Histological tumour grade (Core)

Histological grading provides powerful prognostic information and within each stage grouping there is a relationship between histologic grade and outcome.

All invasive breast carcinomas should be graded. The Nottingham combined histologic grade (Elston-Ellis modification of Scarff-Bloom-Richardson grading system) is the recommended method.[1] It requires some commitment and strict adherence to the accepted protocol. The method involves the assessment of three components of tumour morphology: tubule/acinus/gland formation, nuclear atypia/pleomorphism and frequency of mitoses. Each is scored from 1 to 3. Adding the scores gives the overall histological grade, as shown below. The use of terms such as well differentiated or poorly differentiated in the absence of a numerical grade is inappropriate.

Overall grade

- Grade 1 = Scores of 3–5
- Grade 2 = Scores of 6 or 7
- Grade 3 = Scores of 8 or 9.

Published ratios for grades 1, 2 and 3 are approximately 2:3:5 in symptomatic breast cancer, with about half of all symptomatic cancers assigned as grade 3. Screen detected cancer series are likely to include a smaller proportion of high-grade cases. Poor fixation impairs accurate assessment of mitotic frequency reducing their visibility which can result in a change in grade ratios typically with a larger proportion of grade 2 cases and a lower proportion of grade 3 cases. If audit of grade distribution in symptomatic cancers shows substantially fewer grade 3 cases, or a majority of grade 2 cases, fixation and grading protocols should be reviewed.

Some degree of variation in appearance from one part of a tumour to another undoubtedly occurs; this is particularly true of tumours of mixed type. Assessment of tubular differentiation is made on the overall appearances of the tumour and so account is taken of any variation. Nuclear appearances are evaluated at the periphery and/or least differentiated area of the tumour to obviate differences between the growing edge and the less active centre. The mitotic score is determined by the number of mitotic figures found in representative 10 consecutive high power fields (HPF) in the most mitotically active part of the tumour. Representative field selection is based on fields having appropriate tumour cellularity based on assessment of the overall cellularity of the tumour identified at low magnification scanning. Fields with low or no tumour cells should not be counted. A random meander approach counting only representative fields is recommended. Only clearly identifiable mitotic figures should be counted; hyperchromatic, karyorrhectic, or apoptotic nuclei are excluded. Because of variations in field size, the HPF size must be determined for each microscope and the appropriate point score determined accordingly, which can also be designated as mitoses/mm^2 (see separate section below).

Assessment of grade on needle core biopsies

Histological grade can be assessed on core biopsies using the approach described above. This is of particular value if the patient has pre-operative systemic treatment or if grade in the surgical specimen is not assessable. There is about 70% agreement on grade between core biopsy and subsequent surgical specimen. Usually the histological grade in the surgical specimen is used in preference to the core grade. However, if assessment of grade in the surgical specimen is compromised, for example by poor fixation or pre-operative systemic treatment it is reasonable to use the mitotic count score in the core biopsy. Another alternative is to use the mitotic count score in nodal metastases if interpretation of grade is difficult in the primary carcinoma.

Assignment of Glandular (Acinar)/Tubular differentiation score

All parts of the tumour are scanned, and the proportion occupied by tumour islands showing clear acinar or gland formation or defined tubular structures with a luminal space is assessed semi-quantitatively. This assessment is generally carried out during the initial low power scan of the tumour sections. A tumour in which 75% or more of its area is composed of such structures would score 1 point for gland/tubule formation. A tumour with between 75% and 10% of glandular/tumour area would score 2 points. A tumour with less than 10% gland/tubule formation would score 3 points. These rules apply to tumours with simple gland/tubule formation such as invasive tubular carcinoma, and those exhibiting complex gland formations such as invasive cribriform carcinoma.

In the assessment of gland/tubule formation, only structures in which there are clearly defined central lumens, surrounded by polarised tumour cells, should be counted. This does, however, include larger islands of tumour with central gland formation, as may be seen in mucinous carcinoma or invasive micropapillary tumours. Thus mucinous, micropapillary and pure papillary tumours without, or with <10%, secondary luminal spaces are classified as having no tubular or glandular formation and assigned a score of 3. Papillary structures are also not regarded as glandular/tubular structures. Artefactual 'false' spaces can occur as a consequence of sub optimal fixation and tissue freezing. Such spaces should be excluded from assessment.

Intracytoplasmic lumen formation (intracytoplasmic vacuoles with true luminal microvillar surface, PAS+) does not count as gland formation whatever the size of the intracytoplasmic vacuoles.

Assignment of Nuclear Pleomorphism score

Individual pathologists differ markedly in their approach to nuclear grading, and breast specialists appear to allocate higher grades than non-specialists. Few cancers possess the very bland nuclei warranting an atypia/pleomorphism score of 1, and obvious atypia/pleomorphism should attract a score of 3. The minimum proportion of tumour nuclei which should show marked nuclear atypia/ pleomorphism before a score of 3 is allocated has not been defined, but the finding of an occasional enlarged or bizarre nucleus should not be used to give a score of 3 rather than a score of 2.

Assignment of Mitotic Frequency score

Accurate mitosis counting requires high quality fixation, obtained when fresh specimens are sliced into promptly after surgery and fixed immediately in neutral buffered formalin. This can be achieved without compromising the evaluation of resection margins. Poor quality fixation can result in underscoring of mitotic frequency; optimal fixation is therefore essential.

