UK recommendations for HER2 assessment in breast cancer: an update

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ABSTRACT

The last UK breast cancer (BC) human epidermal growth factor receptor 2 (HER2) testing guideline recommendations were published in 2015. Since then, new data and therapeutic strategies have emerged. The American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) published a focused update in 2018 that reclassified in situ hybridisation (ISH) Group 2 (immunohistochemistry (IHC) score 2+and *HER*2/chromosome enumeration probe 17 (*CEP*17) ratio ≥2.0 and *HER*2 copy number <4.0 signals/cell), as well as addressed other concerns raised by previous guidelines. The present article further refines UK guidelines, with specific attention to definitions of HER2 status focusing on eight key areas: (1) HER2 equivocal (IHC 2+) and assignment of the ASCO/CAP ISH group 2 tumours; (2) the definition of the group of BCs with low IHC scores for HER2 with emphasis on the distinction between IHC score 1+ (HER2-Low) from HER2 IHC score 0 (HER2 negative); (3) reporting cases showing HER2 heterogeneity; (4) HER2 testing in specific settings, including on cytological material; (5) repeat HER2 testing, (6) HER2 testing turnaround time targets; (7) the potential role of next generation sequencing and other diagnostic molecular assays for routine testing of HER2 status in BC and (8) use of image analysis to score HER2 IHC. The two tiered system of HER2 assessment remains unchanged, with first line IHC and then ISH limited to IHC equivocal cases (IHC score 2+) but emerging data on the relationship between IHC scores and levels of response to anti-HER2 therapy are considered. Here, we present the latest UK recommendations for HER2 status evaluation in BC, and where relevant, the differences from other published guidelines.

INTRODUCTION

Human epidermal growth factor receptor 2 (HER2) overexpression in breast cancer (BC) is associated with a poorer prognosis in patients who do not receive adjuvant systemic therapy and is predictive of response to systemic therapies,[1] in particular HER2 targeted treatments.[2–5] Hence, eligibility criteria based on HER2 status have been developed to optimise patient selection for these expensive targeted agents and have evolved over time.[6–8]

Since the publication of the last UK HER2 testing guideline recommendations in BC in 2015,[9] new data have emerged. Focused updates by the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) on HER2 testing were published in 2018[7] to address several concerns that were raised following the ASCO/CAP 2013 guidelines publication[6] and to refine HER2 status definitions in certain situations. Detailed guideline recommendations on preanalytical and analytical variables for HER2 testing are covered in previous publications[6 8–10] and mainly remained unchanged.

At the present time, most countries use a two-tiered system for evaluation of HER2 status with immunohistochemistry (IHC) used for first-line testing and in situ hybridisation (ISH) testing limited to tumours with IHC equivocal (IHC score 2+) status. The use of ISH techniques in the IHC equivocal group determines HER2 status; there are implications for the further workup of tumours showing unusual ISH results. Recent advances, including the description of HER2-Low BC with response to innovative treatments, including antibody drug conjugates (ADCs),[11] and the emergence of newer technologies, are likely to have implications for classification of HER2 status and tumour testing in the future.

This article aims to promote consistency in the approach to HER2 testing and reporting and to further refine definitions of HER2 status. The following areas are addressed: (1) classification of ASCO/CAP ISH group 2 tumours (IHC 2+and *HER2*/ chromosome enumeration probe 17 (*CEP17*) ratio ≥2.0, *HER2* copy number <4.0 signals/cell); (2) HER2-Low BC and refinement of the IHC scoring algorithm with emphasis on the distinction between IHC score 1+ (HER2-Low) and HER2 IHC score 0 (HER2 negative); (3) HER2 heterogeneity; (4) HER2 testing in specific settings including on cytological material, (5) Repeat HER2 testing; (6) HER2 testing turnaround time (TAT) targets; (7) the potential role of new technologies, including next-generation sequencing (NGS) and other diagnostic molecular assays, for testing of HER2 status in BC; and brief comment is also made on (8) image analysis and artificial intelligence approaches. Detailed algorithms for HER2 scoring in clinical practice with some examples of the different IHC staining intensities and patterns are also provided (figures 1–5).

UPDATE ISSUES

ASCO/CAP ISH group 2 breast cancer

The early anti-HER2 treatment clinical trials enrolled patients with HER2 status defined using IHC assays alone and considered patients with both 3+ and 2+ tumour IHC scores as eligible.[2 3 6 12] Subsequent

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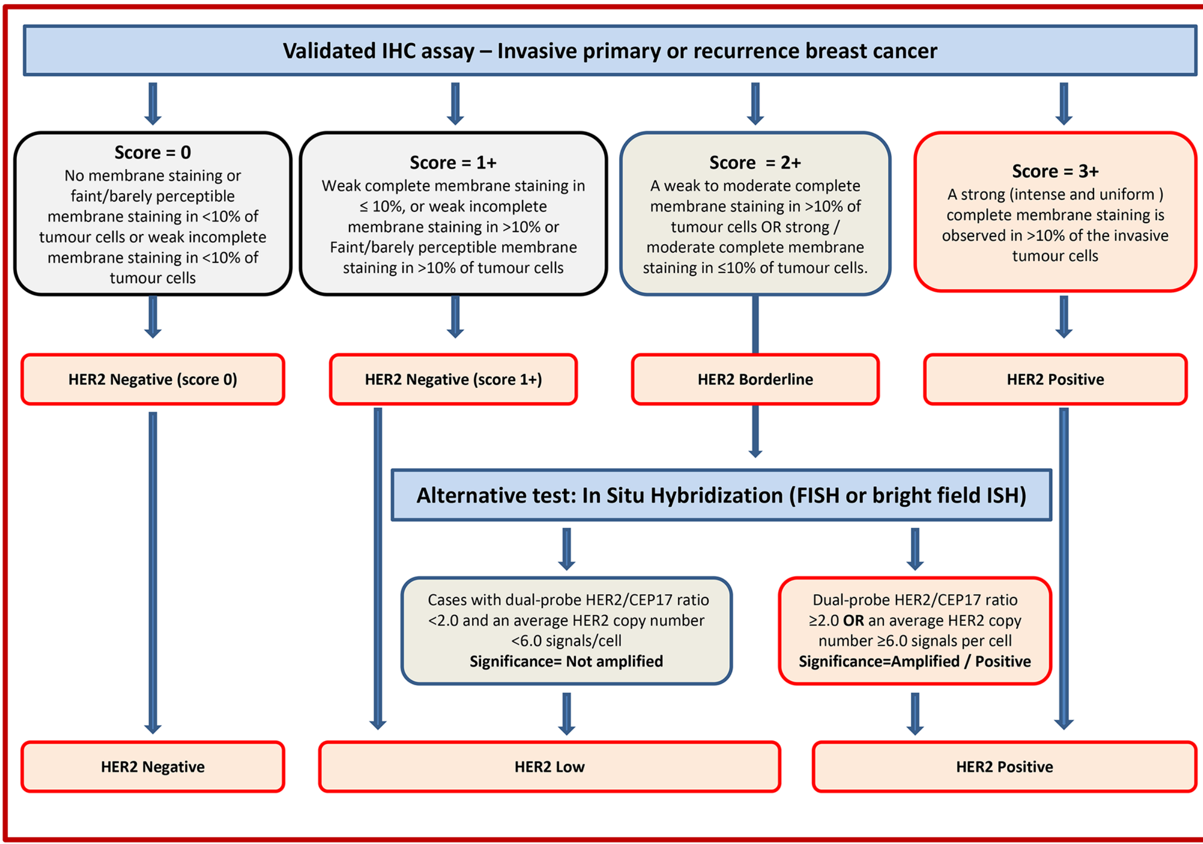

Figure 1 Algorithm for scoring HER2 protein expression in breast cancer by IHC assay using the 4 IHC scores (IHC scores 0–3+) and by the ISH 2 categories (*HER*2 amplified and non-amplified). *HER*2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

analyses indicated that only patients with HER2 equivocal (2+) expression and associated *HER2* gene amplification confirmed by ISH benefited from anti-HER2 therapies.[2 3 13–15] The Food and Drug Administration definition of HER2 positivity was subsequently updated to IHC 3+, or 2+with *HER2* gene amplification, defined as a *HER2/CEP17* ratio≥2.0, regardless of the *HER2* copy number.[14 16 17] This definition was endorsed by the earlier HER2 guidelines in the UK[10 18] and by the ASCO/CAP guidelines in 2007,[8] 2013[6] and the further UK 2015 update[9] where the definition of positivity was also expanded to include tumours with an average *HER2* gene copy number ≥6 signals/nucleus regardless of the *HER2/CEP17* ratio.

