Andrzej Marszałek[1, 2], Maciej Krzakowski[3]

[1]Department of Pathology and Cancer Prevention, Poznan University of Medical Sciences, Poland
[2]Greater Poland Cancer Centre, Poznan, Poland
[3]Department of Lung and Thoracic Cancers, Maria Sklodowska-Curie Institute of Oncology, Warsaw, Poland

Recommendations for testing of predictive marker HER2 in patients with invasive breast cancer

Recommendations in accordance with American Society of Clinical Oncology (ASCO) guidelines from 30th May 2018

Standpoint of National Consultants in: pathology and clinical oncology

Address for correspondence:
Prof. dr hab. n. med. Andrzej Marszałek
Katedra Patologii i Profilaktyki Nowotworów, Uniwersytet Medyczny im. K. Marcinkowskiego w Poznaniu
e-mail: amars@ump.edu.pl

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ABSTRACT

New recommendations for HER2 status (receptor or gene) testing in breast cancer were published following long-term analysis of clinical effectiveness of molecularly targeted treatment based on predictive factor of HER2 status. The new protocols were developed to eliminate equivocal cases, and the new procedure leads to final statement as HER2 positive or HER2 negative. Current testing algorythms are presented.

Key words: HER2, breast cancer, recommendations

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Introduction

Assessment of predictive factors for cancer treatment has become a standard part of pathologic reports. The principles for the preparation of a diagnostic materials — biopsy or post-operative specimens — were published by international societies (World Health Organisation — WHO; International Agency for Research on Cancer — IARC; College of American Pathologists — CAP), adapted to Polish conditions (see standards published by Polish Society of Pathologists; PSP), and are commonly acknowledged.

The technologic development provides broader opportunities for marker assessment. In breast cancer — besides full pathologic evaluation that includes diagnosis of less common histological variants — it is mandatory also to assess the width of cancer-free margins and the presence of extranodal invasion in metastatic lymph nodes. One of the most crucial factors for deciding on further treatment is the evaluation of predictive markers — namely, the presence and percentage of cancer cells with oestrogen receptors (ER), progesterone receptors (PgR), human epidermal growth factor receptors (HER2), and percentage of cancer cells in active phases of the cell cycle evaluated with Ki67 index. All enumerated elements are necessary parts of a pathological report. These markers are usually assessed using immunohistochemistry (IHC). The results of IHC are susceptible to several confounding factors (such as time of cold and warm ischaemia; type, concentration, and temperature of fixative; ratio between amount of fixative and tissue) at the stage of tissue collection, transportation to the pathology unit, and technological processes during final microscopic specimen preparation. The results of IHC staining are influenced by the subjective nature of assessment, es-

pecially when done by an inexperienced observer. IHC staining for ER, PgR, and Ki67 depends on reactions in nuclei and undergoes qualitative assessment, whether the specific marker is present or not. Therefore, the intensity of reaction is less important. However, in the assessment of HER2 expression the situation is clearly different, as discussed below.

HER2 expression

The term HER2 is commonly used to describe the presence of the receptor responsible for transmitting extracellular signalling induced by epidermal growth factor (EGF). The signal from the extracellular part of cell membrane — after the ligand connects with the receptor — is passed down to the cell due to increase of tyrosine kinase activity. Therefore, HER2 receptor can be considered as a member of the tyrosine kinase receptor family. The name HER2 is commonly recognised, but knowledgeable readers might encounter several other synonyms commonly used in literature (c-erbB2, ERBB2, neu, CD340, or transmembrane glycoprotein p185 — the product of *HER2* gene). The activation of HER2 receptor and transduction of signalling require dimerisation — the conjunction of two proteins/peptides in the case of HER receptor. HER2 receptor forms both homodimers and heterodimers with other receptors from the epidermal growth factor receptor (EGFR) family. In normal conditions, HER2 receptor plays a role in embryogenesis and has an influence on cell differentiation, mobility, and intercellular interactions. In breast cancer, HER2 protein expression is used as a predictive marker defining the benefit from targeted therapies. In some cases, the number of *HER2* gene copies is also evaluated. The subjective nature of HER2 expression assessment leads to several modifications of diagnostic guidelines, aiming at improving standardisation and reproducibility. In the last published update by the American Society of Clinical Oncology (from 30th May 2018), the main changes in the diagnostic algorithm and clinical implementation were aimed at the elimination of equivocal results that do not give direct suggestions for targeted treatment efficacy. Details regarding examination protocol and principles of assessment and interpretations are presented herein.