A minimum of 10 HPFs should be counted at the periphery of the tumour, where it has been demonstrated that mitotic activity is greatest on lower power search. If there is variation in the number of mitoses in different areas of the tumour, the least differentiated area (i.e., with the highest mitotic count) should be assessed. If the mitotic frequency score falls very close to a score cut point, one or more further groups of 10 HPFs should be assessed to establish the correct (highest) score. It is recommended that identification of the most mitotically active or least differentiated part of the tumour forms part of the low magnification preliminary assessment of the histological section. If there is no evidence of heterogeneity, mitotic scoring can be carried out at a part of the tumour periphery chosen at random. Fields chosen for scoring are selected during a random meander along the peripheral margin of the selected tumour area. Only fields with a representative tumour burden should be used. The low power scan of the tumour can be used to provide an assessment of the typical tumour to stromal ratio. Only definite mitotic figures (in any phase of the growth cycle) should be counted. Hyperchromatic nuclei and/or apoptotic nuclei should not be scored.

The mitosis score depends on the number of mitoses per 10 HPFs. The size of HPFs of modern microscopes is very variable, so it is necessary to standardise the mitotic count using Table 2 below. Field diameter is a function of the objective lens and the eyepiece, so if either of these is changed this exercise should be repeated. The field diameter of the microscope should be measured using the stage graticule, a Vernier scale or one of the simplified methods detailed below. The scoring category should be assigned from the corresponding line of Table 2. Mitotic counts can also be expressed per mm^2 which may be amenable to digital microscopy assessment.[2]

Modern microscopes have a HPF area which would equate to assessment of an area of approximately 2 mm^2. Using Table 2 it is possible to calibrate a score for 1 mm^2, and to calibrate a digital virtual microscope viewer.

Based on the current grading methodology the cut points for number of mitoses identified in a tumour area of 2 mm^2 is:

- Mitotic score 1: ≤7
- Mitotic score 2: 8-14
- Mitotic score 3: ≥15.

Methods for calculation of field diameter

1. The field diameter can be calculated simply by dividing field number by objective magnification; for example, if the eyepieces give field number 22 when using a x40 objective lens, the field diameter (in mm) is 22/40 = 0.55 mm.
2. Use a clear ruler to measure the diameter of a low-power field. This number can be used to calculate a constant based on the following formula: Eyepiece Magnification x Objective Magnification x Microscopic Field Diameter = A Constant.
   When the value of the constant is known, the diameter of an HPF can be calculated for other objectives by using the following formula: Unknown Field Diameter = Constant/(Eyepiece Magnification x Objective Magnification).
   Half of the field diameter is the radius of the field (*r*), which can then be used to calculate the area of the HPF: 3.1415 x r^2 = Area of Microscopic Field.
3. Use of a calibrated microscope slide.

Table 2: Score categories according to field diameter, area and mitotic count.

Scoring categories of mitotic counts				
Field diameter (mm)	Area (mm^2)	Number of mitoses per 10 fields corresponding to:		
		Score 1	Score 2	Score 3
0.40	1.25	≤4	5 to 9	≥10
0.41	0.132	≤4	5 to 9	≥10
0.42	0.139	≤5	6 to 10	≥11
0.43	0.145	≤5	6 to 10	≥11
0.44	0.152	≤5	6 to 11	≥12

0.45	0.159	≤5	6 to 11	≥12
0.46	0.166	≤6	7 to 12	≥13
0.47	0.173	≤6	7 to 12	≥13
0.48	0.181	≤6	7 to 13	≥14
0.49	0.189	≤6	7 to 13	≥14
0.50	0.196	≤7	8 to 14	≥15
0.51	0.204	≤7	8 to 14	≥15
0.52	0.212	≤7	8 to 15	≥16
0.53	0.221	≤8	9 to 16	≥17
0.54	0.229	≤8	9 to 16	≥17
0.55	0.238	≤8	9 to 17	≥18
0.56	0.246	≤8	9 to 17	≥18
0.57	0.255	≤9	10 to 18	≥19
0.58	0.264	≤9	10 to 19	≥20
0.59	0.273	≤9	10 to 19	≥20
0.60	0.283	≤10	11 to 20	≥21
0.61	0.292	≤10	11 to 21	≥22
0.62	0.302	≤11	12 to 22	≥23
0.63	0.312	≤11	12 to 22	≥23
0.64	0.322	≤11	12 to 23	≥24
0.65	0.332	≤12	13 to 24	≥25
0.66	0.342	≤12	13 to 24	≥25
0.67	0.353	≤12	13 to 25	≥26
0.68	0.363	≤13	14 to 26	≥27
0.69	0.374	≤13	14 to 27	≥28

References

1 Elston CW and Ellis IO (1991). 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. *Histopathology* 19(5):403-410.

2 WHO Classification of Tumours Editorial Board (ed) (2019). *WHO Classification of Tumours, Breast Tumours, 5th Edition*. IARC Publications, Lyon.

3 Royal College of Pathologists and National Coordinating Committee for Breast Pathology (2016). *Pathology reporting of breast disease in surgical excision specimens incorporating the dataset for histological reporting of breast cancer*. Available from: https://www.rcpath.org/profession/guidelines/cancer-datasets-and-tissue-pathways.html (Accessed 26th March 2020).