

REVIEW

# Breast cancer prognostic classification in the molecular era: the role of histological grade

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## Abstract

Breast cancer is a heterogeneous disease with varied morphological appearances, molecular features, behavior, and response to therapy. Current routine clinical management of breast cancer relies on the availability of robust clinical and pathological prognostic and predictive factors to support clinical and patient decision making in which potentially suitable treatment options are increasingly available. One of the best-established prognostic factors in breast cancer is histological grade, which represents the morphological assessment of tumor biological characteristics and has been shown to be able to generate important information related to the clinical behavior of breast cancers. Genome-wide microarray-based expression profiling studies have unraveled several characteristics of breast cancer biology and have provided further evidence that the biological features captured by histological grade are important in determining tumor behavior. Also, expression profiling studies have generated clinically useful data that have significantly improved our understanding of the biology of breast cancer, and these studies are undergoing evaluation as improved prognostic and predictive tools in clinical practice. Clinical acceptance of these molecular assays will require them to be more than expensive surrogates of established traditional factors such as histological grade. It is essential that they provide additional prognostic or predictive information above and beyond that offered by current parameters. Here, we present an analysis of the validity of histological grade as a prognostic factor and a consensus view on the significance of histological grade and its role in breast cancer classification and staging systems in this era of emerging clinical use of molecular classifiers.

## Introduction

Breast cancer is the most common cancer in women and a leading cause of cancer death worldwide. Management of breast cancer relies on the availability of robust clinical and pathological prognostic and predictive factors to guide patient decision making and the selection of treatment options. In early-stage breast cancer, where the use of systemic therapy has to be determined for every patient, the three main prognostic determinants used in routine practice are lymph node (LN) status, tumor size, and histological grade. The Nottingham (Elston-Ellis) modification of the Scarff-Bloom-Richardson grading system, also known as the Nottingham Grading System (NGS) [1], is the grading system recommended by

various professional bodies internationally (World Health Organization [WHO], American Joint Committee on Cancer [AJCC], European Union [EU], and the Royal College of Pathologists (UK RCPATH) [2,3]).

The prognostic relevance of NGS in breast cancer was initially demonstrated in 1991 [1] and has been validated subsequently in multiple independent studies [4-14]. Since NGS has independent but equally powerful prognostic value, it has been combined with LN stage and tumor size to form prognostic indices: the Nottingham Prognostic Index (NPI) [15], which includes NGS and LN stage with equal weighting, and the Kalmar Prognostic Index [4], in which grade is given a higher-weighted value. Owing to the prognostic information provided by NGS, it has also been incorporated in algorithms (for example, Adjuvant! Online [16]) and guidelines (for example, the St. Gallen guidelines [17]) to determine the use of adjuvant chemotherapy. NGS provides a simple, inexpensive, and routinely applicable overview of the intrinsic biological characteristics and clinical behavior of tumors, adding important information to other

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significant and at least partly time-dependent prognostic factors, such as tumor size and LN status.

Although the current well-established clinical and histological factors and some well-defined biological factors (that is, hormone receptors and HER2 expression) show strong association with prognosis and outcome, there are increasing concerns that these variables are limited in their ability to capture the diversity of clinical behaviors of breast cancer and that they would not be sufficient to tailor the therapy to individual patients. In addition, the perceived subjective nature of histopathological assessment of the morphological features such as tumor grade has increased these concerns. The introduction of high-throughput technologies that survey thousands of genes and their products in a single assay, coupled with powerful analytical tools, has opened up new avenues for classifying breast cancer into biologically and clinically distinct groups based on gene expression patterns [18,19] and DNA copy number alterations [20]. However, these expression profiling studies have suggested that molecular tests could perform better than the traditional histopathology and may replace it as the 'gold standard' for prognostication and prediction of response to therapy [21]. Recent studies leading to the development of the 21-gene recurrence score (trade name Oncotype DX; Genomic Health, Inc., Redwood City, CA, USA [22,23]) have highlighted the issue of subjectivity associated with histological grading and called into question the utility of histological grade as a prognostic tool. Regrettably, these results have been perceived as direct evidence that molecular tests provide an objective and reproducible assessment of prognostic features of estrogen receptor (ER)-positive breast cancers but that histopathological analyses are subjective and not reproducible.

Molecular methods undoubtedly provide prognostic and predictive information and may help identify new therapeutic targets, and the interest in molecular classifiers and their potential application is perfectly understandable. However, it is important to understand their limitations and critically evaluate their role in improving breast cancer prognostication above and beyond the traditional variables in a practical and cost-effective way [24,25]. The role of NGS as a simple and cost-effective method of assessment of tumor biology should not be neglected. It is also important to recognize that in countries with limited health resources, access to expensive new technologies may not be possible but that effective cost-efficient methods, such as routine histopathological evaluation, are available for all [26]. In fact, there are numerous lines of evidence to suggest that these molecular tests complement rather than replace the traditional pathological variables, such as NGS, to define the optimal therapy for patients with breast cancer.