Recommendations (standard)

The assessment of HER2 overexpression or amplification (increased amount of protein or gene copy number) should be routinely done as part of the pathologic report. The standards of preparing diagnostic material are as follows.

Tissue material (obtained from core biopsy, mammotome biopsy, or post-surgery specimen) should be fixed in 10% buffered formalin (4% water solution of paraformaldehyde) immediately after acquisition. The fixing time should not exceed 96 hours. No other fixating agents are recommended. The fixed material should be prepared as formalin-fixed paraffin-embedded (FFPE) specimens. Samples should be sliced at 3–4-micrometre intervals and stained by IHC according to standard procedures with usage of both primary antibodies and certified detection systems (with a standardised protocol of assessment as recommended by the manufacturer).

Current principles of pathologic assessment

Implementation of the following pathologic assessment procedures, including IHC and in situ hybridisation techniques (ISH), aims at the elimination of "HER2 equivocal" outcomes. The introduced algorithm should lead to an unambiguous result of either "HER2 positive" or "HER2 negative". Nevertheless, clinicians, relying on their experience, may offer selected patients other treatment schedules. It should be reckoned that, according to novel recommendations, the final result of "HER2 positive" or "HER2 negative" might require an additional commentary in some cases. The presented guidelines do not change the financial regulations of Polish National Health Fund (NFZ, Narodowy Fundusz Zdrowia) regarding HER2-targeted treatment.

The assessment of HER2 protein expression in IHC and ISH is done by a pathologist. If, according to the presented procedures, IHC and/or ISH should be repeated, an additional pathologist responsible for the second assessment should not know the results of the primary evaluation ("blinded analysis").

In ISH procedures, it is recommended to use dual-probe ISH assays (assays that evaluate both aimed elements in a single procedure).

The principles of HER2 expression assessment in IHC procedure are presented in Table 1. Figure 1 presents decision algorithm after IHC evaluation.

If the IHC assessment leads to a result of HER2 2+, the number of *HER2* gene copies should be tested by ISH. Two types of ISH assays are available: with a single-probe (probe aimed only at *HER2* gene) or with double-probe [probe aimed at *HER2* gene and on either chromosome 17 centromere (CEP17, most commonly used) or either *TP53* gene (*HER2*/*TP53* assays simplifies the assessment)]. Due to the situations described below, assays with double-probe are recommended.

In the case of single-probe ISH procedure, the assessment algorithm is presented in Figure 2.

Table 1. Principles of HER2 expression assessment in IHC staining

Result	Description of observed changes	Interpretation
HER2 0	No staining or incomplete membrane staining that is faint or barely perceptible and in ≤ 10% of tumour cells	NEGATIVE outcome
HER2 1+	Incomplete membrane staining that is faint or barely perceptible and in > 10% of tumour cells	NEGATIVE outcome
HER2 2+	Weak to moderate complete membrane staining in > 10% of tumour cells	EQUIVOCAL outcome
HER2 3+	Strong complete membrane staining in > 10% of tumour cells	POSITIVE outcome

