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GUIDELINES

Guidelines for HER2 reporting in breast cancer: Recommendations by the Malaysian Breast Pathology Working Group

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Abstract

This consensus aims to develop a standardised guideline for human epidermal growth factor-2 (HER2) immunohistochemistry interpretation and reporting in Malaysia to support optimal therapeutic decision-making and research compatibility. An expert committee comprising pathologists and oncologists from public, private, and academic institutions convened to review existing international recommendations and taking into the consideration of local healthcare resource variations. The committee aims to harmonise reporting terminology in the reporting of HER2 testing, with emphasis on HER2-low and HER2-ultralow categories. A standardised HER2 reporting is crucial to ensure Malaysian patients benefit equitably from emerging HER2-targeted therapies. We hope this guideline could prepare the national pathology community in leading the evolving landscape of breast cancer management.

Keywords: HER2-low, HER2-ultralow, breast cancer, Malaysia, guideline

INTRODUCTION

The landscape of human epidermal growth factor-2 (HER2)-expression in breast cancer is rapidly evolving. It is no longer confined to a binary system, the recognition of HER2-low and ultralow tumours opens a promising frontier in targeted therapy. Trastuzumab Deruxtecan (T-Dxd) is a newly developed HER2-targeted antibody-drug conjugate (ADC) that targets HER2-positive tumour cells. It delivers a cytotoxic effect directly to tumour cells, even HER2-low cells, leading to DNA damage and cell death. Approximately 60% of human epidermal growth factor receptor 2 (HER2)–negative metastatic breast cancers express low levels of HER2 (IHC 1+ or 2+/ISH negative).[1,2]

DESTINY-Breast04 study found T-Dxd treated HER2-low metastatic breast cancer has better progression-free survival (PFS) and overall survival (OS) which was published in the New England Journal of Medicine.[3] In the PFS, the median survival was 9.9 months with T-DXd versus 5.1 months with chemotherapy, whereas the median OS was 23.4 months with T-DXd versus 16.8 months with chemotherapy.[3] This study challenges the traditional binary classification of HER2 status. It is essential all practicing pathologists need to be aware of this

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update; hence, this guideline is timely to guide the best practice in Malaysia.

In Malaysia, pathologists do not have a consistent way of HER2 score reporting. The possible scenarios could be 1) a simple HER2 positive and HER2 negative, 2) HER2 score 0 (negative), 1 (negative), 2 (equivocal) and 3 (positive), or HER2 score 0 (negative), 1 (negative, incomplete membrane staining in >10% of invasive tumour cells, 2 (equivocal, weak to moderate complete membrane staining in >10% of invasive tumour cells, 3 (positive, strong complete membrane staining in >10% of invasive tumour cells). This expert committee consists of pathologists and oncologists, practicing in the broad sectors of including private, government and university teaching hospitals.

The main purpose of this collaboration was to formalise a national consensus and create a guide to ensure uniformity in HER2 reporting throughout Malaysian pathology services in tandem with current breast cancer management.

Prevalence of HER2 positive breast cancer cases in Malaysia

Globally, HER2-positive account for 15% to 20% of all cases of breast cancer.[4] In a study conducted at a Malaysian tertiary hospital, reported 15% were classified as triple positive (Estrogen Receptor (ER)/ Progesterone Receptor (PR)/HER2-positive), 17.9% were ER/PR negative and HER2 positive, and the remaining were HER2-negative tumours.[5] Taratino *et al.* (2020)[2] showed an estimated prevalence of HER2-low in about 50% of breast cancer. Sandhya *et al*. (2024)[6] focused on HER2-negative tumours and found 29% could be classified as HER2-ultralow and 65% as HER2-low. A multicentre study on breast cancer in China reported 54.5% were HER2-low and 10.6% were HER2-ultralow.[7]

List of commonly used HER2 antibodies

1. Ventana PATHWAY anti-HER2/neu (4B5) (Roche) - Rabbit monoclonal primary antibody
   Link:https://diagnostics.roche.com/global/en/products/lab/her2-neu-4b5-ventana rtd001197.html
2. HercepTest (Dako/Agilent) - Rabbit monoclonal primary antibody
   Link:https://www.agilent.com/en/product/pharmdx/herceptest-kits
3. Bond Oracle CB11 (Leica Biosystems) – Mouse monoclonal primary antibody
   Link:https://www.leicabiosystems.com/sites/default/files/2020-10/Bond_Oracle_HER2_IHC_system_TA9145_ROW-EN-CE_Rev_B.pdf
4. A0485 c-erbB-2 Oncoprotein (Dako/Agilent) - Rabbit polyclonal primary antibody
   Link:1)https://www.citeab.com/antibodies/2390688-a0485-c-erbb-2-oncoprotein;2)https://www.agilent.com/cs/library/packageinsert/public/102441005.PDF