Here, we present an overview of the current evidence of the significance of breast cancer grading in view of the availability of an increasing number of potentially alternative molecular prognostic tests. We present in a pragmatic way a comparison between NGS and recent molecular prognostic tests, taking into account evidence-based clinical and biological significance, cost-effectiveness, practicality of application in different parts of the world, and the impact of this on future plans for improvement in breast cancer prognostication and management.

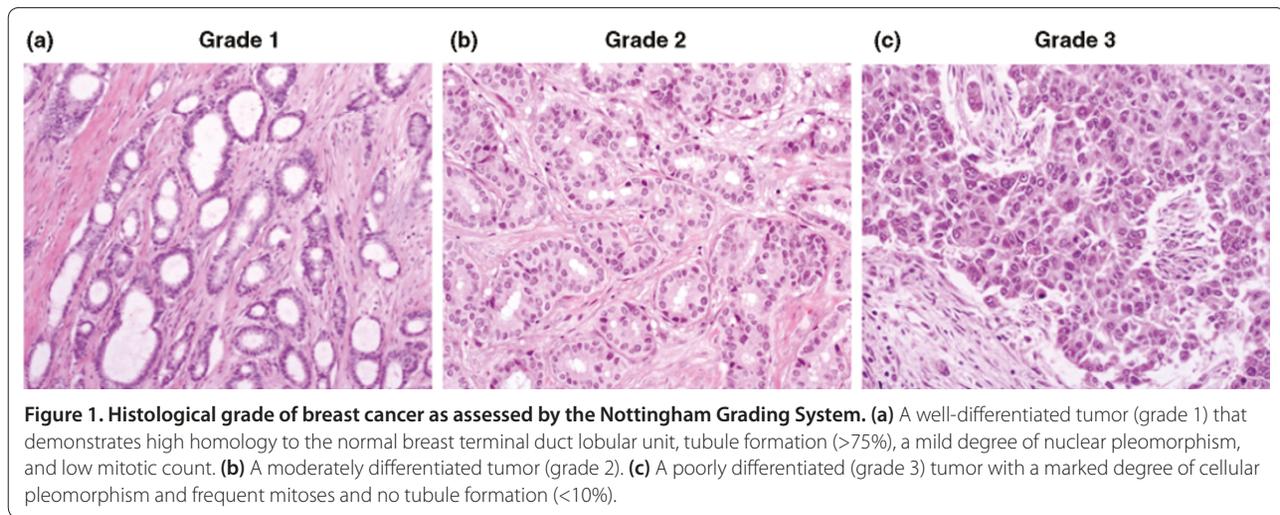
### **What is histological grade?**

Invasive carcinomas are morphologically subdivided according to their growth patterns and degree of differentiation, the latter of which reflects how closely they resemble normal breast epithelial cells. This subdivision is achieved by assessing histological type and histological grade, respectively. Although tumor type provides useful prognostic information, the majority (60% to 75%) of breast cancers have no special type of characteristics (that is, invasive ductal carcinoma of no special type, or NST); those special types that show distinct prognostic significance are relatively uncommon. As a consequence, the role of histological typing in clinical management decision making is currently limited [27].

Histological tumor grade is based on the degree of differentiation of the tumor tissue. In breast cancer, it refers to the semi-quantitative evaluation of morphological characteristics and is a relatively simple and low-cost method, requiring only adequately prepared hematoxylin-eosin-stained tumor tissue sections to be assessed by an appropriately trained pathologist using a standard protocol. NGS is based on the evaluation of three morphological features: (a) degree of tubule or gland formation, (b) nuclear pleomorphism, and (c) mitotic count (Figure 1). For details, see [1,2] and Supplementary Information [28].

### **Histological grade and prognosis**

Multiple independent studies have shown that NGS has prognostic value that is equivalent to that of LN status [29] and greater than that of tumor size [4,15]. In a large study, Henson and colleagues [14], who assessed survival rates of 22,616 cases of breast cancer, demonstrated that patients with histological grade 1, stage II disease had the same survival as those with grade 3, stage I disease. The authors also found that patients with grade 1 tumors of less than 2 cm in size had an excellent prognosis, with 99% 5-year survival, even when they presented with positive LN. These results are supported by a recent study from the Nottingham group [11], which included 2,219 operable breast cancer cases with long-term follow-up. This study has demonstrated that grade is an important determinant of breast cancer outcome and complementary to LN stage



through the ability to influence the outcome of patients in different LN stage categories. These results provide evidence that histological grade, when used in conjunction with LN stage, can improve the prediction of outcome for individual patients and support its inclusion and use in multifactorial indices such as the NPI and Adjuvant! Online. Similar long-term validation has been demonstrated in screen-detected breast cancer in the Swedish Two-County Trial, which demonstrated that tumor grade, LN status, and tumor size at the time of diagnosis have a lasting influence on subsequent survival [10].