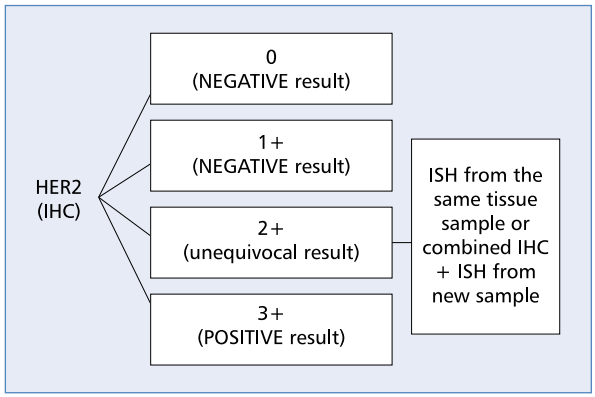

Figure 1. Decision algorithm after immunohistochemical (IHC) evaluation

If a pathologic examination of core needle biopsy specimen shows no presence of HER2 overexpression (negative HER2 test), in situations justified by clinical criteria (such as presence of grade 3 carcinoma), HER2 expression might be re-evaluated from post-operative material. Details regarding clinical decisions will be presented herein.

If *HER2* gene assessment with ISH techniques is required, both ISH and IHC slides should be evaluated by the same pathologist. It is especially crucial in the cases described below:

— A) in cases with HER2/CEP17 ratio (number of *HER2* gene copies per number of chromosome enumeration probe 17) equal to or greater than 2, but with number of *HER2* gene copies lower than 4, combined assessment of both ISH and IHC should be performed from the same tissue sample and, preferably, in the same pathology unit. The combined assessment might result in:
  - Aa) HER2-positive result, if HER2 3+ was obtained in IHC exam;
  - Ab) If the result of re-evaluation of IHC examination is again HER2 2+:
    - o additional assessment of ISH in at least 20 cells in areas with HER2 2+ invasive breast cancer should be performed by a second pathologist, if possible (**Important!** The

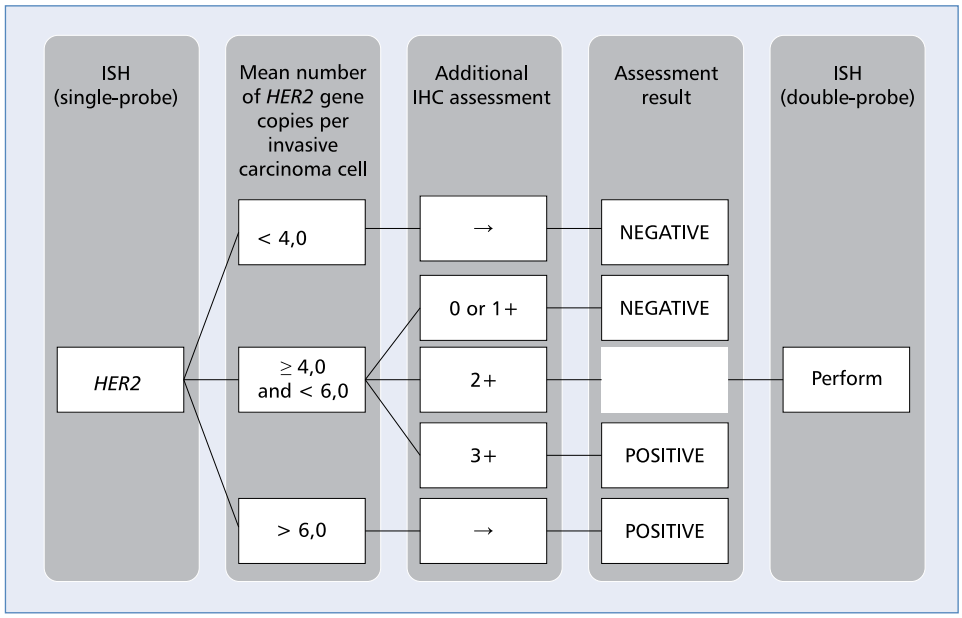

Figure 2. Diagnostic decision algorithm after single-probe (for HER2 gene) in situ hybridisation (ISH) procedures

ISH examination should assess the same areas in which IHC examination showed presence of HER2 2+ invasive breast cancer). The assessment should unequivocally determine HER2 status as positive or negative (**Important!** It is especially important in cases when the HER2/CEP17 ratio is different in comparison with the first assessment). The result of the second assessment is considered to be final,

- If, according to secondary ISH evaluated by a second pathologist, the HER2/CEP17 ratio is equal to or greater than 2 but the number of *HER2* gene copies is lower than 4, then the result should be considered "HER2 negative" with an additional comment;

- Ac) HER2-negative result, if an additional IHC examination result is HER2 0 or HER2 1+.