INTERPRETATION OF HER2 IMMUNO-HISTOCHEMISTRY (Table 1)

Positive

Score 3+: strong, complete circumferential membrane staining observed in >10% of invasive tumour cells (Fig 1A)

Note: Strong intensity staining is defined as equivalent to the intensity of 3+ in the control sample. It will be easily visible using a low power objective

Equivocal

Score 2+: weak to moderate, complete circumferential membrane staining observed in >10% of invasive tumour cells (Fig. 1B)

OR

Strong, complete circumferential membrane staining observed in ≤10% of invasive tumour cells

OR

Micropapillary carcinoma: moderate to strong but incomplete (basolateral or lateral) should be considered as Score 2+

Note: Reflex HER2-in situ hybridisation (ISH) should be performed

Negative

Score 1+: faint/ weak, incomplete membrane staining observed in >10% of invasive tumour cells (Fig. 1C)

Score 0^{+}: faint/ weak, incomplete membrane staining observed in ≤10% of invasive tumour cells

Score 0^{null}: no staining observed (Fig. 1D)

HER2-low

Score 1+ or 2+ with ISH-negative.

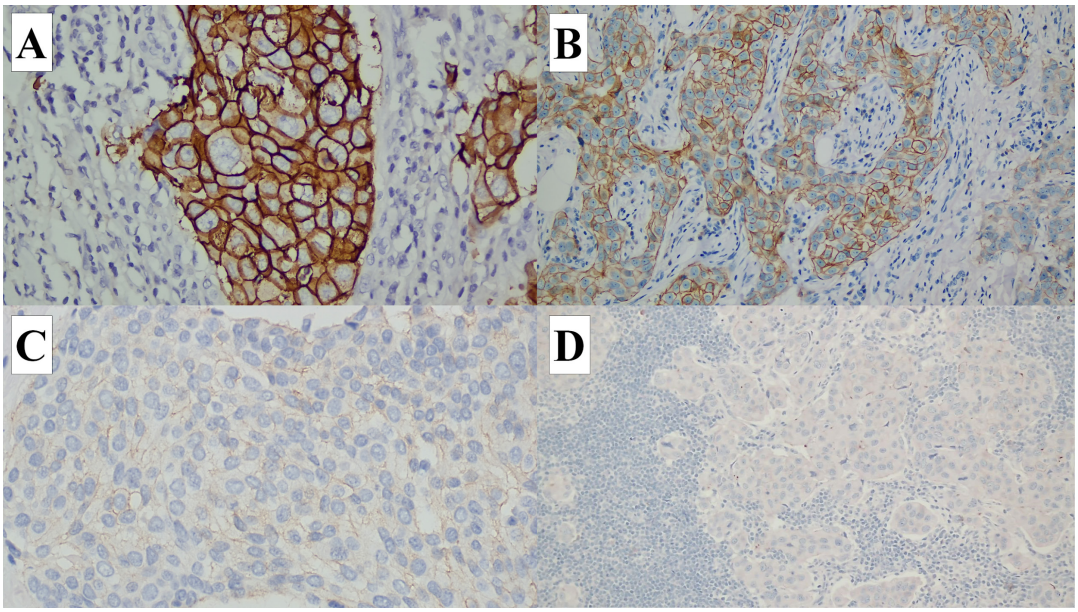

Figure 1. HER2 immunohistochemistry staining pattern. A) HER2 Positive (3+) with strong complete membrane staining (×20). B) HER2 (2+) with moderate complete membrane staining. C) HER2 (1+) with weak incomplete membrane staining. D) HER2 (0) (Negative) complete absent of staining.

Note: HER2-low patients may benefit from trastuzumab deruxtecan (Enhertu) in metastatic breast cancer.

HER2-ultralow
Score 0+: faint/ weak, incomplete membrane staining observed in ≤10% of invasive tumour cells

BEST PRACTICE RECOMMENDATION FOR HER2 IMMUNOHISTOCHEMISTRY REPORTING IN BREAST CANCER

Option 1
HER2: 3+ (Positive)
HER2: 2+ (Equivocal) (Reflex ISH testing)
HER2: 1+ (HER2-low)
HER2: 0+ (HER2-ultralow)
HER2: 0null (Negative)

Option 2
HER2: 3+ (Positive) (strong, complete membrane staining observed in >10% of invasive tumour cells)
HER2: 2+ (Equivocal) (weak/ moderate, complete membrane staining observed in >10% of invasive tumour cells)
HER2: 1+ (HER2-low) (faint/ weak, incomplete membrane staining observed in >10% of invasive tumour cells)
HER2: 0+ (HER2-ultralow) (faint/ weak, incomplete membrane staining observed in ≤10% of invasive tumour cells)
HER2: 0null (Negative) (no/ absent membrane staining)

Option 3
HER2: 3+ (Positive)
HER2: 2+ (Equivocal) (Reflex ISH testing)
HER2: 1+ (Negative)
HER2: 0+/0null (Negative)
Remarks (optional): In the Destiny-Breast 04 and 06 trials, "HER2-low" was considered IHC score 1+ or 2+/ISH-, and "HER2-ultralow" was IHC score 0+. Breast cancers with these staining patterns may be eligible for treatment with trastuzumab-deruxtecan in the metastatic setting.