There is compelling evidence to suggest that histological grade can accurately predict tumor behavior, particularly in earlier small tumors (tumor, node, metastasis [TNM] stage pT1), more than other 'time-dependent' prognostic factors such as tumor size (pT1a, pT1b, and pT1c) [4,9,11,15]. Studies have also demonstrated that grade is an independent prognostic factor in specific subgroups of breast cancer, including ER-positive breast cancer patients who have not [30] or who have received neoadjuvant endocrine therapy [31] and patients with LN-negative [5,11,13,32] or -positive [7,11] breast cancer regardless of ER expression. Recently, Desmedt and colleagues [33] demonstrated that in the ER-positive/HER2-negative tumors (n = 628), only histological grade and the proliferation module retained their association with relapse-free survival (RFS) in the multivariate analysis (hazard ratio [HR] = 2.00, 95% confidence interval [CI] 1.18 to 3.37;  $P = 0.01$ ). In the Nottingham series [11,34], histological grade was an independent predictor of RFS in the ER-positive/HER2-negative tumors (n = 1,077) (HR = 2.13, 95% CI 1.79 to 2.53;  $P < 0.0001$ ). Similar associations between grade and survival were found in (a) the LN-negative subgroup (n = 797), who received only adjuvant hormone therapy

(HR = 1.85, 95% CI 1.46 to 2.34;  $P < 0.0001$ , with rates of 10-year risk of relapse of 7% for grade 1, 14% for grade 2, and 31% for grade 3), and in (b) ER-positive tumors with small-volume LN metastasis (pN1; one to three LNs positive) (n = 316) (HR = 2.07, 95% CI 1.51 to 2.86;  $P < 0.0001$ , with rates of 10-year risk of relapse of 5% for grade 1, 24% for grade 2, and 43% for grade 3) [11,34]. Therefore, histological grade can provide important prognostic information for clinically relevant subgroups in which the benefit of chemotherapy is less certain (for example, LN-negative/ER-positive or in patients with low-volume LN metastatic disease).

We have noted, consistently with the biological and clinical roles of histological grade on breast cancer behavior, an important association between histological grade and pattern of survival. Akin to high-grade lymphoma, high-grade breast cancers tend to recur and metastasize early following diagnosis, typically within the first 8 years; thereafter, breast cancer-related deaths decrease in frequency. Low-grade tumors tend to show a very good outcome, and few (if any) events occur; those that do occur, do so relatively late in the lifetime of the patients. Grade 2 tumors show an intermediate outcome during the early years of follow-up; however, on long-term follow-up, they show an obvious trend for continued recurrence and impaired outcome in the long term [11,35] (Figure 2). In contrast, LN stage, which can provide information on the likelihood of death or survival after breast cancer, shows limited value in predicting the timescale of these events (Rakha EA, Ellis IO, unpublished data). This important observation provides further insight into the appropriate management strategies of patients with breast cancer. High-grade tumors, with their risk of early recurrence and death, require consideration for prompt use of adjuvant chemotherapy, whereas patients with grade 1 tumors, which are almost

invariably ER-positive, could be offered a long-term follow-up with or without a potentially less toxic systemic therapy (that is, endocrine therapy).

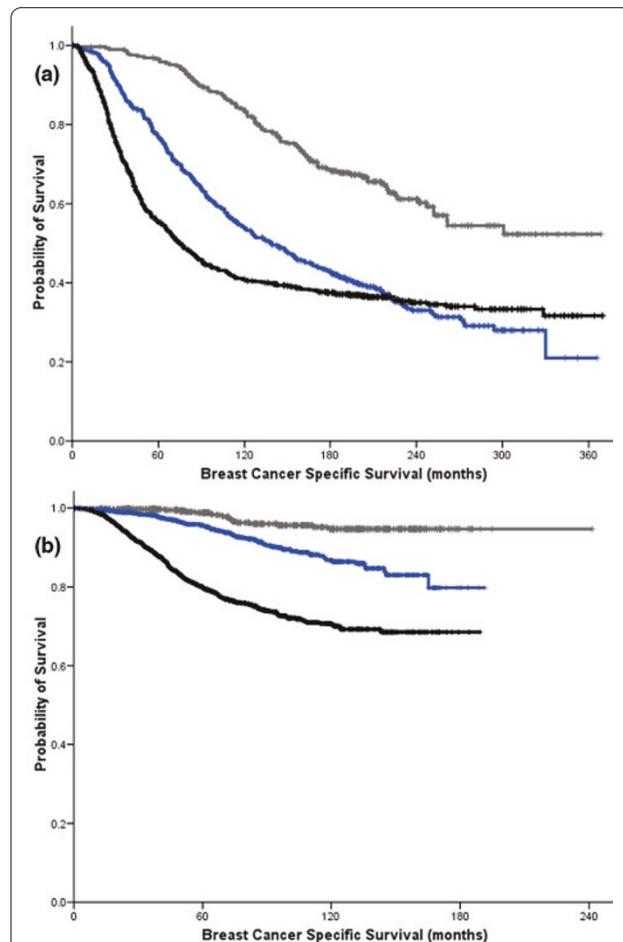
### Histological grade: contentious issues