The ASCO/CAP guideline update in 2018 recommended a change in the status of ISH group 2 (IHC score 2+and *HER2/CEP17* ratio ≥2.0 but *HER2* copy number<4) from HER2 positive (as previously considered) to HER2 negative, unlike group 1 and group 3, which remained classified as HER2 positive.[7] The rationale for this change in terms of survival benefit was based on limited evidence.[7 19 20] To determine whether to adopt this change, the UK National Coordinating Committee for Breast Pathology (NCCBP) undertook a UK-based audit of patients with ISH group 2 tumours who received neoadjuvant chemotherapy (NACT), with or without anti-HER2 therapy, using pathological complete response (pCR) as an indicator for response.[21] Within the limitations of a retrospective study, the audit revealed that patients with group 2 tumours had an essentially similar response to NACT to those with tumours designated as ISH positive (ie, HER2 2+IHC and ISH Group 1: *HER2/CEP17* ratio ≥2.0 and *HER2* copy number ≥4; and HER2 2+IHC and ISH Group 3: *HER2/CEP17* ratio<2 and *HER2* copy number ≥6).[21] Therefore, the decision of the UK NCCBP was to continue to recommend classification of all three ASCO/CAP ISH groups (1, 2 and 3 with prior HER2 IHC 2+scores) as HER2 positive, recognising that the complete response rate to HER2 targeted therapy is limited (about 20%) and significantly less than in patients with HER2 IHC 3+ tumours (>50%).[2 12 21–24]

Therefore, while HER2 IHC score 2+ with low HER2 amplification levels continue to be classified as HER2 positive according to UK guidelines, (ie, unchanged from previous guidance) we recommend that discussion regarding targeted anti-HER2 therapy-based systemic treatment, in the adjuvant and neoadjuvant settings should take place at a multidisciplinary team (MDT) meeting, given the reduced pCR rates in this setting. We believe that decisions regarding treatment and other management plans such as *BRCA* testing (currently young patients whose BC shows triple negative phenotype (oestrogen receptor (ER), progesterone receptor (PR) and HER2 negative) are eligible for familial genetic testing in the UK[25] should also take account of all patient and tumour characteristics and not rely solely on HER2 status. With the increasing use of ADCs in tumours with equivocal IHC results, it is likely that further evidence regarding optimal classification and management will emerge in due course.

Recommendations

- Criteria for HER2 positivity in invasive BC remain unchanged from the previous UK guidelines[9] and include HER2 IHC score 3+, or IHC 2+with ISH evidence of HER2 gene amplification in the form of *HER2/CEP17* ratio ≥2.0 and/ or *HER2* copy number ≥6.0; this includes ASCO/CAP

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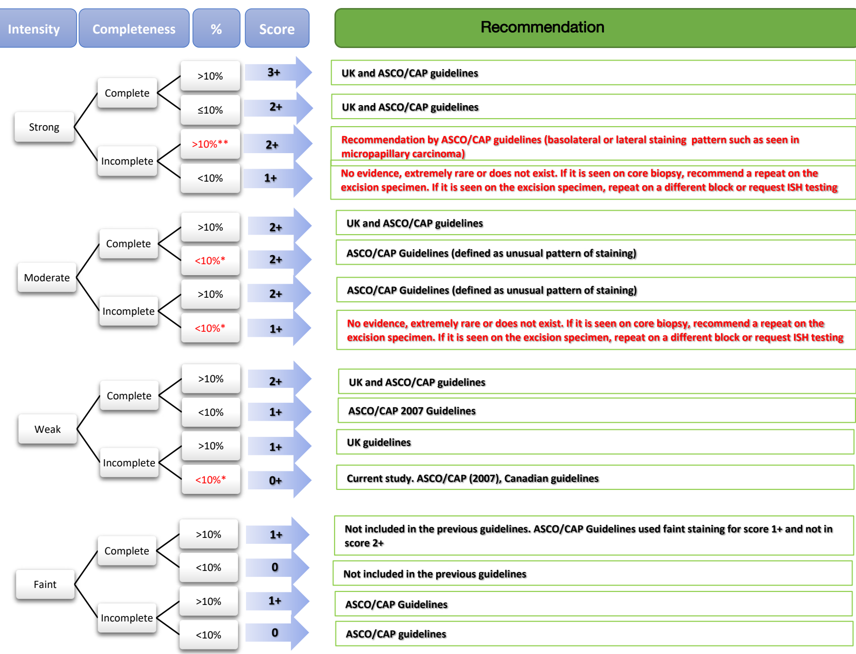

Figure 2 Algorithm for evaluation of HER2 protein expression based on the intensity of staining, pattern of membrane staining and the percentage of staining and the original evidence for each categorisation item. Most of the unusual staining patterns of HER2 IHC are rare but can be encountered in routine practice. When multiple patterns are present, consider those that comprise the highest IHC score. *HER*2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

ISH Group 2 (*HER2/CEP17* ratio ≥2.0 and average *HER2* copy number <4.0).

HER2-low BC and refinement of the IHC scoring algorithm

High concordance is observed between HER2 protein overexpression by IHC and *HER*2 gene amplification by ISH.[26–28] Although occasional tumours that have IHC score 1+or 0 may show borderline HER2 gene amplification if tested using ISH,[29] there is no evidence that these tumours will respond to traditional HER2 targeted therapeutic regimes, and patients with this type of tumour were not included in the initial anti-HER2 therapy clinical trials. Conversely, tumours with unequivocal IHC score 3+are classified as HER2 positive even if ISH shows no evidence of *HER*2 gene amplification.

BC with an HER2 IHC score of 2+and lacking evidence of *HER*2 gene amplification (IHC 2+/ISH−) is currently classified as HER2 negative, similar to tumours with an IHC score of 0 or 1+,[9 30] as patients with these tumours do not benefit from conventional anti-HER2 therapy.[31] Recent data have, however, demonstrated that some of the HER2 directed ADCs confer improved survival outcomes in patients with metastatic BC with low levels of HER2 expression that are currently classified as HER2 negative.[11] This has led to the description of a new category of 'HER2-low' tumours defined as IHC 1+, or IHC 2+ISH non-amplified, which account for 45–64% of all BCs.[32–38]

Identification of HER2-Low BCs is relatively straightforward for those with IHC 2+scores which are ISH negative. These tumours, which show recognisable levels of protein expression that are insufficient to merit a 3+score, are identified as part of the existing well-established HER2 testing protocols to define HER2 positivity. However, the lower limit of protein expression required for response to ADCs is not yet clearly defined. The anti-HER2 ADC clinical trial[11] used the existing ASCO/CAP criteria to define 1+ and to distinguish these from tumours with IHC score 0.

Despite the high overall concordance in the classification of HER2 positive and negative tumours, concordance in distinguishing BCs with IHC scores 1+ and 0 using the existing criteria remains low.[39–41] This is understandable, in view of the traditional lack of clinical relevance such that pathologists have not focused on separating these categories. However, with the introduction of new therapies for patients with IHC score 1+BCs, concordance will hopefully improve with more detailed guidelines and appropriate training.

At least 16 different patterns of HER2 protein expression exist when considering the combination of staining intensity (faint, weak, moderate and strong), membrane completeness (complete vs incomplete) and the cut-off (ie, 10%) to classify the percentage of HER2 staining in the invasive tumour cells into two extent categories (see figures 1 and 2). Pre-existing guidelines have defined HER2 status focussing on the most common staining patterns and, from the limited data available, poor concordance among pathologists in distinguishing HER2-low tumours.[40 42–48] Detailed description of the various expression patterns may improve concordance in the identification and categorisation of these tumours, despite limited clinical evidence (figures 1 and 2). Further evidence-based definitions, particularly of IHC score 1+BC, can hopefully be achieved when the treatment response rate is linked to the expression levels and patterns at individual patient level.

The evaluation of membrane staining intensity is subjective particularly differentiating between faint and weak, scores 0

