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 HER2/chromosome enumeration probe 17 (CEP17) ratio ≥2.0 and HER2 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, 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 20187 to address several concerns that were raised following the ASCO/CAP 2013 guidelines publication9 and to refine HER2 status definitions in certain situations. Detailed guideline recommendations on preanalytical and analytical variables for HER2 testing are covered in previous publications4−6 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),3,4 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/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
analyses indicated that only patients with HER2 equivocal (2+) expression and associated HER2 gene amplification confirmed by ISH benefited from anti-HER2 therapies.\(^2\)\(^3\)\(^13\)\(^14\)\(^15\) 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 as 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,\(^7\) (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...
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. HER2, 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 over-expression by IHC and HER2 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 HER2 gene amplification.

BC with an HER2 IHC score of 2+ and lacking evidence of HER2 gene amplification (IHC 2+/ISH−) is currently classified as HER2 negative, similar to tumours with an IHC score of 0 or 1+.9,10 As patients with these tumours do not benefit from conventional anti-HER2 therapy.11 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.13 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.13–18

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 trial11 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.40–43 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
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, 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. 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 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.

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.
Best practice

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. It is more common in BC with equivocal HER2 protein expression (IHC 2+; approximately 10% of cases) and low level HER2 gene amplification, and may contribute to inaccurate designation of HER2 status.

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. 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.

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.

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 appreciated at ×10 (D), or ×20 (E) but can be seen at ×40 (F).
Best practice

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/CEP17 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 be 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/CEP17 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. 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). With regard to smear preparation, although alcohol fixation has been reported to be very effective in preserving the molecular components, the ASCO/
CAP guidelines recommend formalin fixation. 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. 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. 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 (e.g., 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 (e.g., 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. BC bone metastases showing IHC score 3+ are considered positive. ISH testing...
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/ccribiform, pure low grade adenoid cystic carcinoma, fibromatosis-like metastatic 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 versus grade 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/samples 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.63 64

► 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.65 66 Pathways to achieve fast HER2 TATs in clinical practice have been proposed.69
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. 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. 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. 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, 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 and an increase in HER2 copy number, and to provide a list of genes putatively correlated with HER2 therapy response or resistance. 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.

**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 and recommendations to improve accuracy, precision and reproducibility of HER2 assessment in BC using image analysis tools have been published. 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 testing 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 members 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.
Best practice

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