Despite the utility of histological grade as a prognostic factor for ER-positive disease, there are numerous issues that ought to be considered for the correct use of histological grade in the management of patients with breast cancer [36-39]. These issues are detailed in the following sections.

### Grade and size

The latest (7th edition) AJCC TNM staging system endorsed NGS, but grade was not included in calculating stage [40]. The decision to exclude grade as an element in the TNM staging system, as stated previously [36], is based mainly on the possible interaction between tumor size and histological grade and, in particular, the lack of clear evidence for the role of grade in small tumors (pT1 and pT2). It should be noted that two of the basic principles of breast cancer screening are that outcome of patients with small invasive cancers is good and adverse events are rare. The effect of all known prognostic factors will therefore be limited in such a patient group. However, despite this constraint, there are several lines of evidence that demonstrate the prognostic significance of NGS in small tumors. Studies that examine the prognostic significance of grade in small tumors quoted in the AJCC article by Singletary and colleagues [36] show marked variations in outcome, follow-up times, and number of patients. There was also variation in the grading method used, and information on histological grade was obtained from systematic pathology review, whereas in others, information about grade was abstracted from pathology reports, medical records, or tumor registry databases. These differences in grading systems and study design are expected to lead to different results regarding the prognostic significance not only of grade but also of other variables should they have been assessed. To conclude, although extracting consistent data on the prognostic significance of grade from the different studies cited in the AJCC review [36] is challenging, studies in which modern methods for histological grading were employed have shown that its utility is retained in small tumors [1,5,6,9,11,27,32,41-43]. With the shift that mammographic screening causes in stage distribution, this issue has become increasingly important, with a high proportion of tumors being T1N0M0 at diagnosis, thereby limiting the relevance of TNM staging in routine practice.

In the Nottingham series, development of recurrent disease following diagnosis of grade 1 breast cancer was infrequent, and when observed, the recurrent lesions



**Figure 2. Relationship between histological grade and breast cancer-specific survival. (a)** In the old Nottingham series (1977 to 1989), no systemic therapy was offered to the patients. Of the 1,816 patients, 404 (17.7%) had grade 1 tumors (gray curve), 621 (36.2%) had grade 2 (blue curve), and 791 (46.1%) had grade 3 (black curve) ( $\chi^2 = 97.5, P < 0.0001$ ). **(b)** In the recent Nottingham series (1990 to 2002), systemic therapy was offered to the patients according to Nottingham Prognostic Index and estrogen receptor expression as described previously [11]. Of the 3,579 patients, 677 (18.9%) had grade 1 tumors (gray curve), 1,383 (38.6%) had grade 2 (blue curve), and 1,519 (42.4%) had grade 3 (black curve) ( $\chi^2 = 195.5, P < 0.0001$ ). Analysis of grade 1 and 2 only showed statistical survival difference ( $\chi^2 = 20.7, P < 0.0001$ ). Both series are consecutive and included estrogen receptor-positive and -negative and lymph node-negative and -positive cases [11,75,76].

were either higher-grade tumors or second primaries. The number of patients with grade 1 tumors who developed distant metastasis or died without developing a second event of higher-grade tumor was limited (4%) [44]. This observation implies that grade 1 breast cancer studies that do not include pathology review and evaluation of the second event are likely to overestimate the risk of adverse outcome.

Further justification for the exclusion of grade from TNM [36] is that large tumors (pT3 and pT4) tend to be

high-grade and nearly always carry a recommendation for adjuvant chemotherapy, irrespectively of tumor grade. Although a higher proportion of larger tumors are grade 3 [8,11], some forms of lower-grade breast cancer such as hormone receptor-positive, low-grade invasive lobular cancers frequently present as large mammographically occult tumors and are responsive to hormone therapy. Furthermore, if tumor size/stage alone largely dictates an oncologist's choice of treatment, an argument could be made, at least in many centers, for the irrelevance of other biological variables, including gene expression signatures such as Oncotype DX, 70-gene signature, 76-gene signature, and genomic grade index (GGI). Likewise, although NGS might have limited prognostic value in HER2-positive and triple-negative cancers as most of these tumors are of high grade (grade 3) [30,33], these tumors also typically exhibit poor-prognosis gene signatures [45]. In addition, molecular classifiers such as Oncotype DX and GGI [33] and the MammaPrint (Agendia, Amsterdam, The Netherlands), the last of which is recommended to all patients, have negligible discriminatory power in ER-negative disease [30,33].