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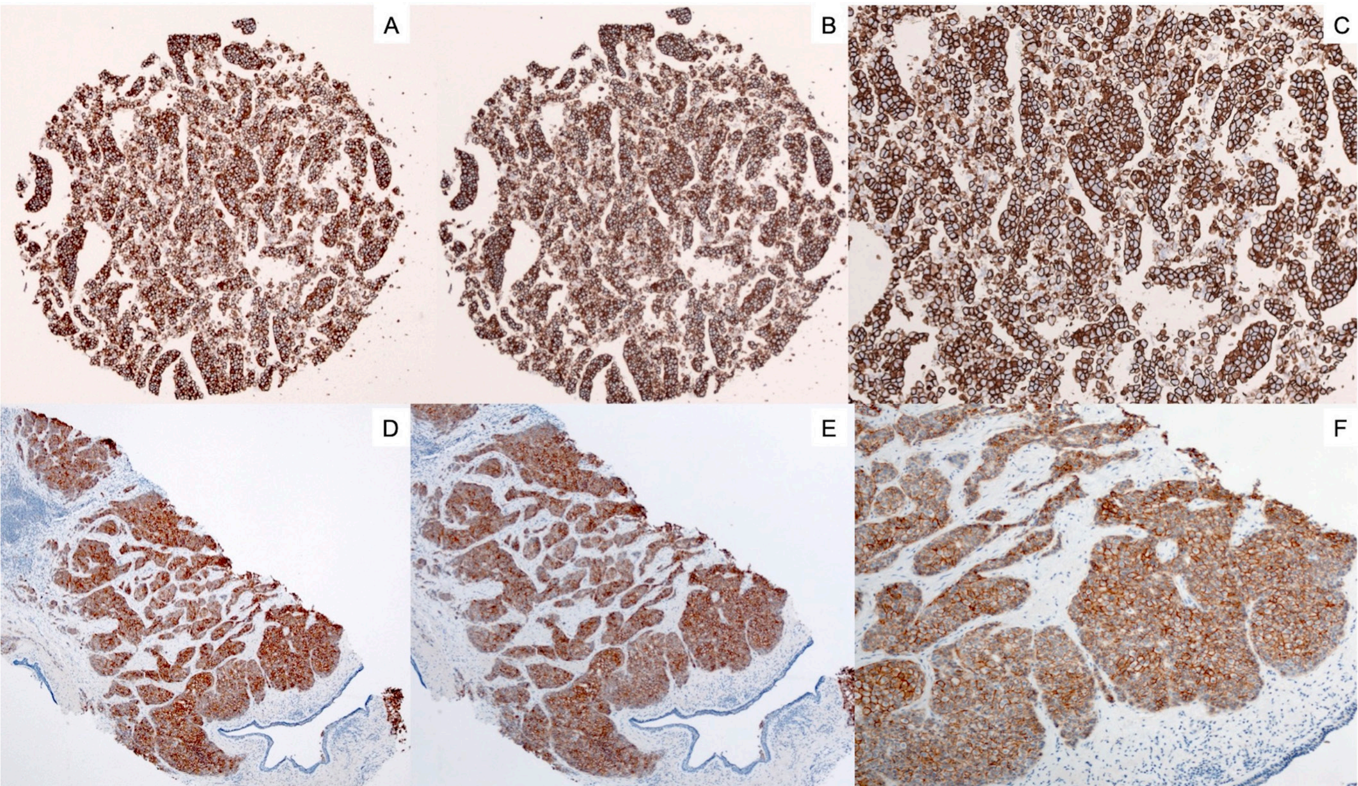

Figure 3 Examples of the 4 HER2 staining intensities that can be recognised at different magnification powers (note that intensity (strong, moderate, weak and faint) is not the same as IHC categories (3+, 2+1+) as these also include completeness and percentage of membrane staining). Strong membrane staining can be recognised at power 2 x (A), and 4 x (B) but it should be confirmed at ×10 (C). (D–F) Show a case of moderate membrane staining intensity associated with some cytoplasmic staining and very focal strong staining. It is difficult to be identified at ×4 (D) but can be appreciated at ×10 (E, F). *HER2*, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

and 1+and scores 1+ and 2+, as the distinction is based on the perceived staining intensity, the width of the DAB chromogen precipitate, and the absolute colour intensity of membrane staining. We recommend focused training, including familiarity with the entire spectrum of expression, to help pathologists to build a consistent recognition pattern with defined thresholds. Comparison with the control preparation is also helpful. Some authors have developed the so-called magnification rule,[49] which uses the different magnifications of the microscope objective lenses; clear-cut intense membrane staining perceived at low magnification (×2 to ×4) corresponds to strong staining (a score 3+ when complete and >10% of the cells) (figure 3). This is the easiest intensity recognition as it resembles the positive control. Comparison with the positive control can be helpful to distinguish strong from moderate intensity. Staining that is unequivocally visible only at x10 most likely corresponds to moderate intensity, whereas staining that is unequivocally visible only at ×20 likely represents a weak intensity (figure 4). Faint barely perceptible staining can only be appreciated at the highest magnification (×40) (figure 4). This rule, which may assist pathologists and lead to increased reproducibility in IHC scoring, is already implemented in some national guidelines.[24] Moderate to intense membrane staining that is non-circumferential with a baso-lateral or lateral pattern may be observed in some tumours, such as invasive micropapillary carcinoma. Reflex ISH testing of such tumours, that is, with strong or moderate intensity but incomplete membrane reactivity (other than if very focal and the majority is clearly 3+), should be considered. In mixed intensity tumours, the highest intensity staining of which comprises complete membrane staining, and its percentage should be used to construct the highest HER2 IHC score.

HER2 testing is currently used as a companion diagnostic in the clinical setting and the introduction of the HER2-low concept does not, we believe, require a change in practice in terms of testing procedures, apart from refinement of the scoring and reporting criteria. Laboratories should continue to use their approved HER2 assays and are not required to change to the HER2-Low clinical trial approved assay, in contrast to novel companion diagnostics such as PD1 and PD-L1 in which new IHC assays have been introduced. It is, however, recognised that different HER2 assays have different sensitivities, particularly at the low end of protein expression. This is expected to be addressed with further concordance studies and at present we recommend that laboratories continue to use their existing well-validated assay until new data on the performance of different assays at the low end of the spectrum of HER2-Low (score 0 vs 1+) emerge and justify a change of assay.

There is no specific correlation of 'HER2-low' with ISH ratios or copy number, hence for laboratories that primarily use ISH as first line rather than the two-tiered approach, the 'HER2-low' category may not be identified. While it could be argued that the current HER2 IHC tests are not optimised to detect low levels of protein expression, it should be noted that the clinical trials that demonstrated efficacy of ADCs in the HER2-low BC used existing assays.

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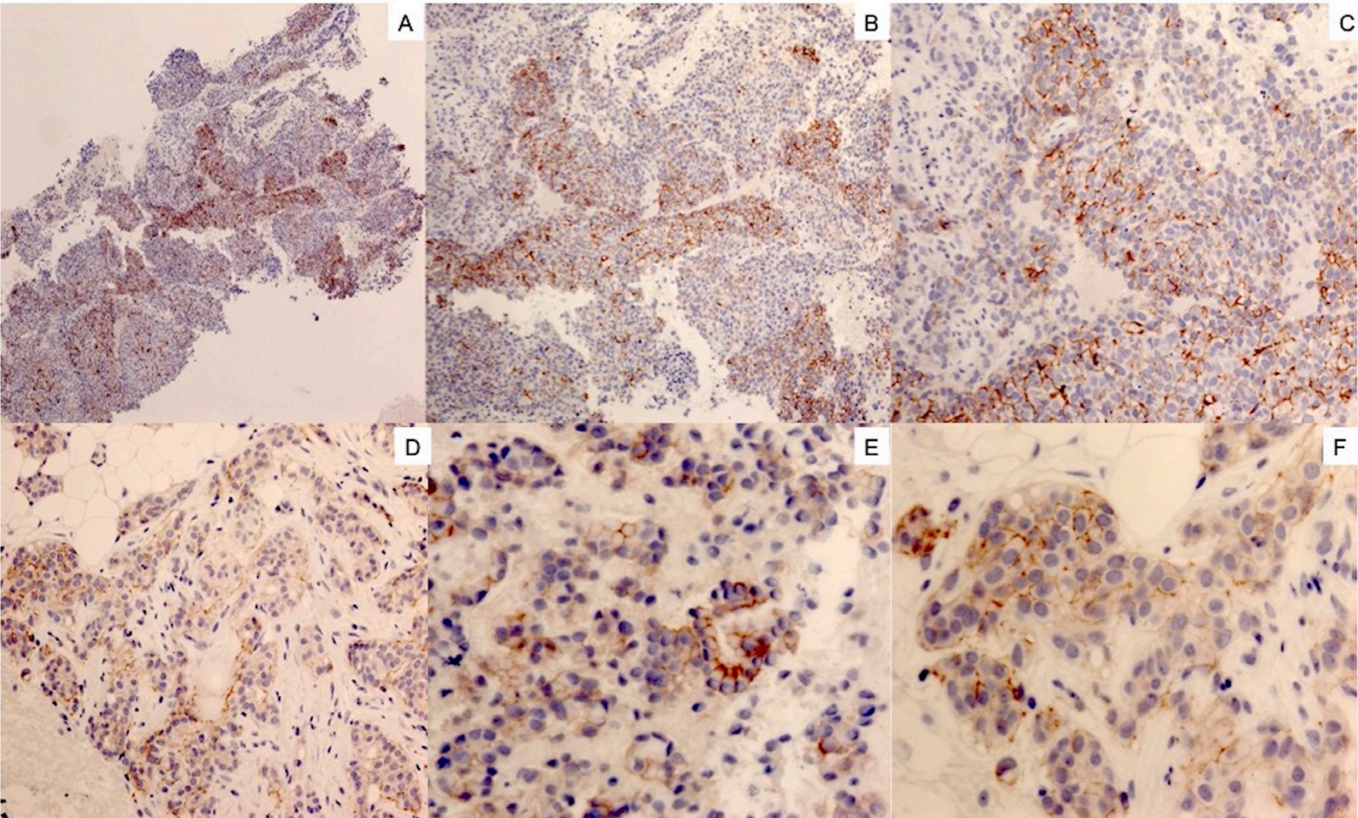

Figure 4 A case with weak complete membrane staining that cannot be appreciated at ×4 (A) or ×10 (B) but can be appreciated at ×20 (C). Note moderate incomplete staining is also seen. Similarly, faint membrane staining cannot be seen at ×10 (D), or ×20 (E) but can be seen at ×40 (F).

