman-Naeser S, Vegelius J *et al.* Analysis of reproducibility of subjective grading systems for breast carcinoma. *J. Clin. Pathol.* 1979; **32**; 979–985.
31. Hopton DS, Thorogood J, Clayden AD, MacKinnon D. Observer variation in histological grading of breast cancer. *Eur. J. Surg. Oncol.* 1989; **15**; 21–23.
32. McDivitt RW, Boyce W, Gersell D. Tubular carcinoma of the breast: clinical and pathological observations concerning 135 cases. *Am. J. Surg. Pathol.* 1982; **6**; 401–411.
33. Ellis IO, Broughton N, Elston CW, Blamey RW. The relationship of histological type to survival and oestrogen receptor status in primary operable breast carcinoma. *J. Pathol.* 1987; **152**; 219A.
34. Ellis PSJ, Whitehead R. Mitosis counting—a need for reappraisal. *Hum. Pathol.* 1981; **12**; 3–4.
35. Mann R, Elston CW, Hunter S *et al.* Evaluation of mitotic activity as a component of histological grade and its contribution to prognosis in primary breast carcinoma. *J. Pathol.* 1985; **146**; 271A.
36. Meyer JS, Hixon B. Advanced stage and early relapse of breast carcinomas associated with high thymidine labelling indices. *Cancer Res.* 1979; **39**; 225–235.
37. Tubiana M, Pejovic MJ, Renaud A *et al.* Kinetic parameters and the course of the disease in breast cancer. *Cancer* 1981; **47**; 937–943.
38. Barnard NJ, Hall PA, Lemoine NR, Kadar N. Proliferative index in breast carcinoma determined *in situ* by Ki67 immunostaining and its relationship to clinical and pathological variables. *J. Pathol.* 1987; **152**; 287–295.
39. Bouzubar N, Walker KJ, Griffiths K *et al.* Ki67 immunostaining in primary breast cancer: pathological and clinical associations. *Br. J. Cancer* 1989; **59**; 943–947.
40. Walker RA, Camplejohn RS. Comparison of monoclonal antibody Ki67 reactivity with grade and DNA flow cytometry of breast carcinomas. *Br. J. Cancer* 1988; **57**; 281–283.
41. Auer G, Eriksson E, Azavedo E, Caspersson T, Wallgren A. Prognostic significance of nuclear DNA content in mammary adenocarcinoma in humans. *Cancer Res.* 1984; **44**; 394–396.
42. Olszewski W, Darzynkiewicz Z, Rosen PP, Schwartz M, Melamed M. Flow cytometry of breast carcinoma. 1. Relation of DNA ploidy level to histology and estrogen receptor. *Cancer* 1981; **48**; 980–984.
43. Tavares AS. Ploidy and histological types of mammary carcinoma. *Eur. J. Cancer* 1967–68; **3**; 449–455.
44. Dowle CS, Owainati A, Robins A *et al.* The prognostic significance of the DNA content of human breast cancer. *Br. J. Surg.* 1987; **74**; 133–136.
45. Lewis S, Locker AP, Todd JH *et al.* Epidermal growth factor receptor expression in human breast carcinoma. *J. Clin. Pathol.* 1990; **43**; 385–389.
46. Lovekin C, Ellis IO, Locker AP, Robertson J, Bell JA, Elston CW, Blamey RW. CerbB2 oncoprotein expression in primary and advanced breast cancer. *Br. J. Cancer* 1991; **63**; 439–443.
47. Cardiff RD. Cellular and molecular aspects of neoplastic progression in the mammary gland. *Eur. J. Cancer Clin. Oncol.* 1988; **24**; 15–20.
48. Van de Vijver MJ, Peterse JL, Modi W *et al.* Neu-protein overexpression in breast cancer: association with comedo-type ductal carcinoma *in situ* and limited prognostic value in stage II breast cancer. *N. Engl. J. Med.* 1988; **319**; 1239–1245.
49. Cox DR. Regression models and life-tables. *J. R. Stat. Soc. (B)* 1972; **34**; 187–220.
50. Todd JH, Dowle C, Williams MR *et al.* A prognostic index in primary breast cancer. *Br. J. Cancer* 1987; **56**; 489–492.
51. Page DL, Anderson TJ, Sakamoto G. Infiltrating carcinoma: major histological types. In: Page DL, Anderson TJ, eds. *Diagnostic Histopathology of the Breast.* Edinburgh: Churchill Livingstone, 1987; 193–235.
52. Rosen PP. The pathological classification of human mammary carcinoma: past, present and future. *Ann. Clin. Lab. Sci.* 1979; **9**; 144–156.
53. Royal College of Pathologists Working Group. *NHS Breast Screening Programme. Pathology Reporting in Breast Cancer Screening,* 1990.