Therefore, we believe that treatment decisions based on TNM staging system, which measures the anatomic extent of the tumor, can be improved by the addition of histological grade, which measures the intrinsic biological features of the tumor and reflects the potential of a carcinoma to metastasize or cause death. Integration of histological grade into the relevant TNM staging system has been accepted for other common epithelial tumors such as adenocarcinoma of the prostate. As the prognostic value of NGS has been proven in operable breast cancer (stages I and II), in which decisions about systemic therapy usage and its regimen need to be made, histological grade could be incorporated in the TNM system to improve its ability to stratify cases into risk-associated subcategories corresponding to grade so that chemotherapy can be potentially be avoided in low-risk groups and considered a high priority for patients in a high-risk category. The maximum benefit of grade assessment would be in the subgroup of patients with ER-positive, LN stage N0 or N1 disease. The current evidence indicates that grading has limited value in advanced or metastatic breast cancer (stages III and VI) and grading is not expected to change treatment decisions and therefore need not be considered in these cases.

#### **Grade and tumor type**

The prognostic value of histological grade has been documented in most tumor types, including invasive lobular carcinomas [46]. Medullary carcinoma might appear to be one subtype in which grading is less significant. By definition, these tumors are of high histological grade

(grade 3) but may have a more favorable prognosis than their histological grade would imply [38]. However, a recent study shows that medullary carcinomas account for less than 1% of breast cancers as a result of the strict criteria required for its recognition and that they do not have a prognosis significantly different than that of other forms of grade 3 ductal carcinoma with prominent inflammation [38]. Importantly, a recent study suggested that the 70-gene prognostic signature may also fail to provide prognostic stratification of patients with some special types of breast cancer. Given that NGS has been shown to provide prognostically relevant information for invasive ductal carcinomas of NST and lobular carcinomas, which together account for greater than 80% of all breast cancers, the systematic inclusion of histological type in breast cancer routine synoptic reports is also advocated.

#### **Grading of needle core biopsy specimens**

Current evidence suggests that histological grading can be assessed relatively reliably whereas other well-established prognostic factors, such as vascular invasion and tumor size, cannot [47,48]. However, some cases may be upgraded when the excision specimen is analyzed following grading of core biopsies (that is, grade I in the core biopsy and grade II in the excision specimen; 30% to 40%). On the other hand, a diagnosis of NGS grade III in a core biopsy is not commonly changed when the excision specimen is graded (5% to 8%). Importantly, changes from grade I in the core to grade III in the excision specimen and vice versa are very rare (0% to 1%) [47,48].

Selection of patients for neoadjuvant therapy requires that prognostic information be available from non-operative diagnostic tumor samples. Amat and colleagues [49] reported that assessment of grade on needle core biopsy (NCB) is a strong predictive factor of response to induction chemotherapy in breast cancer, independently of the type of regimen used. Therefore, despite the limitations associated with the accuracy of grading core biopsies related to tumor sampling issues and visibility of mitotic figures [47,48], assessment of histological grade on NCB can provide information to support preoperative treatment decision making.

#### **Reproducibility of histological grade**

One of the reasons cited in the past for the reluctance to use grading in patient management decisions has been the perceived lack of reproducibility of the method. This may be highlighted by the relatively wide variation in the proportion of each grade in published series. However, a substantial number of studies have reported better levels of inter- and intra-observer concordance [1,27,50-59] (Table 1). The variation in the proportion of each grade reported in the different studies can be explained by the

**Table 1. Inter-observer and intra-observer agreement of breast cancer histological grade.**

Study	Number of cases	Number of readers	Grade	Inter-observer
[32]	613	2	NGS	Kappa 0.69
[8]	52	2	NGS	Kappa 0.54
[55]	425	2	NGS	Complete agreement 76%
[50]	75	6	NGS	Kappa 0.43 to 0.74
[51]	12	600	NGS	Kappa 0.45 to 0.53 (figures after application of guidelines)
[52]		3	NGS	Complete agreement 72.3%; kappa 0.57
[53]	24	21	NGS	Complete agreement 69%; kappa 0.53
[54]	50	5	NGS	Mean polychoric correlation 0.8
[56]	35	13	NGS	Kappa 0.5 to 0.7
[57]	93	7	NGS	Kappa 0.54
[58]	40	3	NGS	Kappa 0.68 to 0.83
[59]	874	2	WHO criteria	Complete agreement 78.1%; kappa 0.66
[61]	50	5	NGS	Complete agreement 83.3%; kappa 0.73