Recommendations

- Previously HER2 IHC scores have been classified as positive (3+), equivocal (2+) and negative (0 or 1+) without a clinically relevant focus on distinguishing between tumours with IHC scores of 0 and 1+. Pathologists should now distinguish between BC with HER2 IHC scores of 0 and 1+and include the actual score in the pathology report.
- It is anticipated that HER2 directed ADCs will be approved for HER2-Low BC in the near future. Once a drug has been approved in clinical practice, the term HER2-low should be included as a descriptive term in pathology reports and includes samples with IHC score of 1+ (following first line IHC testing) as well as those with IHC score of 2+ that lack evidence of *HER2* gene amplification on reflex ISH testing.
- Tumours with unequivocal IHC score 3+ are classified as HER2 positive even if ISH shows no evidence of *HER2* gene amplification. IHC score 3+is defined as strong (intense and uniform) complete membrane staining in >10% of the invasive tumour cells. If pathologist is unsure about the intensity of staining (strong vs moderate), reflex ISH test should be ordered.
- Tumours that have IHC score 1+or 0 are not classified as HER2 positive or equivocal and ISH reflex testing should not be requested. If ISH reflex testing is performed for any reason, detection of *HER2* gene low amplification should not change the IHC test results as HER2 negative.

HER2 heterogeneity

Intratumoural HER2 heterogeneity is observed in a small subset of BC with associated clinical implications.[50 51] It is more common in BC with equivocal HER2 protein expression (IHC 2+; approximately 10% of cases) and low level *HER2* gene amplification,[50 52] and may contribute to inaccurate designation of HER2 status.[53]

At the HER2 protein expression level, heterogeneity is defined as the presence of any aggregate population of cells (subclone) with strong or moderate complete membrane expression in <10% of the tumour; the tumour is considered 2+ and reflex ISH should be ordered. If the subclone of cells shows weak staining in <10% of the tumour in an otherwise negative tumour, the tumour is scored as 1+. Therefore, careful examination of core needle biopsy (CNB) specimens is required to identify topographically distinct tumour cell populations with different HER2 staining patterns.

At the genetic level, three distinct types of HER2 heterogeneity have been described: clustered, mosaic and scattered.[54] The clustered or clonal type displays two topographically distinct tumour cell clones, one harbouring HER2 amplification and the other with normal HER2 status. The clustered type is easier to identify as the tumour cell populations are easily recognised on IHC and ISH preparations. The, more frequent, mosaic type features diffuse intermingling of cells with a spectrum of HER2 protein expression levels and gene copy numbers (figure 6). The scattered type shows isolated *HER2*-amplified cells in a predominantly HER2-negative tumour cell population. These scattered isolated HER2 positive cells often show low levels of HER2 gene amplification and have limited response to anti-HER2 therapy compared with the clustered type.[55]

In tumours with intratumoural heterogeneity, it is important to scan all fields when scoring the ISH slide and to compare with the HER2 IHC slide to detect areas with higher HER2 protein expression and potential *HER2* amplification.

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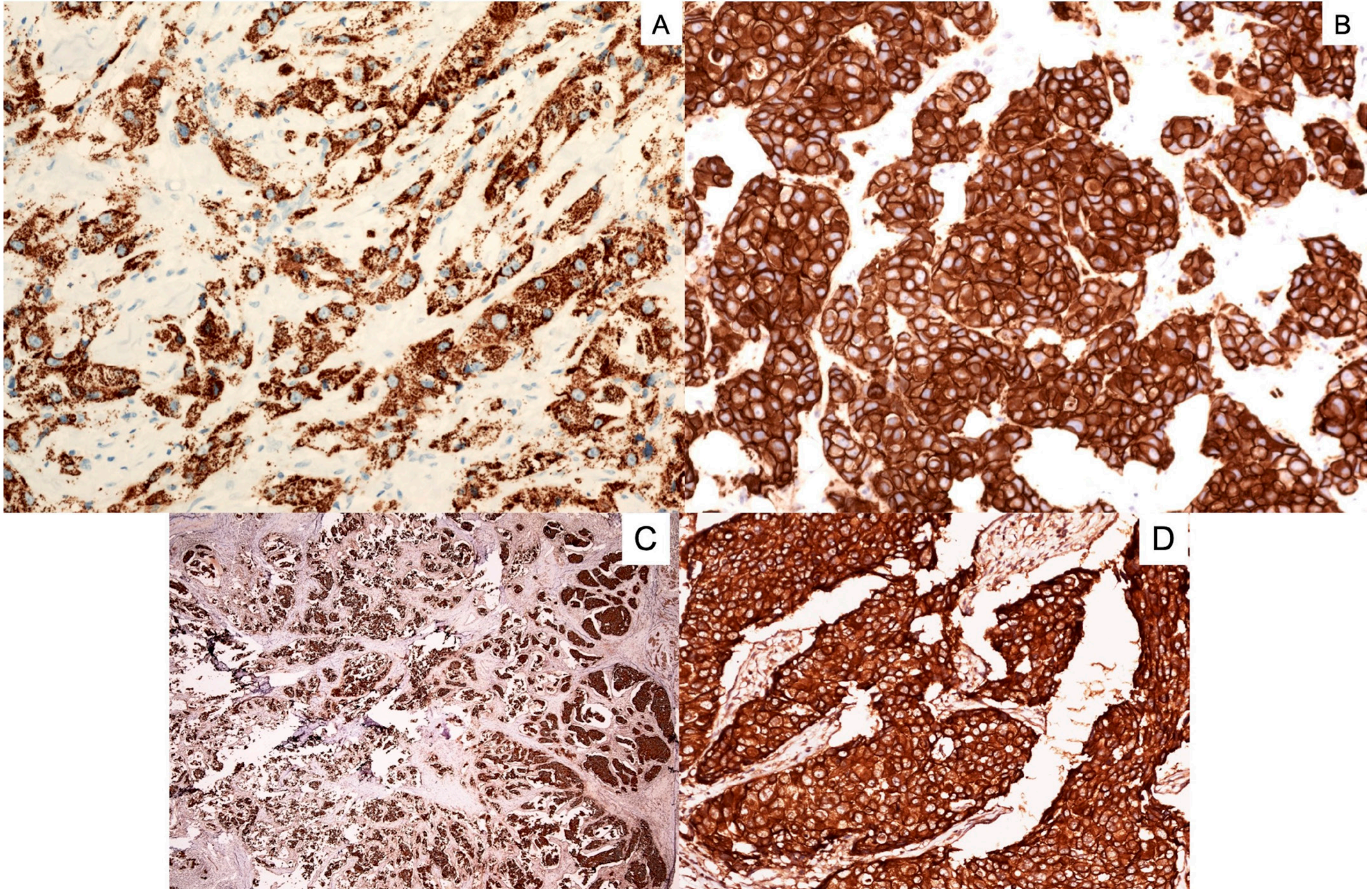

Figure 5 (A) Shows cytoplasmic staining in a case of apocrine carcinoma without membrane staining so it is negative. (B) Shows strong membrane and cytoplasmic expression (×20) which is considered 3+ however, examination of such cases at high power is important to distinguish membrane from cytoplasmic staining. False positive cytoplasmic staining with perimembranous accentuation is seen in (C, D), which demonstrates strong background staining (excessive antigen retrieval). Repeat staining of this case showed negative result.

Recommendations

- In the clustered pattern of HER2 heterogeneity, if the second population of cells with increased *HER2* signals/cell is >5% of tumour cells, separate counting of at least 20 non-overlapping cells should be performed within this population. The *HER2*/*CEP*17 ratio and HER2 copy number should be calculated and reported separately for amplified and non-amplified areas. Tumours with amplified and non-amplified areas are reported as HER2 positive (heterogeneous amplification) and the percentage of the total tumour cell population showing amplification should also be estimated. Retesting of the excision specimen and/or an axillary lymph node metastasis should also be considered, particularly in cases showing a low percentage of amplified clone (around 5%) as this clone may be enriched in the node metastasis or elsewhere in the primary tumour. A similar approach is recommended in the excision specimen if a similar heterogeneous staining pattern is seen.
- In the mosaic pattern, counting additional cells (at least 60 non-overlapping cells in the area of invasion with IHC 2+ staining) is advised and the average copy number and ratio are used to define HER2 status. Having an additional observer(s), blinded to previous ISH result, is also helpful in such cases. If the final ratio is <2.0 and the *HER2* copy number is <6.0, the sample is considered HER2 negative, and a comment added to contextualise the results. Re-testing of the excision specimen and/or an axillary lymph node metastasis should also be considered. A similar approach is recommended in the excision specimen should a similar heterogeneous staining pattern is seen.
- In the scattered pattern, we recommend counting 60 cells in areas containing more amplified cells; tumour HER2 status is defined based on the average *HER2* gene copy number and the *HER2*/*CEP*17 ratio.

