Assessment of HER2 status in breast cancer

Breast cancer continues to rank second, after lung cancer, as a cause of cancer death in women. It is also a leading cause of premature mortality in women.

Breast cancer is the most common cancer in women worldwide. In 2012, deaths from breast cancer accounted for 783 000 years of potential life lost and an average of 19 years of life lost per death.

Even though mortality from breast cancer has declined steadily since 1990, largely due to improvements in early detection and treatment, it remains the most common cause of cancer mortality among women in developing countries and the second most common cause of cancer mortality (lung cancer being first) among women in developed countries.

According to the Cancer Registry of 2003, one in every 31 South African women will develop breast cancer in their lifetime. If diagnosed early, 90% of such women will be alive after five years.

Approximately 15 % to 20 % of women diagnosed with breast cancer have human epidermal growth factor receptor 2 (HER2) positive breast cancers and should be treated with targeted therapies to improve their clinical outcome. The American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) released updated recommendations for HER2 testing in breast cancer in 2013.

The purpose of HER2 testing is to identify patients who could benefit from effective HER2-targeted therapies, which have shown to substantially improve survival in patients with HER2-positive invasive breast cancer.

HER2 IMMUNOHISTOCHEMICAL ANALYSIS

HER2 immunohistochemical (IHC) assays are designed to analyse preserved HER2 protein with a bright field microscope on formalin-fixed, paraffin-embedded (FFPE) clinical tissue sections. However, IHC assays are subjective and semi-quantitative for determining the expression levels of the HER2 protein in breast cancer.

The specificity and sensitivity of HER2 IHC assays are influenced by selections of pretreatment conditions, antibody clones, and signal detection systems. When the threshold for HER2 IHC 2+ cases was reduced from 30 % to 10 % of the tumour cells having incomplete membrane staining, HER2 ISH reflex test frequencies were increased by 59% and the HER2 gene amplification detection of breast cancer cases was increased by 33%.

Because of the subjective nature of HER2 status assessment using HER2 IHC assays, it is possible that HER2 IHC equivocal cases might be determined to be HER2 negative and would not receive further HER2 status assessment.

HER2 *IN SITU* HYBRIDISATION ANALYSIS

HER2 ISH assays are aimed to visualise the HER2 gene with or without a reference target chromosome 17 centromere (CEN17) in FFPE tissue sections of clinical samples. There are multiple signal detection systems approved by the US Food and Drug Administration (FDA) for HER2 ISH assays:

1. Fluorescence in situ hybridisation (FISH).
2. Chromogenic in situ hybridization (CISH).
3. Dual in situ hybridisation (DISH).

Signal preservation in HER2 CISH and DISH slides are superior to HER2 FISH slides and the assessment of the tissue morphology is also easier in CISH and DISH assays.

A major advantage of HER2 ISH assays compared to HER2 IHC assays is that the HER2 status assessment is conducted by quantitative analyses and also includes internal control cells adjacent to tumour cells in the same tissue section.

However, it has been reported that HER2 equivocal cases may result in different HER2 status assessment dependent on which ISH assays are used and the scoring criteria selected. It should be noted that the level of HER2 gene amplification significantly affects pathological complete response to trastuzumab-based neoadjuvant therapy.

COLD ISCHEMIA TIME EFFECTS ON HER2 TESTING

The 2013 ASCO/CAP HER2 testing recommendations state that the time from excision to the initiation of tissue fixation (cold ischaemic time) should be as short as possible and the duration of tissue fixation should be six to 72 hours.

The duration of formalin fixation appears to be less of an issue with HER2 testing results, as up to 96 hours of tissue fixation exposure (more than an over weekend length) did not significantly affect the HER2 testing scores. However, the exact effects of cold ischaemic time on HER2 IHC and HER2 ISH assays are still controversial.

HER2 IHC assays should be performed at institutions that also perform HER2 ISH assay as the primary method of HER2 status assessment, particularly on HER2 ISH negative cases. It is clear that the cold ischaemic time affects the quality of HER2 testing. However, we speculate that variation in published data was caused by, not only the cold ischaemic time, but also the warm ischaemic time and the use of various HER2 testing methods. Thus, further improvements in HER2 testing approaches are required for the accurate diagnosis of breast cancer.

CURRENT HER2 TESTING APPROACH AND TUMOUR HETEROGENEITY

Because previous references suggested that approximately 20% of HER2 testing may not be accurate among breast cancer patients, the ASCO/CAP HER2 testing guideline was introduced in order to improve the quality of HER2 testing in 2007 and was updated in 2013.

Breast cancer is a heterogeneous disease and the intra-tumoural heterogeneity poses an immense challenge to accurate breast cancer diagnostics. Along with the pre-analytic and assay variables discussed previously, HER2 tumour heterogeneity is an unavoidable factor for accurate HER2 status assessment.