NGS, Nottingham Grading System; WHO, World Health Organization.

variation in the grading system used and the difference in the patient cohorts, including age distribution, symptomatic versus screening population, early versus advanced breast cancer groups, and details of tissue fixation. Grading is dependent on a high quality of tissue preservation. Suboptimal levels of tissue fixation lead to disruption and loss of visibility of mitotic figures, one of the three variables assessed in NGS. Assessment of grade in poorly fixed tissue will therefore introduce a bias leading to a reduction in the proportion of cases classified as grade 3 [1,2,60].

Another important point to improve the inter-observer agreement rates is the introduction of guidelines for standardization of pre-analytical parameters, including tissue handling, fixation, and preparation, and of the methods for tumor grading. Differences between centers can be attributed in many cases to differences in the quality of tissue preparation [2,61]. Critical evaluation of these issues and recommendations for good practice have been provided by professional organizations (that is, WHO, EU, UK RCPATH, and the International Union Against Cancer [UICC]) [2]. The use of rigorously optimized and standardized methods in Nottingham has provided a high NGS reproducibility between grading of a recent series [11] and that of an old series published more than two decades ago from the same institution with a similar percentage of cases in each grade (Table 2). Significant improvements in the consistency of histological grading have been observed on a national basis in the UK through the publication of guidelines with linked educational activity and associated external quality assurance (EQA) [51]. These guidelines provide not only information on histological grading methodology but also recommendations on the application of these

methods and guidance on tissue handling. Adherence to these guidelines and participation in EQA are also expected to improve assessment of other important prognostic factors in breast cancer, such as lymphovascular invasion and immunohistochemical determination of other biomarkers. In addition, the current use of NGS is expected to provide consistency among different studies in the future as evidenced from multiple studies from the Nottingham group and other institutions that endorse NGS [2,39,60,62]. However, despite the objective improvements that have been made to breast cancer grading methods, any assessment of morphological characteristics inevitably retains a subjective element and is heavily dependent on the pre-analytical parameters.

It should be noted that the degree of scrutiny of the inter-observer reproducibility histological grade has never been applied to molecular tests in current clinical use. A more detailed reproducibility study of the performance of gene expression studies has not been conducted as of yet. In fact, issues of reproducibility are well recognized in all forms of medical laboratory testing. Despite the undeniable need to improve the inter-observer agreement for histological grade, the criticisms directed against NGS should be tempered by the fact that other parameters used to determine the therapy of patients with breast cancer also suffer from inter-observer variability, including the assessment of small-volume nodal metastases (LN stage), HER2 immunohistochemical and *in situ* hybridization scoring, ER scoring, assessment of vascular invasion, and even the assessment of tumor size.

#### Significance of grade 2 tumors

Mis-assignments of grade I to grade III or vice versa are rarely reported, but grade II tumors usually show the

**Table 2. Proportion of grades among different studies.**

Study	Number of cases	Grade 1	Grade 2	Grade 3
Elston, 1984 [77]	625	17%	37%	46%
Davis <i>et al.</i> , 1986 [78]	1,537	22%	49%	29%
Hopton <i>et al.</i> , 1989 [59]	874	29%	46%	25%
Le Doussal <i>et al.</i> , 1989 [79]	1,262	11%	45%	46%
Balslev <i>et al.</i> , 1994 [80]	9,149	32%	49%	19%
Saimura <i>et al.</i> , 1999 [5]	741	19%	37%	44%
Reed <i>et al.</i> , 2000 [32]	613	25%	41%	35%
Simpson <i>et al.</i> , 2000 [7]	368	22%	45%	33%
Lundin <i>et al.</i> , 2001 [6]	1,554	26%	47%	27%
Frkovic-Grazio and Bracko, 2002 [9]	270	38%	38%	24%
Warwick <i>et al.</i> , 2004 [10]	1,988	23%	37%	40%
Williams <i>et al.</i> , 2006 [26]	1,058	20%	46%	34%
Rakha <i>et al.</i> , 2008 [11]	2,219	18%	36%	46%
Thomas <i>et al.</i> , 2009 [81]	1,650	26%	45%	29%
Blamey <i>et al.</i> , 2009 [12]	16,944	29%	41%	30%

lowest degree of concordance. This is an expected phenomenon of scoring of a biological variable where scores in the overlap regions are usually most difficult to be categorised. The similar example of the problem of reproducibility of classification of a continuous biological variable was noted in the microarray-based gene expression profiling studies [63,64]. For example, in the studies by Sorlie and colleagues [18] and Chang and colleagues [65], only a proportion of cases could be accurately classified into the molecular subtypes, 9% to 15% of tumors could not be assigned as grade 1 and grade 3 by GGI [66], and 19% to 24% of cases showed discordance among different gene expression signatures applied to the same set of tumors [45].