— B) in cases of ISH assessment resulting in a mean number of *HER2* gene copies equal to or higher than 6 with concomitant HER2/CEP17 ratio lower than 2, re-evaluation should be undertaken (or if ISH and IHC were performed from different tissue samples, additional IHC assessment should be done from the FFPE material used for ISH evaluation) with a combined ISH and IHC slide analysis from the same tissue sample, preferably in the same pathology unit. This assessment may result in:

- Ba) HER2-positive result if HER2 3+ was obtained in IHC exam;
- Bb) If the result of re-evaluation of IHC examination is again HER2 2+:
  - additional assessment of ISH in at least 20 cells in areas with HER2 2+ invasive breast cancer should be performed by a second pathologist, if possible (**Important!** The ISH examination should assess the same areas in which IHC examination showed presence of HER2 2+ invasive breast cancer). The assessment should unequivocally determine HER2 status as positive or negative (**Important!** It is especially important in cases when the HER2/CEP17 ratio is changed when compared to the first assessment). The result of the secondary assessment is considered to be final;
  - If, according to secondary ISH evaluated by a second pathologist, the HER2/CEP17 ratio is equal to or greater than 2 but the number of *HER2* gene copies is higher than 6, then the result should be considered "HER2-positive".
- Bc) HER2-negative result with a commentary, if additional IHC examination result is HER2 0 or HER2 1+;

— C) in cases of ISH assessment resulting in a mean number of HER2 gene copies equal to or higher than 4 but lower than 6, with concomitant HER2/CEP17 ratio lower than two, re-evaluation of HER2 status should be undertaken (or if ISH and IHC were performed from different tissue samples, additional IHC assessment should be done from the FFPE material used for ISH evaluation) with a combined ISH and IHC slide analysis from the same tissue sample, preferably in the same pathology unit. This assessment may result in:

- Ca) HER2-positive result if HER2 3+ was obtained in IHC exam;
- Cb) If the result of re-evaluation of IHC examination is again HER2 2+:
  - additional assessment of ISH in at least 20 cells in areas with HER2 2+ invasive breast cancer should be performed by a second pathologist, if possible (**Important!** The ISH examination should assess the same areas in which IHC examination showed presence of HER2 2+ invasive breast cancer). The assessment should unequivocally determine HER2 status as positive or negative (**Important!** It is especially important in cases, when the HER2/CEP17 ratio is changed when compared to the first assessment). The result of the secondary assessment is considered to be final;
  - If, according to secondary ISH evaluated by a second pathologist, the mean number of *HER2* gene copies is equal to or higher than 4 but lower than 6 and concomitant HER2/CEP17 ratio lower than 2, then the results should be considered as "HER2-negative" with a commentary;
- Cc) HER2-negative result with a commentary, if additional IHC examination result is HER2 0 or HER2 1+.

Diagnostic algorithms for the cases described above are presented in Figure 3 to 5.

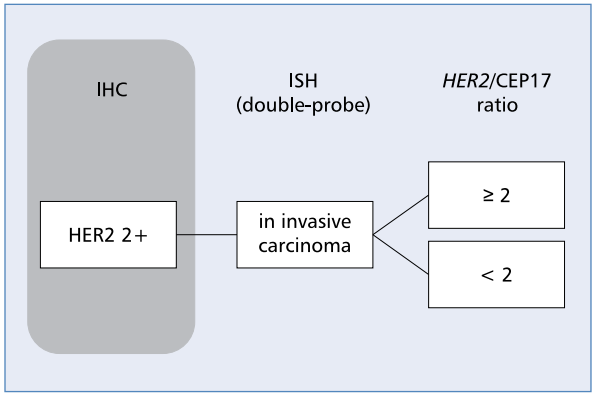

Figure 3. Algorithm for evaluation of *HER2* gene copy number in ISH examination using double-probe assays (for *HER2* gene and chromosome 17 centromere; CEP17)