HER2 tumour heterogeneity describes a cancer with different tumour cell populations (including obvious HER2 amplification status) in the same patient and this creates difficulty in treating breast cancer patients with a single targeted therapy.

There are two main types of HER2 tumour heterogeneity:

- Clustered HER2 amplification showing a congregated HER2 amplified tumour cell population bordered by non-HER2 amplified tumour cells.
- Mixed HER2 amplification showing dispersed HER2 amplified tumour cells among non-HER2 amplified tumour cells.


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HER2 tumour heterogeneity is therefore a challenge for accurate HER2 status assessment of breast cancer as well as other cancers, particularly gastric cancer that is notorious for HER2 tumour heterogeneity. However, the HER2 heterogeneity guidelines are based on expert opinions, not clinical outcome data.

Some studies have reported that HER2 positive breast cancer patients with HER2 genetic heterogeneity had significantly shorter disease-free survival times compared to without heterogeneity and that HER2 heterogeneity was an independent predictor of breast cancer clinical outcome to trastuzumab therapy.

Future studies are needed to define the significance of HER2 heterogeneity for treating breast cancer patients with HER2-targeted therapies and therefore a guideline is required for clinically relevant heterogeneity data collections to improve breast cancer patient care.

DEVELOPMENT OF HER2 GENE-PROTEIN ASSAY

HER2 IHC and HER2 ISH assays are used for the same purpose: Selecting breast cancer patients for HER2-targeted therapy. However, HER2 IHC and HER2 ISH assays detect biologically different targets, namely HER2 protein overexpression for the target of HER2 therapy and HER2 gene amplification (with or without a CEN17 control probe) correlated to HER2 protein overexpression, respectively. Each assay has advantages and disadvantages.

The major discordant results of HER2 protein and HER2 gene statuses are caused by tumour heterogeneity, mainly detected in HER2 equivocal cases. In general, HER2 status analyses of equivocal cases require both HER2 IHC and HER2 ISH assays, unless different tissue blocks of breast cancer patients are available for retesting with the same IHC or ISH test.

Therefore, our concept was to combine HER2 IHC and HER2 ISH assays into one assay: The HER2 gene-protein assay (GPA) so that the HER2 protein and HER2 gene status can be determined more accurately at the individual cell level in heterogeneous breast cancer.

We presented feasibility study data of the automated tricolor HER2 GPA application at the joint meeting of the Japan Society of Histochemistry and Cytochemistry and the Histochemistry Society in 2006.

This version of the HER2 GPA application was for HER2 IHC with NBT/BCIP detection (purple), HER2 gene ISH with diaminobenzidine (DAB) detection (brown dots), and CEN17 with fast red (red dots). This was the first attempt to perform an automated tricolor HER2 GPA with breast cancer cases. The next full publication of a dual HER2 GPA application was by Ni *et al* in 2007.

The third publication of dual HER2 GPA with DAB detection (brown color) for both HER2 gene and HER2 protein was published by Reisenbichler *et al* in 2012. These investigators compared two versions of their HER2 GPA protocols:

- HER2 IHC followed by HER2 CISH.
- HER2 CISH followed by HER2 IHC.

They reported that the HER2 CISH signal was not obtainable when the CISH was performed after HER2 IHC. Thus the authors focused on optimising a GPA in which the HER2 CISH assay was conducted before the HER2 IHC assay.

±15%-20%

of women diagnosed with breast cancer have HER2 positive breast cancers

We published the first bright field tricolor HER2 GPA assay development and performance analyses with xenograft tumours and breast cancer tissue microarrays in 2012.

This version of the HER2 GPA allows for the visualisation of HER2 protein with DAB detection (brown), HER2 gene with silver detection (black dots), and CEN17 with fast red detection (red dots). This HER2 GPA is a fully automated protocol that uses FDA approved HER2 IHC and HER2 DISH assays.

In order to develop the bright field tricolor HER2 GPA protocol, we first established a dual color ISH assay for HER2 gene and CEN17 targets and this dual color ISH assay became the HER2 DISH assay. The HER2 IHC assay was already approved by the FDA, so it was decided not to alter the protocol.

Therefore HER2 IHC had to be performed using DAB detection prior to HER2 DISH in order to maintain the sensitivity, dynamic range, and familiar brown staining of HER2 IHC.

We did encounter unacceptable background staining as well as difficulty obtaining ISH signals, but these challenges were overcome allowing us to successfully combine HER2 IHC and HER2 DISH as one assay on the same tissue section.

The goal in developing the HER2 GPA was solely for more accurate diagnosis of breast cancer patients for HER2-targeted therapy.

The performance of our tricolor HER2 GPA assay has been evaluated further on breast cancer, gastric cancer, and lung cancer. A publication by Li *et al*, which focused on HER2 equivocal cases, reported that the HER2 GPA divided breast cancer cases accurately into HER2 positive, equivocal, and negative statuses and that it aided in the identification of HER2 heterogeneity as well.