In the latest meeting of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer (2009) [17], it was recommended that grade 1 and grade 3 be taken into consideration for the assessment of indications of adjuvant chemotherapy. Grade 2 was regarded as being similar to other parameters of intermediate-risk significance, such as tumor size of between 2 and 5 cm, low numbers (one to three) of involved LNs, and intermediate scores on multigene assays, and it was inferred that they do not provide a definitive indication of risk with respect to the clinical decision of whether to give or withhold chemotherapy. However, it was also noted that the presence of these intermediate-risk criteria usually tips the balance toward the use of chemotherapy [17].

The advantage of applications of molecular tests as complements to grade is particularly evident with respect to grade 2 tumors. Several attempts have been made to improve biological and clinical significance of histological

grading by classifying grade 2 tumors into two distinct subclasses: a grade 1-like subgroup, which has an excellent outcome and may not require adjuvant chemotherapy, and a grade 3-like subgroup, which comprises tumors that behave in a way similar to high-grade cancers and need a more aggressive systemic treatment. Examples of these studies include the application of GGI to subclassify histological grade 2 into two molecular subclasses (GGI1 and GGI3) [66] or the use of proliferation biomarkers such as MIB1 (Ki67) expression (Rakha EA, Ellis IO, unpublished data). However, the clinical usefulness and the cost-benefit ratios of these studies need to be further evaluated if they are to be translated into routine practice worldwide, particularly in countries with limited resources.

### Grade and molecular profiling

Recent profiling studies of breast cancer have emphasized the relevance of tumor biology in governing breast cancer behavior and hence the importance of histological grade. Tumors of different histological grades show distinct molecular profiles at the genomic, transcriptomic, and immunohistochemical levels. These results suggest that the majority of high-grade tumors are unlikely to stem from the progression of low-grade cancers and that grade 1 and 3 breast tumors are probably different diseases [67].

Gene expression studies have demonstrated that histological grade better reflects the molecular makeup of breast cancer than LN status and tumor size do [68,69]. Sotiriou and colleagues [66] developed a 97-gene classifier that can accurately identify cases diagnosed as NGS I or NGS III. Their studies have shown an association between a 'gene signature' developed to recapitulate

histological grade (GGI) of breast cancer and patient outcome, independently of LN status or tumor size [66]. This assay is currently being commercialized in Europe (MapQuant Dx; Ipsogen, Marseille, France). When the prognostic performance of GGI was compared with the Oncotype DX [22] and 70- and 76-gene signatures, a similar separation in distant metastasis-free survival between low- and high-risk groups by the three signatures was found [45]. Another group has similarly demonstrated that the genetic grade signature (RNA-based) remained significantly associated with disease recurrence in most cases.

However, recent meta-analyses of microarray-based expression profiling studies have demonstrated that the prognostic impact of the signatures investigated stems from the proliferation-related genes [30,33]. In fact, when several of the published signatures were divided into partial signatures composed of proliferation-related genes and genes not related to proliferation, the latter failed to show prognostic significance, whereas the prognostic power of some signatures even improved by the removal of genes not related to proliferation [30]. Most importantly, in numerous studies using molecular signatures, histological grade remained an independent prognostic factor for ER-positive tumors even after the inclusion of gene signatures in the multivariate models [22,25].

There are several lines of evidence to suggest that the objective contribution of gene signatures above and beyond the current clinicopathological parameters is limited. Dunkler and colleagues [70] demonstrated that the explained variation of prognosis (that is, the proportion of patients whose prognosis is determined solely by a given parameter) by prognostic signatures is limited (for example, 3% for the 70-gene signature) when grade and other clinicopathological variables such as LN stage, patient age, and ER status are included in the survival models [30,70,71]. It is important to mention, however, that there are relatively few head-to-head comparisons of NGS versus molecular signatures and that most of them so far have a competitive tone to them. Studies that combine molecular assays and NGS in a balanced manner would be warranted.

### **Grade in the era of molecular profiling tests**

Prognostic molecular tests for patients with breast cancer, including Oncotype DX [22] and MammaPrint [72], have already been approved for clinical use. Undoubtedly, these assays support breast cancer prognostication and can be used as a complement to the well-established variables currently used in routine practice [73], as recently recommended in the St. Gallen guidelines [17].