HER2 assessment on cytological material and bone

CNBs are considered the most appropriate samples for tumour HER2 testing, with a high level of concordance with surgical resections, including results for the HER2-Low BC category.[56] The advantages of CNB specimens include fixation that is often superior to that in surgical samples, as well as more timely availability of results for clinical management. Biopsies are also suitable for testing metastatic lesions providing there is adequate tumour tissue present.

Cytological specimens represent another source of tumour material and may be the only material available from metastatic sites. Several studies have evaluated the determination of HER2 status in cytological BC material. Methodologies vary between publications and the most marked differences in performance are observed between sample types (smears, liquid based cytology (LBC), cytospins and cell blocks).[57] With regard to smear preparation, although alcohol fixation has been reported to be very effective in preserving the molecular components, the ASCO/

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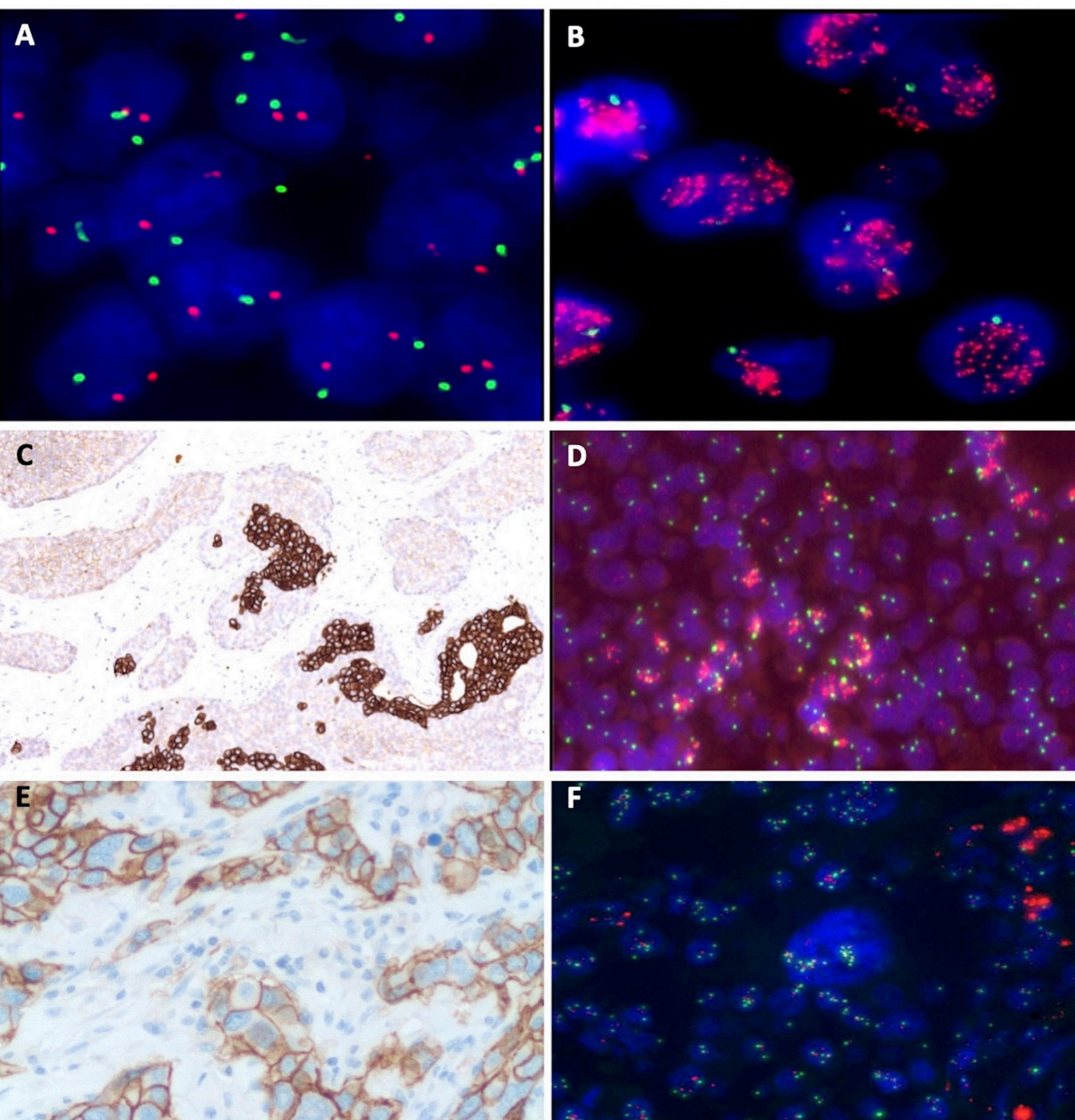

Figure 6 Representative FISH images from cases with normal *HER2* gene copy number (non-amplified) (A), and increased *HER2* gene copy number (amplified) (B). Figures C and D show a case with clustered pattern of HER2 protein overexpression (C) with a focus of strong complete membrane staining among a dominant clone that shows a score of 1+ staining. Figure D shows the corresponding FISH image with clustered pattern of *HER2* gene amplification. Figures E and F represent a case of HER2 IHC (E) showing mosaic type heterogeneity in copy number and ratios (F). Red signals represent *HER2* gene, green signals represent *CEP17*. Only signals within invasive tumour cell nuclei are considered. Occasional bizarre (multinucleated) cells as seen in high grade metaplastic breast carcinomas should be avoided in the counting as these may bias the overall count. For ISH, 4–6 µm FFPE sections are recommended. Thinner sections (<3 µm) may lead to false negative results for *HER2* gene amplification. FISH, fluorescence in situ hybridisation; *HER2*, human epidermal growth factor receptor 2; IHC, immunohistochemistry; FFPE, Formalin-Fixed Paraffin-Embedded.

CAP guidelines recommend formalin fixation.[6] For LBC, most studies focused on the use of the ThinPrep system and either CytoLyt or PreserveCyt as fixatives, both methanol based, and used a heat-based method for antigen retrieval.[57] Cell blocks prepared from cytological material are often considered to be the best approximation of histology as samples are embedded in paraffin and the antigen retrieval procedures are the same as those used for histology. Although the methods of sample fixation have varied in published studies to date, most authors have used formaldehyde.[57] If there is doubt regarding the quality of HER2 staining in cytological material, particularly if the fixative used is questionable or if there is scanty material only present, ISH testing should be carried out. Assessment of adequacy of the sample is complex and multifactorial. A limited number of cells (eg, 20) with strong circumferential membrane staining can be considered adequate for scoring and the tumour is designated HER2 positive. For tumours that appear negative on initial studies, it may be necessary to count a greater number of cells and/or further workup (eg, status of the primary tumour, reflex ISH test or repeat biopsy if possible).

Bone is a common metastatic site for BC, representing an important site of sampling for re-evaluation of HER2 status. The quality of both IHC and ISH is affected by decalcification methodology. Acetic acid and hydrochloric/formic acid are not recommended and EDTA-based decalcification methods are superior to other decalcification techniques particularly with regard to HER2 IHC and ISH testing performance.[58 59 60] BC bone metastases showing IHC score 3+ are considered positive. ISH testing

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is recommended in bone deposits that do not demonstrate an IHC 3+ score (ie, 2+, 1+ or 0) to avoid false negative results due to the possible effects of decalcification. Optimal decalcification remains a priority as the efficacy of ISH testing may also be compromised by the mode of decalcification although to a lesser extent than IHC.

Recommendations

- Cell blocks prepared from cytological material are considered to be the best approximation of histology as samples are embedded in paraffin and the antigen retrieval procedures are the same as those used for histology. If there is doubt regarding the quality of HER2 staining in cytological material, ISH testing should be carried out.
- For bone samples, EDTA-based decalcification methods are superior to others; consideration should be given to undertaking ISH in samples that are not clearly 3+ positive.

Repeat HER2 testing

Repeat testing on the same tumour block/sample is advised if a technical issue during IHC staining or ISH testing is suspected. Repeat testing on a different tumour block/sample should be performed where there is insufficient tumour present in the initial block/sample tested, as judged by the reporting pathologist (eg, <100 viable tumour cells). Repeat testing is also advised if carcinoma in a second specimen shows a different morphology to that in the original specimen, for example, grade 1 in the CNB versus grade 3 in the excision specimen or a clearly different tumour type in the excision specimen or lymph node metastasis compared with the prior CNB specimen. In tumours with multiple invasive foci, testing additional tumour foci should be based on the similarity to the index tumour mass. If multiple invasive tumour foci are present in the excision specimen and they all show the same morphology (type and grade), testing the main tumour mass can be considered sufficient. However, if the other tumour foci show different morphology (type and or grade), these should be tested.