Chenard *et al* demonstrated a 25% time saving in scoring a breast cancer case by a single HER2 GPA slide scoring protocol compared to a typical IHC followed by a HER2 DISH testing protocol. Therefore, the use of the HER2 GPA approach can optimize laboratory workflow and deliver faster, more accurate HER2 status assessment for patient management.

Stålhammar *et al* identified another advantage of the HER2 GPA – a pathologist can determine an area for HER2 DISH scoring based on HER2 IHC staining at a lower magnification.

All publications conclude that the HER2 GPA is an ideal test for HER2 equivocal cases that require additional HER2 testing. Furthermore, the HER2 GPA method allows for the detection of tumour cell populations with amplified HER2 gene signals, without overexpression of the HER2 protein in HER2 positive, equivocal, and negative clinical cases

This new type of HER2 gene/protein discordant phenomenon at individual cell levels has been termed 'HER2 micro-heterogeneity' and has been proposed as a new category of HER2 heterogeneity.

It has also been confirmed that HER2 micro-heterogeneity exists in xenograft tumours of a HER2 positive cell line. Further studies are required to examine the HER2 micro-heterogeneity hypothesis as heterogeneity status may play a significant role in determining appropriate treatment choices for HER2 positive breast cancer patients.

French novelist Marcel Proust said: "The real act of discovery consists not in finding new lands, but in seeing with new eyes" and the HER2 GPA approach might be a new eye to see HER2 tumour heterogeneity in breast cancer.

Because HER2 IHC and HER2 ISH test results are the decision making factors in selecting breast cancer patients for a targeted therapy, the simultaneous analyses of HER2 gene and HER2 protein status by the HER2 GPA provides pathologists better eyes to assess the HER2 status at individual cell level in difficult cases.

Sponsored article.

REFERENCE

Nitta H, Kelly BD, Allred C *et al*. The assessment of HER2 status in breast cancer: the past, the present, and the future. *Pathology International*, 2016. SF

Multiple choice questions

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1 What is the percentage of breast cancers that are HER2 positive?

a. 12%-21%. A
b. 15%-20%. B
c. 15%-21%. C
d. 15%-20.5%. D

2 Describe the author's tricolour HER2 GPA assay protocol.

a. HER2 IHC is performed using detection prior to HER2 DISH. A
b. HER2 IHC is performed using detection prior to HER2 FISH. B
c. HER2 IHC is performed using detection prior to HER2 CISH. C
d. None of the above. D

3 What advantages do ISH assays offer IHC assays?

a. ISH assay analysis is not quantitative and therefore does not include internal control cells adjacent to tumour cells in the same tissue section. A
b. ISH assay analysis is quantitative and also includes internal control cells adjacent to tumour cells in the same tissue section. B
c. ISH assay analysis is quantitative and also includes internal control cells adjacent to tumour cells in the same lymphatic section. C
d. None of the above. D

4 What factors affect the specificity and sensitivity of HER2 IHC?

a. HER2 IHC is affected by pretreatment conditions. A
b. HER2 IHC is affected by antibody clones. B
c. HER2 IHC is affected by signal detection systems. C
d. All of the above. D

5 Besides pre-analytic and assay variables, what poses additional challenges in breast cancer diagnostics?

a. Tumour consistency. A
b. Intra-tumoural heterogeneity. B
c. Tumour growth and intra-tumoural heterogeneity. C
d. Tumour heterogeneity and intra-tumoural heterogeneity pose additional challenges. D

6 What challenges does tumour heterogeneity pose?

a. Accurate HER2 status assessment of breast cancer and creates difficulty in treating breast cancer patients with a single targeted therapy. A
b. Difficulty in treating breast cancer patients on multiple therapy. B
c. Accurate HER2 status assessment of breast cancer and creates difficulty in treating breast cancer patients with on multiple targeted therapy. C
d. None of the above. D

7 Discordance between HER2 IHC and HER2 ISH results are caused by?

a. Tumour location. A
b. Tumour heterogeneity. B
c. Tumour size. C
d. Tumour spread. D

8 Discordant results are evident is these cases?

a. HER2 ambigous cases. A
b. HER2 equivocal cases. B
c. HER2 misleading cases. C
d. All of the above. D

9 Who published the second publication on the HER2 GPA application?

a. Nietze *et al* in 2009. A
b. Niam *et al* in 2007. B
c. Ni *et al* in 2010. C
d. None of the above. D

10 What advantages does the HER2 GPA assay offer?

a. More accurate diagnosis of breast cancer patients for HER2-targeted therapy A
b. Aids in the identification of HER2 heterogeneity and demonstrates time saving. B
c. A pathologist can determine an area for HER2 DISH scoring based on HER2 IHC staining at a lower magnification. C
d. All of the above. D

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