The cost of MammaPrint and Oncotype DX [74] is orders of magnitude higher than that of histological

grading. Oncotype DX has undergone health economic evaluation in the US and has been reported to be cost-beneficial through reduction of widespread use and appropriate targeting of use of adjuvant chemotherapy. In countries or centers where chemotherapy is less widely prescribed or where targeting is based on other tests, there may be a reduced benefit and justification of the test cost [26]. There is a trend in the research community not to consider cost-effectiveness when promoting the use of a newly developed molecular test, even though costs typically are taken into consideration when evaluating new interventions [26,74]. The costs of these modern assays are likely to remain high, and it should be borne in mind that there are still many parts of the world that do not and will not have ready access to these costly tests. Therefore, histological grading, when carried out properly on well-fixed specimens, provides a simple, inexpensive, and highly accurate alternative method for assessing tumor biological characteristics and patient prognosis and identifying patients at high and low risk for adverse outcomes. In addition, the cost and availability are not the only factors limiting the routine applicability of currently approved or recommended molecular prognostic assays as there may also be some skepticism of the scientific rigor of industry-sponsored cost-benefit economic models.

Given that grade has been shown by multiple independent groups to be prognostic and that the levels of inter-observer agreement have increased with the adoption of NGS, it is rather surprising that clinical practice has changed with molecular tests that have not been as comprehensively tested but has not changed with NGS. Possibly, this stems from the purported objectivity of molecular tests and the denounced subjectivity of histopathological analysis [25]. However, molecular tests also suffer from subjectivity in terms of the biostatistical approaches employed, the stability of the molecular subgroups identified by the tests, and the reproducibility of assays performed with cell extracts without careful microdissection of tumor cells (that is, contamination with normal breast epithelial cells or proliferating stromal cells may change the results of molecular tests based on the assessment of ER- and proliferation-related genes) [25]. Therefore, it should be recognized that both molecular assays and NGS have their own strengths and weaknesses, which vary in different situations. Both can provide valuable prognostic information and both should complement rather than compete with each other and this should be understood when they are used for patient management decision making. When used in combination, molecular tests such as GGI are potentially important in the subclassification of grade 2 breast tumors. However, the application of molecular tests to known grade 1 and grade 3 breast cancers in the treatment

decision-making process may need further validation and confirmation of any additional prognostic value and cost benefit.

## Conclusions

There is an international consensus that NGS should be considered the 'gold standard' for breast cancer grading. The adoption of the objective criteria of NGS has been shown to overcome many of the previous problems of reproducibility of grading, problems that resulted from using a variety of approaches. To provide a consistent and uniform way of assessing histological grade and to improve its reproducibility, consensus criteria and guidelines have been published with critical evaluation of these issues and recommendations for good practice [2]. Strict adherence to these criteria is expected to improve consistency and reproducibility of breast cancer grading among different institutions. Histological grading, when adequately carried out, provides a simple, inexpensive, and highly accurate method for assessing tumor biological characteristics and patient prognosis. This is of particular importance for breast cancer patients in parts of the world where access to new molecular technology is not currently available. Molecular assays and NGS should complement rather than compete with each other. We conclude that the assessment of histological grade is an important determinant of breast cancer prognostication and should be incorporated in staging systems and in algorithms to define therapy for patients with breast cancer.

## Take-home messages

The Nottingham Grading System, when adequately carried out, provides a simple, inexpensive, accurate, and validated method for assessing patient prognosis.

Consensus criteria for histological grading and recommendations for good practice have been published [2,51] and should be followed.

The Nottingham Grading System is a validated alternative to molecular tests in parts of the world where access to new molecular technology is not currently available or likely to become available in the near future.

Assessment of histological grade is an important determinant of breast cancer prognostication and should be incorporated in algorithms to define therapy for patients with breast cancer.

## Search strategy and selection criteria

Literature databases, including PubMed, Medline, and the Cochrane Library, were searched for articles published from 1980 to 2009 in English. The keywords used for the search were 'breast cancer', 'grade', 'histologic(al) grade', 'molecular profile', and 'reproducibility' in relation to biology, prognosis, prediction, and patient

outcome. Articles published before 1980 or in another language were also considered if they were commonly referenced or were highly regarded older publications. The search also included the references list for these articles and selected additional articles and webpages that were judged to be relevant. Data from publications submitted as abstracts were excluded.

## Abbreviations

AJCC, American Joint Committee on Cancer; CI, confidence interval; EQA, external quality assurance; ER, estrogen receptor; EU, European Union; GGI, genomic grade index; HR, hazard ratio; LN, lymph node; NCB, needle core biopsy; NGS, Nottingham Grading System; NPI, Nottingham Prognostic Index; NST, no special type; RFS, relapse-free survival; TNM, tumor, node, metastasis; UK RCPATH, Royal College of Pathologists; WHO, World Health Organization.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

EAR and JSR-F performed the literature review and helped write the first draft of the manuscript. SB and IOE helped write the first draft. All authors contributed to the writing and approval of the final and revised drafts of the manuscript.

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