DISCUSSION

Addressing HER2 intratumour heterogeneity
HER2 intratumoral heterogeneity is the co-existence of at least two distinct clones of tumour cells with varying HER2 expression in the same tumour. It can be further divided into genetic and non-genetic types. Genetic heterogeneity is subcategorised as 1) Clustered type: 2 distinct areas with different HER2 amplification. 2) Mosaic type: Intermingling of tumour cells

Table 1: Interpretation of HER2 immunohistochemistry

HER2 Score	Staining intensity	Staining pattern	Percentage	Interpretation
3+	Strong/ intense	Complete circumferential membrane	>10% of invasive tumour cells	Positive
2+	Weak to moderate	Complete circumferential membrane	>10% of invasive tumour cells	Equivocal (Reflex ISH testing)
	Strong	Complete circumferential membrane	≤10% of invasive tumour cells	
1+	Faint/ weak	Incomplete membrane	>10% of invasive tumour cells	Negative/ HER2-low
0^{+}	Faint/ weak	Incomplete membrane	≤10% of invasive tumour cells	Negative/ HER2-ultralow
0^{null}	No staining	No membrane staining	0% of invasive tumour cells	Negative

with different HER2 amplification. 3) Scattered type: Isolated HER2 amplified tumour cells in a predominantly non-amplified tumour. Non-genetic heterogeneity is defined as tumour cells with HER2 gene amplification without HER2 protein expression, intermixed with tumour cells with concordant HER2 amplification and protein expression.[8,9]

When the 3+ HER2 expression is >10% as a distinct clustered separate population from non 3+ overexpressed background, it is reported as Positive (3+). The IHC score of the non 3+ areas is also reported. It is suggested to use College of American Pathologists (CAP) guideline in reporting clustered heterogeneity (see below). If ISH testing is performed, it should be counted at the 3+ areas, rather than an average of the overall 3+ and non 3+ areas. If 3+ staining areas is ≤10%, the result is interpreted as HER2 equivocal (2+).

Clustered Heterogeneity (discrete separate 3+ populations)

□ Not applicable
□ Not identified (3+ staining is homogenous throughout the tumour)
□ Present (distinct 3+ and non 3+ areas/ staining population)

Specify percentage of 3+ and non 3+ staining areas

3+: _____ 2+: _____ 1+: _____ 0: _____

Causes of false positive HER2 immunohistochemistry

1. Edge artifact – Cells at the edge of the tissue samples might stain stronger due to better fixation at the edge; hence, preserving the antigen. The concentration of antibody may be more concentrated at the edge, and tissue at the edge of the sample dry faster.
2. Cytoplasmic and granular staining – It may be mistaken as membrane staining or obscure membrane staining (Figs. 2A and 2B).
3. Basolateral staining in micropapillary carcinoma – may be mistakenly classified as HER2 (1+) staining due to incomplete membrane staining (Fig. 2C).
4. Misinterpretation of ductal carcinoma in situ (DCIS) as HER2-positive. Therefore, it is important to first examine the H&E stain slide before performing HER2 interpretation. HER2 scoring must be performed only of the invasive component.
5. Absence of control sample – It is crucial to have at least a HER2-positive (3+) tissue sample as control. This also helps in the comparison between sample and control tissues for HER2 (3+) areas. Control tissue with HER2 (3+) is used as the benchmark.
6. Sclerosing adenosis around DCIS that mimic invasive carcinoma may be mistakenly scored as HER2 (3+) positive.

Causes of false negative HER2 immunohistochemistry

1. Prolonged cold ischaemia time – The American Society of Clinical Oncology/ College of American Pathologists (ASCO/ CAP) recommends that cold ischaemia time is kept under 1 hour. The study showed cold ischaemia time of >4 hours resulted in minimal IHC staining changed.[10]