If there is a mismatch between the tumour type and the expected HER2 status, review of H&E histology should be performed and repeat of the HER2 test (or confirmation by ISH) should be considered. For example, pure invasive tubular/cribriform, pure low grade adenoid cystic carcinoma, fibromatosis-like metaplastic carcinoma and low-grade adenosquamous carcinoma are typically HER2 negative, as are grade 1, ER and PR positive invasive BC of No Special Type and tubulo-lobular carcinoma. If these appear to be positive on IHC, confirmation by ISH may be prudent.

Given the high concordance rate of HER2 status between NCB and resection specimens, routine retesting of the excision specimens is not required. However, in NACT setting (chemotherapy with or without anti-HER2 therapy), clinically actionable alterations in HER2 have been reported in 1%–9% of residual tumours with subsequent implications for further management decisions.[61 62] This has led to considerable variation in the percentage of tumours that are retested following NACT with varied results.[61 63] Loss is more common than gain and higher rates of change are observed with IHC compared with ISH and following anti-HER2 therapy compared with chemotherapy alone.[62] Potential reasons for the change in the HER2 status post-NACT include true biological effects such as interactions between signalling pathways and selective response due to intra-umoural heterogeneity as well as technical factors such as fixation and sampling error that may produce false negative or false positive results (figure 5). Retesting of HER2 post NACT may be considered, particularly if pretreatment HER2 status was negative, the tumour in the CNB specimen was limited (although ideally these patients should have undergone repeat testing/biopsy at diagnosis to obtain an optimal tumour sample before commencing treatment) or the residual tumour shows significantly different morphology to the tumour in the pretreatment CNB. Clinical judgement is required as this does not refer to post-treatment changes commonly induced by NACT, such as increased pleomorphism or lower mitotic frequency but, as in the primary surgery setting, includes tumours with mixed histological patterns and with components not seen on the prior CNB specimen. In patients whose BC changes to HER2 positive post-NACT, anti-HER2 therapy can be initiated.[64] The value of repeat HER2 testing following neoadjuvant endocrine therapy has not been demonstrated and retesting of HER2 in this setting is currently not justified.

In all IHC score 2+ tumours, a reflex test on the same sample using ISH should be carried out. If this is not possible (eg, if there is insufficient tumour remaining), a new test on an alternative sample using both IHC and ISH should be performed.

If the specimen ISH test result is borderline and close to the ISH ratio threshold for positivity (*HER2* copy number 4.0 to <6.0 and/or a ratio 1.8 to <2.0), there is a high likelihood that repeat testing on the same or even on a different tumour block/sample may result in different result (ie, just the other side of a cut-point) by chance alone. In those samples, a larger number of cells should be counted, and preferably a second assessor should also score the sample. If the final result remains as borderline negative after such repeat testing, it is recommended that the result is accepted as negative without further testing; in other words, there is no benefit in reassessing all blocks exhaustively.[7]

Recommendations

- Pathologists should repeat HER2 testing in certain situations that have the potential to change the results, to ensure the validity and reliability of the test.
- Retesting of HER2 status post NACT should be considered if pretreatment HER2 status was unknown; the tumour in the CNB specimen was limited and HER2 was negative; or the residual tumour in the resection specimen shows either significantly different morphology to that in the pretreatment CNB or is composed of multiple tumour foci with varying morphology. Retesting post NACT may also be considered if no pathological response to anti-HER2-based therapy is observed in patients whose tumours were classified as HER2 positive pretreatment, to assist decision making regarding further tailored adjuvant therapy.[65 66]
- If repeat testing is performed and if a HER2 positive tumour changes to HER2 negative in the post-NACT sample, reviewing the pretreatment HER2 status is appropriate as a quality assurance measure.

HER2 turnaround time

Timely availability of HER2 results, including ISH results when required, is essential for treatment decisions and for identifying BC patients for NACT and/or 'window of opportunity' trials and further genomic profiling. The availability and recording of HER2 results in all newly diagnosed invasive BC at the initial preoperative MDT meeting, based on core biopsy assessment, avoids delays and the need for further discussion (https://www.england.nhs.uk/cancer/faster-diagnosis/#fds),.[67 68] Pathways to achieve fast HER2 TATs in clinical practice have been proposed.[69]

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Early request of HER2 IHC in clinically/radiologically malignant tumours at the time of processing or immediate request after histological confirmation of invasive BC and frequent staining runs of HER2 IHC in laboratories can expedite the availability of the HER2 results in >70% of cases.[70] For tumours that require ISH testing, the result will take longer. Regardless of the testing model used; local, central, 'hub and spoke', or mixed, laboratories should audit and streamline the pathway to ensure rapid availability of the results to the clinical team.

Recommendations

- ► Measures to achieve as fast as possible HER2 turnaround times should be implemented and delays in provision of HER2 results to the preoperative MDT meeting should be minimised.

HER2 testing and NGS

A high correlation between *HER2* gene status and HER2 mRNA levels is well documented.[71] Several techniques have been applied for HER2 mRNA analysis, including quantitative procedures, such as real-time quantitative PCR (qPCR) methods. However, these techniques are not widely used in clinical practice and are not currently recognised as alternative methods to assess HER2 status in diagnostic practice.

Gene expression tests such as Oncotype Dx and Prosigna are currently used in some ER positive/ HER2 negative BCs. The Oncotype Dx test provides a value for HER2 mRNA levels, and Prosigna provides an intrinsic subtype, for example, HER2-enriched. It should be noted that there is only a modest correlation between intrinsic subtype established by gene expression profiling and clinical HER2 receptor status; up to a third of HER2-enriched tumours are HER2 negative using currently accepted methodologies and approximately one third of HER2 positive tumours are classified as non-HER2-enriched.[72,73] Nonetheless, if there is discordance with the clinical gene expression test result, the HER2 test (IHC+/−ISH) should be reviewed, and if the original HER2 status was established on CNB, repeat HER2 testing should be considered on the excision specimen (ideally the same block that was submitted for genomic testing). Despite the correlation between the HER2-enriched molecular class and response to anti-HER2 therapy,[72] the final HER2 result in discordant cases should be based on the currently approved assays (IHC+/−ISH) after validation of the results. The results of genomic assays should not be used to define a tumour HER2 status as positive or negative for clinical management.

NGS techniques such as whole genome sequencing, are increasingly used in the clinical setting. NGS techniques may help to identify ERBB2-activating mutations[74] and an increase in HER2 copy number, and to provide a list of genes putatively correlated with HER2 therapy response or resistance.[75] However, its use in clinical practice to define HER2 status remains to be assessed; the reliability of the NGS assay for detecting HER2 status, the criteria for defining HER2 positivity and evidence supporting NGS as predictor of response to anti-HER2 therapy compared with the existing approved assays are all required. In tumours in which the HER2 NGS results do not align with those of approved assays (IHC and/or ISH), HER2 status is determined according to the results of the approved assays.

Recommendations

- ► HER2 status should only be defined for clinical management using the currently approved assays, that is, IHC+/−ISH.
- ► Additional assays, which, for example, provide *HER2* mRNA levels or NGS, may be valuable for quality assurance of diagnostic results.

HER2 assessment using image analysis and artificial intelligence algorithms

The introduction of digital pathology and whole slide imaging technology facilitates the use of image analysis and application to quantify HER2 expression on IHC-stained slides. Comparative studies have shown these computer-aided scoring methods to be as accurate and reproducible as pathologists[76–78] and recommendations to improve accuracy, precision and reproducibility of HER2 assessment in BC using image analysis tools have been published.[76] Clearly, validated procedures are required before implementation of this technology and quality control/assurance is mandatory. The reliability of automated image techniques in evaluating unusual patterns of HER2 staining remains to be determined but regular audit and performance monitoring has the potential to reduce risk of misclassification. There is no convincing evidence so far to support the use of artificial intelligence algorithms in routine HER2 reporting and more research is required. However, this will be reviewed if new evidence emerges.

Recommendations

- ► Image analysis and artificial intelligence algorithms applied to digitised slides are not yet an approved method for routine reporting of HER2 status but will be reviewed if new evidence emerges.

Comments

The current HER2 guideline update panel are member of the National Coordinating Committee of Breast Pathology (NCCBP) of the UK. The panel, which also included external experts (PHT and JS), represents the UK NCCBP. The NCCBP is responsible for coordinating QA procedures and guidance, in particular reviewing and recommending standards in the UK. NCCBP also provides guidelines and advice on pathological examination of breast tissues to achieve a high level of accuracy and consistency in reporting breast lesions. The update panel conducted a formal and comprehensive review of the peer-reviewed literature published since 2015 to revise and update the HER2 guideline recommendations as appropriate. After careful consideration of the available evidence and expert opinions, the Panel revised the update items to arrive at the best available evidence-based recommendations in addition to expert consensus supported in practice. The guideline was circulated in draft form among the Panel and was released to the other UK NCCBP members for comment and the final version was approved by all. Variation in the recommendations was resolved by discussion of the Panel and a consensus was reached.