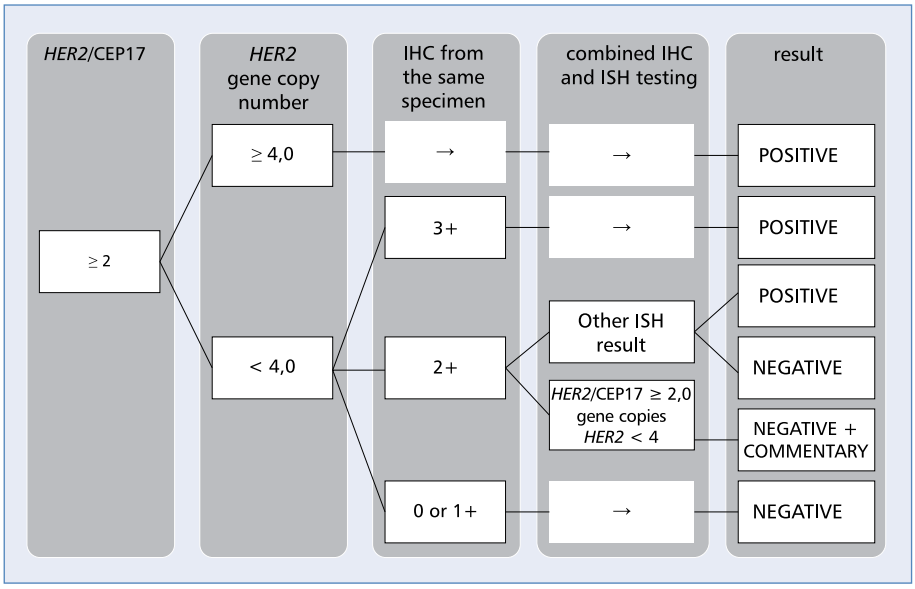

Figure 4. Algorithm for diagnosis and evaluation of *HER2* gene copy number in cases with *HER2*/CEP17 ratio equal or greater than 2

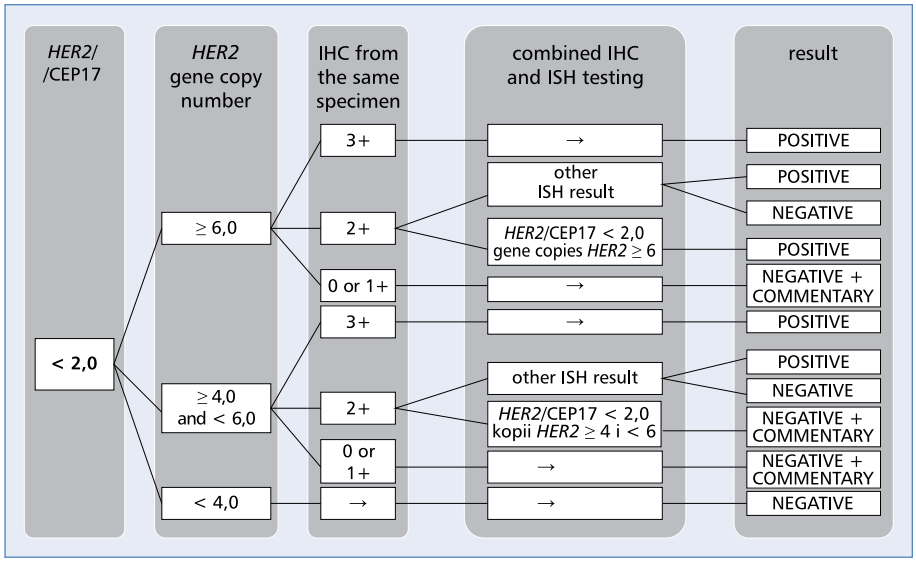

Figure 5. Algorithm for diagnosis and evaluation of *HER2* gene copy number in cases with *HER2*/CEP17 ratio lower than 2