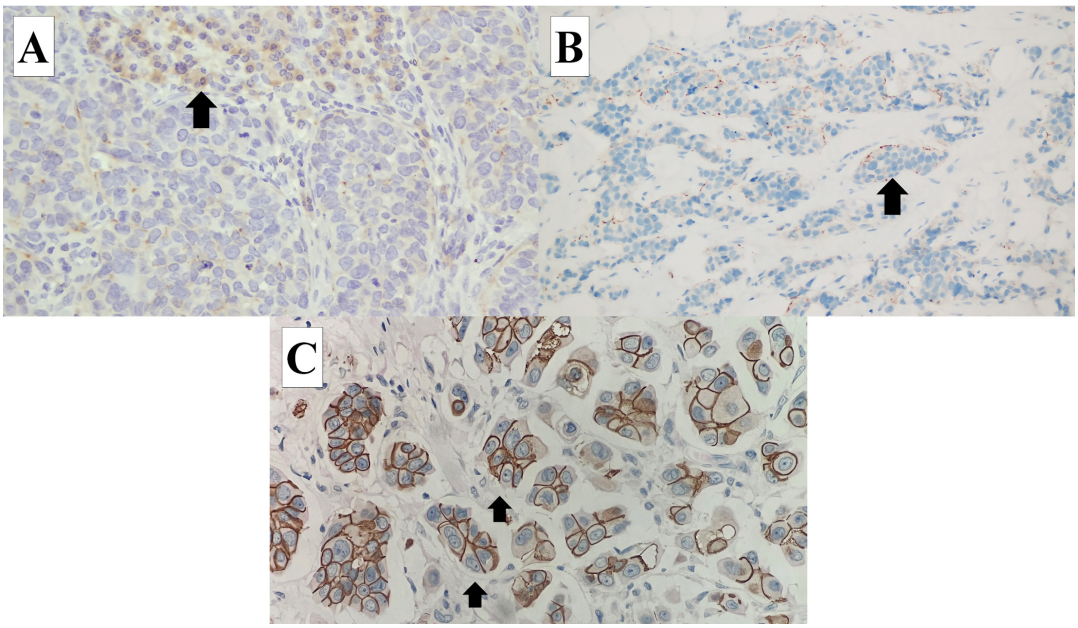

Figure 2. Pitfalls in HER2 immunohistochemistry. A) HER2 with cytoplasmic staining pattern (×20). B. HER2 with granular staining pattern (×10). C) HER2 with basolateral staining pattern in micropapillary carcinoma.

2. Tumour heterogeneity in small biopsy tissue sample – In HER2-Negative (0null) of highly grade and negative to weak ER/PR expression in a biopsy sample, repeat testing on subsequent excision sample should be considered.
3. Improper antibody selection and concentration.

Situations to consider repeat HER2 assessment on excision specimen

1. Histological discordance with HER2 positivity on core biopsy sample: a) Grade 1 invasive carcinoma, b) Special type of breast carcinoma, examples tubular carcinoma, cribriform carcinoma, mucinous carcinoma and lobular carcinoma.
2. Small volume of tumour in the core biopsy sample.
3. The morphology of tumour is histologically different between core biopsy and excision samples.
4. HER2 scoring is suboptimal due to artefact and fixation issue.
5. Post-neoadjuvant treated breast cancer: There is no established guidelines. A repeat HER2 testing could be considered if significant amount of residual tumour or the residual tumour has a distinctly different morphology from the pre-treatment tumour.

GENERAL RECOMMENDATIONS

1. At present, ASCO/CAP guidelines do not recommend the incorporation of HER2 low and HER2 ultra-low terminology in the histopathology report.
2. Tissue is to be placed in buffered 10% formalin within 1 hour (ideally) or not more than 4 hours of surgery and is to be fixed for at least 6 hours.
3. A HER2-Positive (3+) tumour tissue sample is to be included as control. The intensity of staining of control will be used as the benchmark for HER2 Positive (3+) in the test sample.
4. Laboratories should consider participating in the QAP on HER2 IHC testing for both technical and diagnostic categories.
5. HER2 expression is to be considered only of the invasive component.
6. Basolateral staining in micropapillary carcinoma should be considered as HER2 Equivocal (2+), instead of 1+.
7. Cytoplasmic and granular staining should not be considered in HER2 score assessment.
8. Cytology samples are not recommended for HER2 testing due to fixation issues and inability to distinguish invasive carcinoma cells from in-situ carcinoma cells.

FUTURE CONSIDERATIONS

1. With the advancement of artificial intelligence, whether to consider using AI-assisted HER2 IHC interpretation. The determination of percentage of staining may be more accurate and improve turnaround time. The use of AI may reduce the inter- and intra-observers' variability in interpreting HER2 (0null, 0+ and 1+).
2. Local validation of AI-assisted HER2 IHC interpretation can be explored through national QAP programs.
3. Studies should investigate whether there is a difference in HER2 expression in biopsy versus excision, metastatic and recurrent tissue samples. This will provide insight as to whether there is a need to repeat testing.
4. Studies should compare other biomarkers such as PDL1, PI3K, AKT, PTEN and microRNAs changes in relation to HER2, to establish the cell-cell signalling pathways in breast cancer.

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