Guideline disclaimer

The clinical practice guidelines and other guidance published herein are provided to assist in clinical decision making and the information therein should not be relied on as being complete or accurate, nor should it be considered as inclusive of all HER2 treatments or methods of care or as a statement of the standard of care. This information does not mandate any particular course of medical care and is not intended to substitute for the independent professional judgement, as the information does not account for individual variation among patients.

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REFERENCES

1 Yamauchi H, Stearns V, Hayes DF. When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. *J Clin Oncol* 2001;19:2334–56.
2 Slamon DJ, Leyland-Jones B, Shak S, *et al*. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;344:783–92.
3 Vogel CL, Cobleigh MA, Tripathy D, *et al*. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2002;20:719–26.
4 Untch M, von Minckwitz G, Gerber B, *et al*. Survival analysis after neoadjuvant chemotherapy with trastuzumab or lapatinib in patients with human epidermal growth factor receptor 2-positive breast cancer in the GeparQuinto (G5) study (GBG 44). *J Clin Oncol* 2018;36:1308–16.
5 Johnston SRD, Hegg R, Im S-A, *et al*. Phase III, randomized study of dual human epidermal growth factor receptor 2 (HER2) blockade with lapatinib plus trastuzumab in combination with an aromatase inhibitor in postmenopausal women with HER2-positive, hormone receptor-positive metastatic breast cancer: alternative. *J Clin Oncol* 2018;36:741–8.
6 Wolff AC, Hammond MEH, Hicks DG, *et al*. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of clinical Oncology/College of American pathologists clinical practice guideline update. *J Clin Oncol* 2013;31:3997–4013.
7 Wolff AC, Hammond MEH, Allison KH, *et al*. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of clinical Oncology/College of American pathologists clinical practice guideline focused update. *J Clin Oncol* 2018;36:2105–22.
8 Wolff AC, Hammond MEH, Schwartz JN, *et al*. American Society of clinical Oncology/College of American pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 2007;25:118–45.
9 Rakha EA, Pinder SE, Bartlett JMS, *et al*. Updated UK recommendations for HER2 assessment in breast cancer. *J Clin Pathol* 2015;68:93–9.
10 Ellis IO, Bartlett J, Dowsett M, *et al*. Best practice no 176: updated recommendations for HER2 testing in the UK. *J Clin Pathol* 2004;57:233–7.
11 Modi S, Jacot W, Yamashita T, *et al*. Trastuzumab Deruxtecan in previously treated HER2-Low advanced breast cancer. *N Engl J Med* 2022;387:9–20.
12 Cobleigh MA, Vogel CL, Tripathy D, *et al*. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol* 1999;17:2639–48.
13 Sawaki M, Ito Y, Tada K, *et al*. Efficacy and safety of trastuzumab as a single agent in heavily pretreated patients with HER-2/neu-overexpressing metastatic breast cancer. *Tumori* 2004;90:40–3.
14 Romond EH, Perez EA, Bryant J, *et al*. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005;353:1673–84.
15 Kaufman B, Mackey JR, Clemens MR, *et al*. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III tandem study. *J Clin Oncol* 2009;27:5529–37.
16 Joensuu H, Kellokumpu-Lehtinen P-L, Bono P, *et al*. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. *N Engl J Med* 2006;354:809–20.
17 Piccart-Gebhart MJ, Procter M, Leyland-Jones B, *et al*. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005;353:1659–72.
18 Ellis IO, Dowsett M, Bartlett J, *et al*. Recommendations for HER2 testing in the UK. *J Clin Pathol* 2000;53:890–2.
19 Press MF, Sauter G, Buyse M, *et al*. Her2 gene amplification testing by fluorescent in situ hybridization (fish): comparison of the ASCO-College of American pathologists guidelines with fish scores used for enrollment in breast cancer international Research group clinical trials. *J Clin Oncol* 2016;34:3518–28.
20 Ballard M, Jalikis F, Krings G, *et al*. 'Non-Classical' HER2 fish results in breast cancer: a multi-institutional study. *Mod Pathol* 2017;30:227–35.
21 Rakha EA, Miligy IM, Quinn CM. Retrospective observational study of HER2 immunohistochemistry in borderline breast cancer patients undergoing neoadjuvant therapy, with an emphasis on Group 2 (HER2/CEP17 ratio >/=2.0, HER2 copy number. *Br J Cancer* 2021.
22 Krystel-Whittemore M, Xu J, Brogi E, *et al*. Pathologic complete response rate according to HER2 detection methods in HER2-positive breast cancer treated with neoadjuvant systemic therapy. *Breast Cancer Res Treat* 2019;177:61–6.
23 Zeng J, Edelweiss M, Ross DS, *et al*. Triple-Positive breast carcinoma: histopathologic features and response to neoadjuvant chemotherapy. *Arch Pathol Lab Med* 2021;145:728–35.
24 Franchet C, Djerroudi L, Maran-Gonzalez A, *et al*. [2021 update of the GEFPICS' recommendations for HER2 status assessment in invasive breast cancer in France]. *Ann Pathol* 2021;41:507–20.
25 Familial breast cancer: classification, care and managing breast cancer and related risks in people with a family history of breast cancer. London 2019.
26 Couturier J, Vincent-Salomon A, Nicolas A, *et al*. Strong correlation between results of fluorescent in situ hybridization and immunohistochemistry for the assessment of the ErbB2 (HER-2/neu) gene status in breast carcinoma. *Mod Pathol* 2000;13:1238–43.
27 Yaziji H, Goldstein LC, Barry TS, *et al*. Her-2 testing in breast cancer using parallel tissue-based methods. *JAMA* 2004;291:1972–7.
28 Lebeau A, Deimling D, Kaltz C, *et al*. Her-2/Neu analysis in archival tissue samples of human breast cancer: comparison of immunohistochemistry and fluorescence in situ hybridization. *J Clin Oncol* 2001;19:354–63.
29 Memon R, Prieto Granada CN, Harada S, *et al*. Discordance between immunohistochemistry and in situ hybridization to detect HER2 Overexpression/Gene amplification in breast cancer in the modern age: a single institution experience and pooled literature review study. *Clin Breast Cancer* 2022;22:e123–33.
30 Wolff AC, Hammond MEH, Allison KH, *et al*. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of clinical Oncology/College of American pathologists clinical practice guideline focused update. *Arch Pathol Lab Med* 2018;142:1364–82.
31 Fehrenbacher L, Cecchini RS, Geyer CE, *et al*. Nsabp B-47/NRG oncology phase III randomized trial comparing adjuvant chemotherapy with or without trastuzumab in high-risk invasive breast cancer negative for HER2 by fish and with IHC 1+ or 2. *J Clin Oncol* 2020;38:444–53.
32 Tarantino P, Hamilton E, Tolaney SM, *et al*. HER2-low breast cancer: pathological and clinical landscape. *J Clin Oncol* 2020;38:1951–62.
33 Agostinetto E, Rediti M, Fimereli D, *et al*. HER2-Low breast cancer: molecular characteristics and prognosis. *Cancers* 2021;13. doi:10.3390/cancers13112824. [Epub ahead of print: 05 06 2021].
34 Horisawa N, Adachi Y, Takatsuka D, *et al*. The frequency of low HER2 expression in breast cancer and a comparison of prognosis between patients with HER2-low and HER2-negative breast cancer by HR status. *Breast Cancer* 2022;29:234–41.
35 Saura C, Thistlethwaite F, Banerji U. A phase I expansion cohorts study of SYD985 in heavily pretreated patients with HER2-positive or HER2-low metastatic breast cancer. *American Society of Clinical Oncology* 2018..