Current recommendations regarding re-evaluation of HER2 status due to discrepancies between HER2 examination and histological type of invasive breast cancer

In some situations, especially when pathologic assessment and IHC examination are performed in different pathology units, discrepancies between obtained and expected results might be seen in selected clinical conditions. Considering clinical trial details and epidemiological data, it is known that some types of invasive breast cancer are mostly HER2-positive or HER2-negative. If the mentioned discrepancy arises, the algorithms presented below should be used (Figs. 6–8).

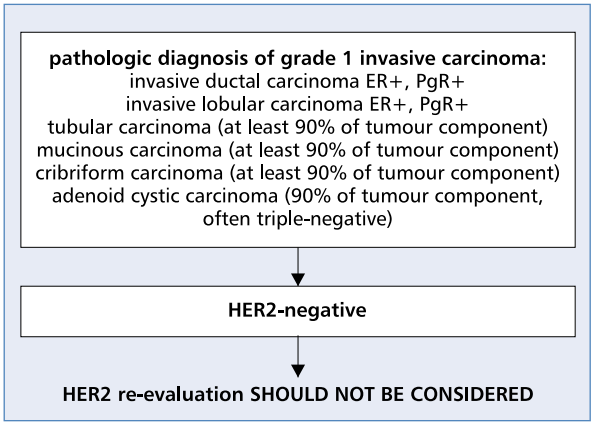

Figure 6. If immunohistochemical (IHC) examination results in an HER2-negative outcome, re-examination of HER2 is discouraged in the situations described below

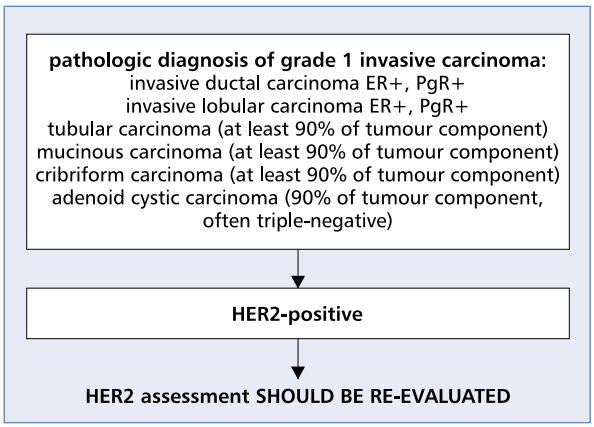

Figure 7. If immunohistochemical (IHC) results in an HER2--positive outcome, the presented clinical factors justify re-examination of HER2 status

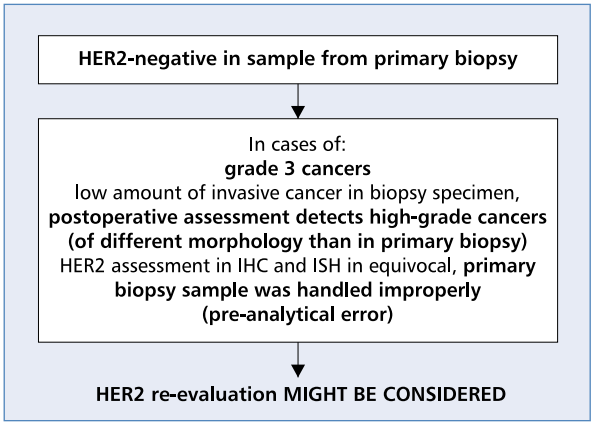

Figure 8. If immunohistochemical (IHC) examination from primary biopsy results in an HER2-negative outcome, the presented factors might justify re-examination of HER2 status in post-operative specimens

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