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36 Rinnerthaler G, Gampenrieder SP, Greil R. Her2 directed antibody-drug-conjugates beyond T-DM1 in breast cancer. *Int J Mol Sci* 2019;20. doi:10.3390/ijms20051115. [Epub ahead of print: 05 Mar 2019].
37 Modi S, Park H, Murthy RK. Antitumor activity and safety of trastuzumab deruxtecan in patients with HER2-low–expressing advanced breast cancer: results from a phase Ib study. *Journal of Clinical Oncology*20201887.
38 Zhang X, Huang AC, Chen F, *et al*. Novel development strategies and challenges for anti-HER2 antibody-drug conjugates. *Antib Ther* 2022;5:18–29.
39 Jørgensen JT, Winther H, Askaa J, *et al*. A companion diagnostic with significant clinical impact in treatment of breast and gastric cancer. *Front Oncol* 2021;11:676939.
40 Canda T, Yavuz E, Özdemir N, *et al*. Immunohistochemical HER2 status evaluation in breast cancer pathology samples: a multicenter, Parallel-Design concordance study. *Eur J Breast Health* 2018;14:160–5.
41 Md Pauzi SH, Masir N, Yahaya A, *et al*. Her2 testing by immunohistochemistry in breast cancer: a multicenter proficiency ring study. *Indian J Pathol Microbiol* 2021;64:677–82.
42 Boyraz B, Ly A. Discerning subsets of breast cancer with very low and absent HER2 protein expression. *Hum Pathol* 2022;127:50–5.
43 Perez EA, Suman VJ, Davidson NE, *et al*. Her2 testing by local, central, and reference laboratories in specimens from the North central cancer treatment group N9831 intergroup adjuvant trial. *J Clin Oncol* 2006;24:3032–8.
44 Fernandez A, Liu M, Bellizzi A. *Examination of low HER2 expression in breast cancer. presented at: 2021 San Antonio breast cancer symposium*. San Antonio, TX: Abstract P1-02-02, 2021.
45 Grimm EE, Schmidt RA, Swanson PE, *et al*. Achieving 95% cross-methodological concordance in HER2 testing: causes and implications of discordant cases. *Am J Clin Pathol* 2010;134:284–92.
46 Dybdal N, Leiberman G, Anderson S, *et al*. Determination of HER2 gene amplification by fluorescence in situ hybridization and concordance with the clinical trials immunohistochemical assay in women with metastatic breast cancer evaluated for treatment with trastuzumab. *Breast Cancer Res Treat* 2005;93:3–11.
47 Paik S, Bryant J, Tan-Chiu E, *et al*. Real-world performance of HER2 testing--National Surgical Adjuvant Breast and Bowel Project experience. *J Natl Cancer Inst* 2002;94:852–4.
48 Roche PC, Suman VJ, Jenkins RB, *et al*. Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. *J Natl Cancer Inst* 2002;94:855–7.
49 Scheel AH, Penault-Llorca F, Hanna W, *et al*. Physical basis of the 'magnification rule' for standardized Immunohistochemical scoring of HER2 in breast and gastric cancer. *Diagn Pathol* 2018;13:19.
50 Seol H, Lee HJ, Choi Y, *et al*. Intratumoral heterogeneity of HER2 gene amplification in breast cancer: its clinicopathological significance. *Mod Pathol* 2012;25:938–48.
51 Lee HJ, Kim JY, Park SY, *et al*. Clinicopathologic significance of the intratumoral heterogeneity of HER2 gene amplification in HER2-positive breast cancer patients treated with adjuvant trastuzumab. *Am J Clin Pathol* 2015;144:570–8.
52 Allison KH, Dintzis SM, Schmidt RA. Frequency of HER2 heterogeneity by fluorescence in situ hybridization according to cap expert panel recommendations: time for a new look at how to report heterogeneity. *Am J Clin Pathol* 2011;136:864–71.
53 Marchiò C, Dell'Orto P, Annaratone L, *et al*. The dilemma of HER2 Double-equivocal breast carcinomas: genomic profiling and implications for treatment. *Am J Surg Pathol* 2018;42:1190–200.
54 Hanna WM, Rüschoff J, Bilous M, *et al*. Her2 in situ hybridization in breast cancer: clinical implications of polysomy 17 and genetic heterogeneity. *Mod Pathol* 2014;27:4–18.
55 Rye IH, Trinh A, Saetersdal AB, *et al*. Intratumor heterogeneity defines treatment-resistant HER2+ breast tumors. *Mol Oncol* 2018;12:1838–55.
56 Miglietta F, Griguolo G, Bottosso M, *et al*. Evolution of HER2-low expression from primary to recurrent breast cancer. *NPJ Breast Cancer* 2021;7.
57 Pinto D, Schmitt FC. Immunohistochemistry applied to breast cytological material. *Pathobiology* 2022;89:343–58.
58 Washburn E, Tang X, Caruso C, *et al*. Effect of EDTA decalcification on estrogen receptor and progesterone receptor immunohistochemistry and HER2/neu fluorescence in situ hybridization in breast carcinoma. *Hum Pathol* 2021;117:108–14.
59 Schrijver WAME, van der Groep P, Hoefnagel LD, *et al*. Influence of decalcification procedures on immunohistochemistry and molecular pathology in breast cancer. *Mod Pathol* 2016;29:1460–70.
60 van Es SC, van der Vegt B, Bensch F, *et al*. Decalcification of breast cancer bone metastases with EDTA does not affect ER, PR, and HER2 results. *Am J Surg Pathol* 2019;43:1355–60.
61 Sahoo S, Krings G, Chen Y-Y, *et al*. Standardizing pathologic evaluation of breast carcinoma after neoadjuvant chemotherapy. *Arch Pathol Lab Med* 2022. doi:10.5858/arpa.2022-0021-EP. [Epub ahead of print: 17 Aug 2022].
62 Shaaban AM, Provenzano E. Receptor status after neoadjuvant therapy of breast cancer: significance and implications. *Pathobiology* 2022;89:297–308.
63 Katayama A, Miligy IM, Shiino S, *et al*. Predictors of pathological complete response to neoadjuvant treatment and changes to post-neoadjuvant HER2 status in HER2-positive invasive breast cancer. *Mod Pathol* 2021;34:1271–81.
64 Harbeck N. Neoadjuvant and adjuvant treatment of patients with HER2-positive early breast cancer. *Breast* 2022;62 Suppl 1:S12–16.
65 Robertson S, Rönnlund C, de Boniface J, *et al*. Re-Testing of predictive biomarkers on surgical breast cancer specimens is clinically relevant. *Breast Cancer Res Treat* 2019;174:795–805.
66 Wang R-X, Chen S, Jin X, *et al*. Weekly paclitaxel plus carboplatin with or without trastuzumab as neoadjuvant chemotherapy for HER2-positive breast cancer: loss of HER2 amplification and its impact on response and prognosis. *Breast Cancer Res Treat* 2017;161:259–67.
67 Francis A, Bartlett J, Rea D, *et al*. Viewpoint: availability of oestrogen receptor and HER2 status for the breast multidisciplinary meeting discussion; time to get it right. *Eur J Surg Oncol* 2016;42:994–8.
68 Evidence reviews for adjuvant systemic therapy planning. *Early and locally advanced breast cancer: diagnosis and management: evidence review C*. London, 2018.
69 Shaaban AM, Purdie CA, Bartlett JMS, *et al*. Her2 testing for breast carcinoma: recommendations for rapid diagnostic pathways in clinical practice. *J Clin Pathol* 2014;67:161–7.
70 Dodson A, Parry S, Ibrahim M, *et al*. Breast cancer biomarkers in clinical testing: analysis of a UK national external quality assessment scheme for immunocytochemistry and in situ hybridisation database containing results from 199 300 patients. *J Pathol Clin Res* 2018;4:262–73.
71 Baehner FL, Achacoso N, Maddala T, *et al*. Human epidermal growth factor receptor 2 assessment in a case-control study: comparison of fluorescence in situ hybridization and quantitative reverse transcription polymerase chain reaction performed by central laboratories. *J Clin Oncol* 2010;28:4300–6.
72 Prat A, Pascual T, De Angelis C, *et al*. HER2-Enriched subtype and ErbB2 expression in HER2-positive breast cancer treated with dual HER2 blockade. *J Natl Cancer Inst* 2020;112:46–54.
73 Kensler KH, Sankar VN, Wang J, *et al*. PAM50 molecular intrinsic subtypes in the nurses' health study cohorts. *Cancer Epidemiol Biomarkers Prev* 2019;28:798–806.
74 Cocco E, Javier Carmona F, Razavi P, *et al*. Neratinib is effective in breast tumors bearing both amplification and mutation of ErbB2 (HER2). *Sci Signal* 2018;11. doi:10.1126/scisignal.aat9773. [Epub ahead of print: 09 Oct 2018].
75 Ross DS, Zehir A, Cheng DT, *et al*. Next-Generation assessment of human epidermal growth factor receptor 2 (ErbB2) amplification status: clinical validation in the context of a hybrid capture-based, comprehensive solid tumor genomic profiling assay. *J Mol Diagn* 2017;19:244–54.
76 Bui MM, Riben MW, Allison KH, *et al*. Quantitative image analysis of human epidermal growth factor receptor 2 immunohistochemistry for breast cancer: guideline from the College of American pathologists. *Arch Pathol Lab Med* 2019;143:1180–95.
77 Yue M, Zhang J, Wang X, *et al*. Can AI-assisted microscope facilitate breast HER2 interpretation? A multi-institutional ring study. *Virchows Arch* 2021;479:443–9.
78 Aeffner F, Zarella MD, Buchbinder N. Introduction to digital image analysis in Whole-slide imaging. *A White Paper from the Digital Pathology Association. J Pathol Inform* 2019;10:9.
79 Yu J, Wang Q, Xue P, *et al*. A model for the impact of FFPE section thickness on gene copy number measurement by fish. *Sci Rep* 2019;9.

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